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The application of voxel-based methods to magnetic resonance imaging in the study of psychiatric disorder.

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The application of voxel-based methods to magnetic resonance imaging in the study of psychiatric disorder

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A thesis submitted to the Royal College of Surgeons in Ireland in fulfilment of the requirement for the degree of Doctor of Philosophy, January 2012

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Professor Kieran C Murphy and
Professor Declan GM Murphy
Signed thesis declaration

I declare that this thesis, which I submit to RCSI for examination in consideration of the award of a Doctor of Philosophy degree (PhD) is my own personal effort. Where any of the content presented is the result of input or data from a related collaborative research programme this is duly acknowledged in the text such that it is possible to ascertain how much of the work is my own. I have not already obtained a degree in RCSI or elsewhere on the basis of this work. Furthermore, I took reasonable care to ensure that the work is original, and, to the best of my knowledge, does not breach copyright law, and has not been taken from other sources except where such work has been cited and acknowledged within the text.

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Appendix 1: Publications -


(Sundram and Murphy, 2011a). Schizophrenia and 22q11.2 deletion syndrome. Encyclopaedia of Life Sciences. Chichester, UK: John Wiley & Sons, Ltd (http://www.els.net/)

(Sundram et al., 2010b). White matter microstructure in 22q11 deletion syndrome: a pilot diffusion tensor imaging and voxel-based morphometry
study of children and adolescents. *Journal of Neurodevelopmental Disorders* 2(2): 77-92


(Sundram et al., 2011). White matter microstructural abnormalities in the frontal lobe of adults with antisocial personality disorder. *Cortex (in press)*

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Summary of thesis

While there are a number of psychiatric disorders as classified by the major international coding systems, however, the application of modern neuroimaging methods has only been utilised on a limited basis with some disorders receiving more research attention than others. Consequently, psychiatric phenotypes that have been relatively understudied are investigated further in this thesis. These disorders correspond to psychiatric disorder in: 22q11 deletion syndrome, temporal lobe epilepsy, antisocial personality disorder and Asperger syndrome. Subjects with each of these diagnoses were recruited and then compared to healthy matched controls using the application of novel whole-brain voxel-based analyses to their magnetic resonance imaging data whereby white matter integrity and/or brain tissue volume was assessed in each experimental study of this thesis.

In Study 1, young people with 22q11 deletion syndrome were found to have significant differences in both white matter microstructure and volume. Additionally, there was preliminary evidence that within 22q11 deletion syndrome, some regional differences in fractional anisotropy were associated with allelic variation in COMT and with schizotypy. In Study 2, while significant grey and white matter volume deficits were found in temporal lobe epilepsy with comorbid psychosis, these abnormalities encompassed not only the medial temporal lobe structures but also extended to lateral temporal and extratemporal regions whereby some of the deficits also overlapped with those found in schizophrenia.
In Study 3, reduced fractional anisotropy was found in antisocial personality disorder and psychopathy in tracts of interhemispheric, posterior brain and frontal lobe networks. Additionally, fractional anisotropy deficits in the frontal lobe demonstrated a significant negative correlation with psychopathy measures. Finally, in Study 4, adults with Asperger syndrome were specifically recruited and found to not only demonstrate impairments in white matter microstructural integrity in regions relevant to social skills and behaviour but also in more widespread white matter networks.
Publications, presentations and awards

Peer reviewed journal publications

**Sundram F**, Deeley Q, Sarkar S, Daly E, Latham R, Craig M, Raczek M, Fahy T, Picchioni M, the UK AIMS network, Barker GJ and Murphy DGM. White matter microstructural abnormalities in the frontal lobe of adults with antisocial personality disorder. *Cortex (in press)*


Peer reviewed book chapters


Published conference abstracts


International conference oral presentations


White matter microstructure in children with Velocardiofacial Syndrome: A Diffusion Tensor Imaging and Voxel Based Morphometry study. 6th International Conference on 22q11.2DS, Utrecht, The Netherlands Jun 2008

National conference oral presentations

Neuroanatomical correlates of psychosis in temporal lobe epilepsy: a voxel-based morphometry study. Annual meeting of the Royal Academy of Medicine in Ireland Biomedical Sciences Section, Jun 2010

White matter microstructural abnormalities extend beyond the frontal lobe in antisocial personality disorder: a pilot diffusion tensor imaging study. Senior
Registrars oral presentation at the annual Royal Academy of Medicine in Ireland, Psychiatry Section meeting, Nov 2010, Dublin

Neuroanatomical correlates of psychosis in temporal lobe epilepsy: a voxel-based morphometry study. Senior Registrars oral presentation at the annual Royal Academy of Medicine in Ireland, Psychiatry Section meeting, Dec 2009, Dublin

White matter microstructure in children with Velocardiofacial Syndrome: A Diffusion Tensor Imaging and Voxel Based Morphometry study. Royal College of Surgeons in Ireland Annual Research Day 2008 meeting, Dublin

White matter microstructure in children with Velocardiofacial Syndrome: A Diffusion Tensor Imaging and Voxel Based Morphometry study. Registrars oral presentation at the annual Royal Academy of Medicine in Ireland, Psychiatry Section meeting 2008, Dublin

Poster presentations

Sundram F, Deeley Q, Sarkar S, Daly E, Latham R, Craig M, Fahy T, Picchioni M, the UK AIMS network, Barker GJ and Murphy DGM. White matter microstructural abnormalities in the frontal lobe of adults with antisocial personality disorder: a pilot diffusion tensor imaging study. Molecular Medicine Ireland Clinician Scientist Annual Meeting, July 2011, Dublin

Sundram F, Deeley Q, Sarkar S, Daly E, Latham R, Craig M, Fahy T, Picchioni M, the UK AIMS network, Barker GJ and Murphy DGM. White matter microstructural
abnormalities in the frontal lobe of adults with antisocial personality disorder: a pilot diffusion tensor imaging study. Royal College of Surgeons in Ireland Annual Research Day meeting, Apr 2011, Dublin

**Sundram F, Deeley Q, Sarkar S, Daly E, Latham R, Craig M, Fahy T, Picchioni M, the UK AIMS network, Barker GJ and Murphy DGM.** White matter microstructural abnormalities in the frontal lobe of adults with antisocial personality disorder: a pilot diffusion tensor imaging study. 19th European Congress of Psychiatry (European Psychiatric Association), Mar 2011, Vienna, Austria

**Sundram F, Murphy DG, Murphy KC.** White matter microstructure in children with Velocardiofacial Syndrome: A Diffusion Tensor Imaging and Voxel Based Morphometry study. Sheppard Prize Annual meeting, Feb 2008, Beaumont Hospital, Dublin

**Awards**


Winner of the Psychiatry Registrars’ Prize for best oral presentation - White matter microstructure in children with Velocardiofacial Syndrome: A Diffusion Tensor Imaging and Voxel Based Morphometry study. Annual Royal Academy of Medicine in Ireland, Psychiatry Section meeting 2008, Dublin, Ireland
My sincere thanks to all my family, collaborators and study participants who have helped me achieve the completion of this thesis. It has been a long process and would not have been possible without your sustained input and support all these years.

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I am extremely thankful to Prof Kieran Murphy for providing me the opportunity and support to gain further training in neuroimaging in London and continued encouragement after my return to Dublin. I truly appreciate the regular meetings and emails to enable the realisation of the various studies in this thesis as well as the constructive feedback over the years and throughout the thesis writing phase. I would like to express my deepest gratitude to Prof Declan Murphy who facilitated my tight integration with his research group at London and who enabled recruitment of participants and analysis of the neuroimaging data at his research lab. Through his critical lens and witty feedback, my skills in manuscript preparation and completion have also taken shape! It was an invaluable experience and I truly enjoyed my time
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List of abbreviated terms

2-D - two-dimensional
3-D - three-dimensional
6-D - six-dimensional
22q11DS - 22q11 deletion syndrome
AC-PC - anterior commissure-posterior commissure plane
AD - axial diffusivity
ADC - apparent diffusion coefficient
ADD/ADHD - attention deficit hyperactivity disorder
ADI-R - autism diagnostic interview - revised
ADOS - autism diagnostic observation schedule
ADPEAF - autosomal dominant partial epilepsy with auditory features
AIMS - Autism Imaging Multicentre Study
ANOVA - analysis of variance
ANCOVA - analysis of covariance
ASPD - antisocial personality disorder
ASQ - autism screening questionnaire
BET - Brain Extraction Tool
BOLD - blood oxygenation level-dependent
BPAD - bipolar affective disorder
CHARGE syndrome - coloboma of eye, heart defects, atresia of the nasal choanae, retardation of growth, genital abnormalities, and ear abnormalities
CHD7 - chromodomain helicase DNA-binding protein-7
COMT - catechol-O-methyltransferase
CNV - copy-number variation
CT/CAT - computed tomography
DSM-IV - Diagnostic and Statistical Manual of Mental Disorders 4th edition
DALY - disability-adjusted life year
DAMP - deficits in attention, motor control and perception
DARTEL - Diffeomorphic Anatomical Registration Through Exponentiated Lie Algebra
DNA - deoxyribonucleic acid
DT-MRI - diffusion tensor MRI
DWI - diffusion-weighted imaging
EEG - electroencephalography
EPI - echo-planar imaging
FA - fractional anisotropy
FISH - fluorescence in situ hybridisation
fMRI - functional MRI
FSIQ – full scale IQ
FSL - Functional Software Library
GABRB3 - gamma-aminobutyric acid (GABA) A receptor, beta 3
HARDI - high angular resolution diffusion imaging
HDR syndrome - hypoparathyroidism, deafness and renal dysplasia
HFA - high-functioning autism
ICD-10 - International Classification of Diseases 10th revision
IQ - intelligence quotient
IR-SPGR - inversion recovery prepared spoiled gradient recalled acquisition in the steady state
MD - mean diffusivity
Met-COMT - Methionine-COMT
MLPA - multiplex ligation-dependent probe amplification
MMR - measles, mumps, and rubella
MNI - Montreal Neurological Institute
MR - magnetic resonance
MREC - Multicentre Research Ethics Committee
MRI - magnetic resonance imaging
MRS - magnetic resonance spectroscopy
MTS - mesial temporal sclerosis
OPCRIT - Operational Criteria Checklist for Psychotic Illness
PCL - Psychopathy Checklist
PCL-R - Psychopathy Checklist - Revised
PCL-SV - Psychopathy Checklist - Short Version
PET - positron emission tomography
PFC - prefrontal cortex
PRODH - proline dehydrogenase
RA - relative anisotropy
RD - radial/transverse diffusivity
ROI - region of interest
SD - standard deviation
SDQ - strengths and difficulties questionnaire
SNP - single nucleotide polymorphism
SPECT - single photon emission computed tomography
SPGR - spoiled gradient recalled acquisition in the steady state
SPM - statistical parametric mapping
SPSS - Statistical Package for the Social Sciences
TBSS - tract-based spatial statistics
TBX1 - T-box 1
TDR - typically deleted region
TE - echo time
TLE - temporal lobe epilepsy
TLE+psychosis - TLE and associated psychosis
TR - repetition time
Val-COMT - Valine-COMT
VBM - voxel-based morphometry
VCFS - velo-cardio-facial syndrome
vmPFC - ventromedial PFC
VSD - ventricular septal defect
VR - volume ratio
WAIS-R - Wechsler Adult Intelligence Scale - Revised
WISC-III - Wechsler Intelligence Scale for Children-III
XBAMM - Brain Activation and Morphometric Mapping
Chapter 1:

The application of voxel-based methods to magnetic resonance imaging in the study of psychiatric disorder

This chapter is divided into four components. It starts with a background of magnetic resonance imaging techniques that are currently available to the neurosciences. This is then followed by a description of various magnetic resonance acquisition paradigms and in particular, conventional and diffusion tensor magnetic resonance imaging. Additionally, voxel-based analytical techniques for the study of magnetic resonance images are detailed. Following these, descriptions are provided on the major neuroanatomical divisions of the brain and currently recognised white matter pathways that overall serve as a neuroanatomical guide for all the experimental studies in subsequent chapters.

Additionally, the relevant literature is assessed with regard to psychiatric disorders that have been relatively understudied where the focus of this thesis is assessment of such disorders through the modern magnetic resonance imaging techniques outlined earlier. Finally, the literature is examined with specific focus on the phenotypes studied in this thesis which correspond to psychiatric disorder in: 22q11 deletion syndrome, temporal lobe epilepsy, antisocial personality disorder and Asperger syndrome.
1.1. Introduction

The last four decades have witnessed significant advances in the neurosciences which have permitted the introduction of new technologies for understanding the neurobiology and evolution of psychiatric disorders (Abou-Saleh, 2006; Stahl and Niculescu, 2002). Among these developments, the advancement of computing, physics, mathematics and clinical imaging sciences hold much promise in unravelling the aetiology of psychiatric disorders.

Currently, neuroimaging in psychiatry is broadly divided into assessment of structure, function, electrophysiology and connectivity of cerebral tissue. Structural methods encompass the following neuroimaging techniques: computed tomography (CT or CAT) and magnetic resonance imaging (MRI) while magnetic resonance spectroscopy (MRS), functional MRI (fMRI) and emission tomography (which includes positron emission tomography (PET) and single photon emission computed tomography (SPECT)) represent the functional methods. Electroencephalography (EEG) provides electrophysiological measurements of brain activity while diffusion MRI is the only method presently available for the assessment of cerebral connectivity in-vivo among all the neuroimaging modalities. Overall, only MRI acquisition and analytical techniques are utilised in this thesis while an overview of MRI and diffusion MRI is provided below.

1.1.1. Magnetic resonance imaging (MRI)
The underlying principles of MRI were first described in the 1940s by two independent authors (Bloch et al., 1946; Purcell et al., 1945) who were subsequently awarded the Nobel prize in Physics in 1952. Thereafter, the nuclear magnetic relaxation times of tissues and tumours were first shown to differ in 1971 and provided impetus to investigate medical disorders based on nuclear magnetic resonance rather than ionising radiation (Damadian, 1971).

While MRI permits the visualisation of the internal structure and function of the brain and is similar in some respects to CT, there are several intrinsic differences between these modalities. CT utilises ionising radiation whereas MRI uses nuclear magnetic resonance through a powerful longitudinal magnetic field ($B_0$) to align the nuclear magnetisation of hydrogen atoms of water in the body. Given that hydrogen is abundantly found in tissues and provides the strongest signal, it is the element most commonly imaged with MRI. The nucleus of a hydrogen atom is composed of a single spinning proton with a positive charge that generates a small magnetic field and therefore a magnetic dipole. Radiofrequency fields are used with MRI to systematically alter the alignment of this magnetisation through pulses of radiofrequency (Larmor frequency) thereby causing the hydrogen nuclei to spin in unison. Radiofrequency pulses also tip over the acquired longitudinal magnetisation by 90 degrees into the transverse plane and when the magnetic field is removed, the excited protons emit energy which can be detected by the receiver coil of a magnet. This signal decay termed free induction decay can subsequently be manipulated by additional magnetic fields so as to build up enough information to reconstruct an image of the brain (Budinger and Lauterbur, 1984; Lauterbur, 1973).
The magnetic resonance appearance of brain tissue is dependent on a number of factors including the intrinsic tissue properties which are described through measures of relaxation time (T1 and T2), spin density and spectral and flow shifts (Hendee and Morgan, 1984). When hydrogen nuclei align with the magnetic field producing their own net longitudinal magnetisation (i.e. in the same direction as $B_0$), this alignment (which is a relaxation towards the equilibrium state) occurs with a time constant T1. T2 on the other hand refers to a time constant for decay of transverse magnetisation (in a plane perpendicular to $B_0$). These and other settings such as echo times (TE), flip angles and repetition times (TR) can be adjusted via imaging protocols to determine the exact nature of the image obtained. TE corresponds to the time between radiofrequency pulse and measurement while TR represents the time between successive radiofrequency pulses. Amongst others, potential imaging acquisition sequences include spin-echo, fast spin-echo, gradient-echo, echo-planar imaging (EPI) and inversion recovery that each vary in terms of these settings.

Overall, MRI has certain clinical disadvantages relative to CT - for instance CT remains the gold-standard neuroimaging modality for assessing acute trauma and cerebral vascular haemorrhage. Also, as CT is less expensive than MRI, it is more widely available and accessible. Moreover, CT has fewer contraindications in comparison to MRI whereby metallic implants or pacemakers can be drawn towards the powerful magnets used during scanning sessions with the latter. However, as CT utilises ionising radiation, there are transient and permanent health risks associated with radiation exposure (Brenner and Hall, 2007) such as an increased risk of cancer mortality and as a result, may restrict its safe use in the research setting.
Additionally, MRI provides a higher degree of resolution than CT between the different soft tissue classes of the body thereby making it particularly useful in psychiatric, neurological, musculoskeletal, oncology and cardiovascular imaging. Further, lesions such as brain cysts, tumours, necrosis and white matter changes are readily detectable with MRI. MRI also enables identification of changes in brain regions such as the brain stem and cerebellum that are difficult to detect with CT. Consequently, MRI studies have been able to define the neural substrates of major psychiatric disorders and have provided a neuroanatomical basis to guide other imaging methodologies (Fleck et al., 2008) such as connectivity studies with diffusion MRI (which is covered in more detail in the Section 1.1.3.).

1.1.2. Conventional MRI analytical methodology

Following image acquisition and post-processing, MRI analytical techniques may be applied that are usually based on manual or computerised methodology. The earliest methods for examining structural brain anomalies were based on subjective qualitative assessments rather than objective quantitative study where for example a T2-weighted image (which provides good tissue contrasts) would be used to identify lesions. Qualitative methods are particularly useful when there are gross structural abnormalities, however, abnormalities are less likely to be detected when scattered diffusely. Therefore, quantitative studies are currently recommended for comparing brain anatomy between groups as the objectivity that these afford enable meaningful comparison between studies, provide detail on diffuse structural abnormalities and permit replication. There are currently two main techniques used in quantitative
analysis with several approaches subsumed within these: 1) region of interest (ROI); and 2) voxel-based morphometry (VBM). A more detailed description of VBM is provided later (see Section 1.1.2.2.).

After image acquisition and usually as part of post-processing (but prior to image analysis), a set of further steps is required to prepare MR images for computerised analysis. These may include scalp, skull and meningeal stripping thereby leaving only the underlying brain intact for analysis. Additionally, the brain may be rotated to align it with a particular plane e.g. anterior commissure-posterior commissure plane or the image brightness or contrast may be adjusted; these parameters may be adjusted manually or automatically depending on the image analysis protocol. For instance, these parameters may be adjusted manually when they are used in conjunction with ROI studies or alternatively, these may be computerised/automated as in the case with modern VBM. Next, overviews are provided of ROI and VBM methods as applied to MRI data where arguments are provided for using VBM in this thesis.

1.1.2.1. Region of interest (ROI) analyses of conventional MRI data

ROI methods are often used when investigators have *a priori* hypotheses and therefore, assessments are confined to a limited set of brain regions. These methodologies are conceptually simple and are usually conducted through manual steps such as delineating the ROI through hand tracing or point counting on a per
pixel basis or alternatively, via semi-automated techniques such as stereology where a three-dimensional (3-D) grid of fixed dimensions is placed on the entire brain and subsequently the volumes of structures of interest are calculated by the manual marking of pixels falling within each two-dimensional (2-D) slice of the structure of interest by a rater. The volume of the structure of interest which corresponds to the total number of marked pixels is then automatically calculated by computer software.

Advantages of ROI analyses include the ability to analyse a discrete structure or parts of a structure and when differences are found, these findings are robust. However, the methodology is time-consuming and dependent on intra/inter-rater reliability (Fleck et al., 2008). Further, biases may result especially due to variations in identifying neuroanatomical landmarks and therefore, results may not be easily reproducible. As the method is labour-intensive and time-consuming, this limits the overall number of structures that can be investigated at any one time. Further limitations are that it is not possible to concurrently assess a large number of subjects and thus, sample sizes tend to be restricted. Also, ROI methods are not able to identify diffuse or subtle brain abnormalities.

1.1.2.2. Voxel-based morphometry (VBM) analyses of conventional MRI data

Currently, the analytical approaches involved in computational neuroanatomy are broadly divided into those that examine differences in brain shape and those that assess differences in the local composition of brain tissue after macroscopic
differences in shape have been controlled for (Ashburner and Friston, 2000; Wright et al., 1995). The former uses deformation fields that map any individual brain onto a standard reference template so as to characterise neuroanatomy whereas the latter compares images on a per voxel basis after the deformation fields have been used to spatially normalise images. When images are computerised, pixels are used to describe 2-D image data while voxels (volumetric pixel) represent volume data in 3-D space.

Computational neuroanatomic techniques can either use the deformation fields themselves for analysis or alternatively, these fields may be utilised to spatially normalise images. These normalised images can then be entered into an analysis comparing regional brain differences. When comparing groups, deformation-based morphometry uses deformation fields to identify differences in the relative positions of structures within subjects’ brains, whereas tensor-based morphometry refers to methodology that localises differences in the local shape of brain structures (Ashburner and Friston, 2000). VBM is an example of a computerised statistical analytical method developed for use with MRI which is automated, unbiased and comprehensive and is classified as a tensor-based morphometry approach. The software package most often used with VBM is statistical parametric mapping (SPM; http://www.fil.ion.ucl.ac.uk/spm/). VBM uses Jacobian determinants and allows a comparison of parenchymal tissues and cerebrospinal fluid throughout the whole brain thereby enabling the identification of subtle and regionally-specific spatial brain differences between groups that may be masked by an apparently ‘normal’ total volume.
Performing a VBM analysis involves several steps, the first of which involves the spatial normalisation of high-resolution images from all subjects being studied into the same stereotactic space and this also permits for correction of global brain shape differences between subjects. SPM performs a 12-parameter affine transformation followed by a non-linear registration using a mean squared difference matching function (Ashburner and Friston, 2000). During the process of normalisation, the template image used could involve a single MRI scan or can alternatively be created by averaging a number of different MRI scans that have been placed in the same stereotactic space.

Spatial normalisation is then followed by segmentation of subject brains into their various constituent tissue classes e.g. grey matter, white matter and cerebrospinal fluid usually based on prior probability brain maps as well as voxel intensity (as in SPM) to define and classify each tissue type at individual voxels. To assist with the segmentation process, the intensity of the structural MR images is often corrected for inhomogeneities caused by technical issues associated with the MRI head coil (Malhi and Lagopoulos, 2008). Modulation then corrects for changes in volume induced by non-linear normalisation by multiplying voxel intensities by a modulation matrix derived from the normalisation step. Modulation enables inferences to be made about tissue volume rather than tissue density or concentration. Smoothing is then performed on the respective tissue segments to improve the signal-to-noise ratio and to enable the data to be more normally distributed where a convolution with an isotropic Gaussian kernel is applied.
Finally, voxel-wise statistical tests are performed to compare the smoothed images where parametric or non-parametric statistics based on the general linear model may be applied to the data (Ashburner and Friston, 2000; Bullmore et al., 1999; Nichols and Holmes, 2002). Statistical comparisons made at the level of each individual voxel between the brains of subject groups under study often involve complex statistical models and corrections for multiple comparisons are also performed during a VBM analysis using the theory of Gaussian random fields so as to reduce the reporting of false positives for instance by using correction based on family-wise error or false discovery rate methods (Friston et al., 1994; Genovese et al., 2002). Significant regions of difference are localised and typically reported in standard space for instance by using the Talairach coordinate system (Talairach and Tournoux, 1988). Following the finding of statistically significant results, it is possible to make inferences relating to the measurement of interest. Refer to Fig. 1.1 for an overview of the steps involved in VBM.

In summary, VBM is able to identify regional differences in the tissue content of grey or white matter and has several advantages over ROI approaches. VBM is an automated and comprehensive technique that allows an unbiased examination of the entire brain on a per voxel basis. It is less time-consuming and provides for increased objectivity and is able to identify subtle brain differences. It also permits a hypothesis-free survey of the whole brain while enabling the identification of spatially specific and subtle brain differences between groups which may then lead to hypothesis generation.
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Fig. 1.1 Overview of voxel-based morphometry processing steps (adapted from http://www.fil.ion.ucl.ac.uk/spm/doc)
1.1.3. Diffusion MRI

Diffusion MRI is the term that refers to magnetic resonance methods where the contrast mechanism uses the property of Brownian or random motion of water molecules (Schaefer et al., 2000) to reproduce intrinsic features of tissue microstructure (Pierpaoli et al., 1996) whereby the diffusion of water molecules is a passive process driven by the ambient temperature (Parker, 2004). When water molecules move within tissues, free diffusion of these molecules is often not occurring as they encounter a number of barriers to diffusion; these include cell membranes and myelin sheaths and therefore what is observed as regarded as an apparent diffusion (Parker, 2004; Taylor et al., 2004). Diffusion MRI provides significantly more data than conventional MRI and enables 3-D in-vivo images of biological tissues involving diffusion of water molecules.

Diffusion-weighted sequences can be applied to high-performance magnetic field gradients (Schaefer et al., 2000) where the signal observed in a diffusion-weighted image is determined by the apparent diffusion coefficient (ADC) and a weighting factor \( b \) that is applied as part of the magnetic field gradient. The \( b \) value is proportional to the product of the diffusion time interval and the square of the strength of the diffusion gradient (Hagmann et al., 2006). Most studies have shown a \( b \) value of between 900 and 1,500 s mm\(^{-2}\) to be suited for human brain diffusion imaging when considering acquisition time and noise-independent measurements (Armitage and Bastin, 2001; Xing et al., 1997) where most have used 1,000 s mm\(^{-2}\). Diffusion MRI is complementary to conventional MRI datasets and currently, EPI...
sequences are most frequently used due its short acquisition time, greater resistance to variability in phase between application of diffusion-encoding gradients and reduced susceptibility to subject motion (Abe et al., 2010). Diffusion MRI which was developed in the 1990s (Basser et al., 1994a; Filler, 2009; Le Bihan, 1991; Moseley et al., 1990; Richards et al., 1992), provides for a comprehensive means of characterising the random motion or diffusivity of water molecules in-vivo (Jones et al., 2002a) and it is possible with diffusion MRI to examine the diffusion of water especially along white matter pathways of the brain and currently, the field of diffusion MRI is divided into two distinct groups of application: diffusion-weighted imaging (DWI) and diffusion tensor MRI (DT-MRI).

DWI represents a simpler model of assessing the diffusion process where each image voxel possesses an image intensity that reflects a single best measurement of the rate of water diffusion at that particular 3-D location. DWI is especially useful when the tissue of interest displays mainly isotropic (i.e. equal in all directions) water movement for example, the grey matter of the cerebral cortex or subcortical nuclei, where the diffusion rate appears to be constant regardless of the axis of measurement (Lansberg et al., 2001). When measuring water molecule motion with DWI, usually the ADC is calculated (Le Bihan et al., 1986; Schaefer et al., 2000) which is a quantitative measure of the degree of restriction of water molecules and represents a spatial average of the diffusion coefficient over an image voxel (Dong et al., 2004).

DT-MRI on the other hand is a technique for evaluating white matter through the preferred direction of water molecule diffusion termed anisotropy but through directionally independent image acquisitions (Basser et al., 1994a; b). DT-MRI is a
more complex imaging modality relative to DWI where each voxel in a DT-MRI image is usually described by one or more pairs of parameters: a preferred direction of diffusion and a rate of diffusion which are described in terms of 3-D space (Filler, 2009). Diffusivity and anisotropy measures that are most often used with DT-MRI are fractional anisotropy (FA; directional diffusion along and coherence of axons) or mean diffusivity (MD; omni-directional diffusion) (Abe et al., 2010). Other less-often used measures are relative anisotropy (RA; magnitude of anisotropic part of tensor divided by isotropic part), volume ratio (VR; ratio of the ellipsoid volume to the volume of a sphere of radius \( r \)), axial diffusivity (AD; parallel diffusion) and radial/transverse diffusivity (RD; perpendicular diffusion).

MD is a scalar measure of total diffusion within a voxel which is calculated by division of the sum of the eigenvalues of the diffusion tensor (which correspond to the magnitude of diffusion in three orthogonal directions) by three which reflects change in cell density and extracellular space (Rugg-Gunn et al., 2001). FA on the other hand is a measure of the degree of anisotropy or directionality within a given voxel where values range from 0 (perfectly isotropic diffusion) to 1 (perfectly anisotropic diffusion) (Pierpaoli and Basser, 1996). FA also reflects the structural integrity and geometry within axonal fibres (Neil et al., 2002). From FA measures, it is possible to determine not only white matter tract orientation, trajectory and integrity but also characterisation of phenotype, brain development and unnoticed subtle changes on conventional T2-weighted MRI (Chenevert et al., 1990; Horsfield and Jones, 2002; Mori and Zhang, 2006).
DT-MRI is ideally suited for assessment of white matter in cerebral tissue and is predicated on the fact that anatomical structures such as cell membranes, myelin sheaths, as well as intracellular micro-organelles act as barriers to the free diffusion and movement of water (Malhi and Lagopoulos, 2008). As these structures restrict the free-flow of water molecules in white matter, DT-MRI assesses the preferential diffusion of water molecules along a particular axis aligned with the tissue’s internal structure e.g. the arrangement of axons in parallel bundles within myelin sheaths. However, the measured rate of diffusion as well as principal axis of diffusion will differ depending on the direction from which observations are measured. Therefore DT-MRI is usually acquired in at least six non-collinear directions where images are obtained with a different orientation of the diffusion sensitising gradients.

A pulsed diffusion magnetic gradient is applied during scan acquisition such that nondiffusion weighted images are obtained at each slice location together with multiple diffusion-weighted acquisitions from several orientations. Also during scan acquisition, images are synchronised with the cardiac cycle so that pulsatile variations in water molecule movement are taken into account. After DT-MRI acquisition, the properties of each voxel is calculated by vector or tensor mathematics from the different diffusion-weighted acquisitions and it is possible then to reconstruct the brain’s underlying neuronal microstructure. To enhance the estimation of DT-MRI-derived parameters, recent reports have suggested approximately 30 diffusion-encoding directions defined by the electrostatic repulsion algorithm combined with a ratio of the total number of diffusion-weighted images to nondiffusion-weighted images equal to six, provides robust determination of FA, MD, and tensor orientation (Abe et al., 2010; Jones, 2004; Zhu et al., 2009).
Overall, DT-MRI represents an approach which corresponds to a move from assessing 'lesion in a region' to examining microstructural and connectivity deficits within neural networks (Bandettini, 2009). As DT-MRI data contain a significant amount of information, it is currently possible to generate not only computerised maps of white matter fibres through fibre in-vivo tractography but also voxel-wise maps of DT-MRI derived parameters (Basser et al., 2000; Shergill et al., 2007). The predominant diffusion direction can be used as an input to fibre tracking algorithms (Dong et al., 2004) and currently, fibre-tracking methods enable the reconstruction of individual white matter tracts in-vivo (Basser et al., 2000; Conturo et al., 1999; Mori et al., 1999). Although fibre-tracking allows the assessment of a single tract along its entire course, methods investigating white matter tracts on a whole brain level have been recently made possible in combination with voxel-based analytical techniques thereby allowing a better understanding of white matter connectivity through assessment of multiple white matter tracts concurrently (Bandettini, 2009).

1.1.4. DT-MRI analytical methodology

Typically, with diffusion-weighted imaging in tissues that demonstrate isotropic diffusion, measurement is concerned with assessing the full amount of water molecule diffusion within a given voxel. This contrasts with DT-MRI where not only the total amount of water molecule diffusion is investigated but also the principal axis this diffusion is occurring along. See Fig. 1.2 for examples of isotropic and anisotropic diffusion in tissue.
Gradient Axis Parallel

Gradient Axis Perpendicular

Isotropic and anisotropic diffusion of water molecules in diffusion magnetic resonance imaging. Shades of gray intensity indicate the intensity of the magnetic field, which varies across the image plane because of the imposed pulsed magnetic field gradient. Water diffuses in all directions in most nonneural tissues (isotropically) but diffuses preferentially along the long axis of nerves (anisotropically). When all of the water molecules in a tissue experience identical magnetic field strength despite diffusion movements, the magnetic resonance signal from that tissue remains bright relative to the signal decay in surrounding isotropically diffusing tissue water. This is the situation on the left where the magnetic gradient is oriented perpendicular to the nerve. In the situation on the right, the water molecules in nerve move preferentially to different positions in the gradients more rapidly than in the nonneural tissue so that the signal remains brighter from nonneural tissue.

Fig. 1.2 Isotropic and anisotropic diffusion of water in tissue (adapted from (Filler, 2009))
Although DT-MRI is especially used in the assessment of white matter pathways, however, there are difficulties associated with examining the true course of a nerve fibre. For instance, the path of nerve tracts is often neither linear nor perpendicular to the axis of measurement but is frequently curved instead. Additionally, the orientation from which measurements are taken from can obscure the true course of a nerve fibre as the apparent diffusivity of water molecules within regions of the brain is strongly dependent on the direction in which measurements are observed from (Chenevert et al., 1990). Therefore, by acquiring an MRI dataset with diffusion-weighting applied in several different orientations, DT-MRI provides for a rotationally invariant estimate of diffusivity where the diffusion tensor is estimated within each image voxel (Basser et al., 1994a; Jones et al., 2002a).

Mathematical geometry algorithms are used with DT-MRI to determine the principal direction of a nerve fibre within any given voxel in 3-D space that is described in three orthogonal planes (x, y and z axes). Mathematical models usually involve tensor matrices e.g. a 3 x 3 symmetric matrix that is defined by nine elements that correspond to the mobility of water molecules along each direction in 3-D space and correlation between these directions (Le Bihan et al., 2001). With DT-MRI, six diffusion-weighted acquisitions are required as a minimum to complete a second rank tensor analysis (Filler, 2009; Hagmann et al., 2006) where tensor ranks are used to describe biophysical properties that are examined with tensor mathematics.

For example, temperature is regarded as a rank 0 tensor as it is a scalar measure with no directionality. However, if a property were to have directionality, it is usually described as a vector containing both direction and magnitude for instance a
mechanical force occurring in 3-D space is reported as a tensor of rank one. In order to describe the relationship between two vectors, a second rank tensor is required e.g. the relationship between the Brownian motion of water molecules and their displacement which may be due to free diffusion or barriers to diffusion. With DT-MRI analyses, a construct known as a dyad is usually explored which may consist of a combination of a scalar quantity and two directions or alternatively being composed of two vectors (Filler, 2009). See Fig. 1.3a where the diffusion tensor (D) is described by nine diffusion coefficients in a 3 x 3 tensor matrix (Le Bihan et al., 2001).
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\[
D = \begin{pmatrix}
D_{xx} & D_{xy} & D_{xz} \\
D_{yx} & D_{yy} & D_{yz} \\
D_{zx} & D_{zy} & D_{zz}
\end{pmatrix}
\]

**Fig. 1.3a** A typical diffusion tensor 3 x 3 matrix

\[
D = \begin{pmatrix}
D_{xx} & D_{xy} & D_{xz} \\
D_{yx} & D_{yy} & D_{yz} \\
D_{zx} & D_{zy} & D_{zz}
\end{pmatrix} \xrightarrow{\text{diagonalisation}} \begin{pmatrix}
D_1 & 0 & 0 \\
0 & D_2 & 0 \\
0 & 0 & D_3
\end{pmatrix}, v_1, v_2, v_3
\]

**Fig 1.3b** Tensor diagonalisation (D_1, D_2 and D_3 = eigenvalues; v_1, v_2 and v_3 = eigenvectors) adapted from (Reiser et al., 2008)
Basser and colleagues described the diffusion in each voxel as corresponding to a diffusion ellipse which represents in 3-D space the diffusion distance covered by water molecules in a given diffusion time; the diffusion ellipse is usually defined by at least six gradient measurements where the ellipsoid contains detailed information about microscopic tissue structure (Basser et al., 1994a; b; Le Bihan et al., 2001; Parker, 2004; Taylor et al., 2004). With current DT-MRI acquisition techniques, it is possible to acquire DT-MR images with more than six diffusion sensitising gradients thereby increasing the accuracy in assessing white matter pathways (Le Bihan et al., 2001). However, interpreting tensor data is not straightforward, as the 3-D information they contain cannot be easily represented via 2-D images. This has led to the derivation of scalar measures (as mentioned in Section 1.1.3.) such as FA, MD, VR and AD which enable conventional 2-D mapping (Abe et al., 2010; Basser and Pierpaoli, 1996; Kanaan et al., 2005).

Papadakis et al. (1999) have reported that FA is able to map diffusion anisotropy with the greatest detail as well as signal-to-noise ratio whereas VR provides the strongest contrast between low- and high-anisotropy areas but at the expense of increased noise contamination and decreased resolution in anisotropic regions while RA is of intermediate quality (Papadakis et al., 1999). Given the relative advantages of using FA over other DT-MRI derived parameters, FA will be the measure that is assessed in all studies involving DT-MRI in this thesis.

Additionally with DT-MRI analyses, the degree of FA is considered alongside eigenvectors and eigenvalues (Basser et al., 1994a; b) where eigenvectors are used in the study of differential equations, linear algebra, linear transformations and
matrices. Eigenvectors are described as having both direction and magnitude and are defined as a non-zero vector that is mapped by a given linear transformation of a vector space onto a vector that is the product of the original vector multiplied by a scalar. Eigenvectors are also considered to have eigenvalues that describe their length. Eigenvectors $e_1$, $e_2$ and $e_3$ (also known as $v_1$, $v_2$ and $v_3$) have eigenvalues which are called $\lambda_1$, $\lambda_2$ and $\lambda_3$ (also known as $D_1$, $D_2$ and $D_3$) respectively which are additionally known as diffusivities or diffusion coefficients. See Fig. 1.4 for an example of a diffusion ellipsoid with eigenvectors and eigenvalues in 3-D space.

Although in DT-MRI the primary eigenvector is often the most relevant as it indicates a white matter fibre's principal orientation (Lin et al., 2001), through tensor diagonalisation (finding the three axes (i.e. orientation and length) that define the ellipsoid by transforming the off-diagonal elements to zero; see Fig. 1.3b), it is possible to estimate the orientation of white matter fibres by assessing all the eigenvectors of the diffusion tensor namely, the primary eigenvector ($e_1$) in conjunction with orthogonal eigenvectors ($e_2$ and $e_3$). With the directional information available through the DT-MRI, it is possible to examine the routes of cerebral fibre pathways via in-vivo fibre tractography (Catani et al., 2002; Conturo et al., 1999) or whole-brain voxel-based analyses which are described in more detail next.
Diagram of the diffusion ellipsoid showing the principle diffusivities: $D_1$, $D_2$, and $D_3$. The eigenvectors are the arrows that point along the direction of $D_1$, $D_2$, and $D_3$. The ellipsoid is rotated from the scanner coordinate system ($X$, $Y$, $Z$) by angles ($\rho$, $\theta$, $\phi$).

Fig. 1.4 Diffusion ellipsoid (adapted from (Taylor et al., 2004))
1.1.4.1. In-vivo fibre tractography analyses of DT-MRI data

Tractography is a technique applied to DT-MRI data that enables the reconstruction and visualisation of white matter tracts in-vivo; it also allows for the examination of the connectivity of these white matter fibres with regions of the brain (Basser et al., 2000). Tractography techniques can usually be divided into local and global approaches. Local tractography techniques are based on the preferred direction of water diffusion which is provided by the diffusion tensor in each image voxel from a starting or 'seed' region (Ciccarelli et al., 2008) while global approaches identify the best path between two points of interest. The main assumption that underpins diffusion tractography is that the dominant direction of water molecule motion (which is the primary eigenvector of the diffusion tensor) aligns with the principal orientation of the fibres in an image voxel (Le Bihan et al., 2001).

With tractography, DT-MRI derived parameters such as FA can be used to make comparisons of white matter integrity between subject groups by delineating complete tracts in each individual brain. As individual anatomical differences can be taken into account and because only the voxels within tracts are analysed, partial volume effects are minimised. However, tractography has inherent limitations such as discrepancies between the scale of axonal fibre diameter and image voxel size (i.e. low spatial resolution), noise contained in the diffusion data as well as imaging artefacts (Ciccarelli et al., 2008; Le Bihan et al., 2006). Further considerations are that the technique may not be able to identify fibre pathways clearly when there is more than one fibre population or where fibres cross with each other, converge or diverge. In these scenarios, fibre tracking tends to retain topology such that tracts
arising from an ipsilateral locus continue to remain ipsilateral beyond a merging point although in reality, fibres may have crossed over to a contralateral trajectory (Basser et al., 2000; Wakana et al., 2004). The interpretation of tractography data also requires experience and *a priori* knowledge of white matter pathways and given that the knowledge of white matter anatomy is gradually being established, this poses additional difficulties.

**1.1.4.2. Whole-brain voxel-based analysis of DT-MRI data**

Details of VBM for use with conventional MRI data and analysis of brain volumes has been provided earlier (in Section 1.1.2.2.). Recently, these techniques have been adapted for use in the comparison of voxel-wise maps of DT-MRI derived parameters such as FA between subjects (Kakeda and Korogi, 2010). Currently, there are two principal methods used in the analysis of FA maps; these are ROI and whole-brain voxel-based analyses where the majority of previous studies have employed ROI methodology (Kanaan et al., 2005; Park et al., 2004; Snook et al., 2007). While the ROI approach requires *a priori* hypotheses to define expected regions where differences may be found, the ROI method however introduces biases such as the definition of ROIs which may vary within and between raters. The whole-brain voxel-based strategy on the other hand represents an objective explorative method that permits the identification of unanticipated areas of neuroanatomical differences given that the entire brain is assessed (Barnea-Goraly et al., 2003; Gong et al., 2005; Park et al., 2004; Schmithorst et al., 2002).
Usually for voxel-based analyses of whole-brain FA maps, the SPM software package which is used with VBM (see Section 1.1.2.2.) can be also utilised in the normalisation of DT-MRI data. When DT-MRI data is acquired, both nondiffusion-weighted \((b = 0)\) and diffusion-weighted images are obtained during the same scanning session where the former acts a reference measure of signal intensity (Basser and Pierpaoli, 1998). However, as SPM does not supply either a standard FA or MD registration template for DT-MRI data, the nondiffusion-weighted images are normalised to EPI or T2-weighted templates (provided by SPM) in standard space during the image registration step. The derived mapping parameters from the registration of each subject’s nondiffusion-weighted images are then applied to their FA images. Subsequently, these FA images are averaged to create a study-specific FA template which is smoothed with an 8-mm isotropic Gaussian kernel and then used to re-register all FA images. Normalisation using a 2-stage approach with \(b = 0\) and FA data is analogous to ‘optimised VBM’ (Good et al., 2001) and enables images to be aligned accurately and therefore minimises registration errors. Indeed, the steps employed for normalisation attempt to reduce inaccurate registration which would otherwise misattribute differences by Type 1 error and is thus an important issue to address (White et al., 2008).

Given that normalised FA images still contain grey and white matter and cerebrospinal fluid tissue classes, tissue segmentation and masking is performed so as to isolate these various compartments and in particular to delineate white matter. This serves to increase the accuracy by minimising edge-effects and limiting statistical analyses to those voxels falling within white matter but also reduces the total number of statistical tests run and therefore minimises potential biases and
error. With regard to the size of the smoothing filter subsequently applied to maps of DT-MRI derived parameters, there is currently no consensus but a size 2-3 times the voxel size is suggested as per VBM (Abe et al., 2010). Analysis of FA maps can be conducted using parametric or non-parametric statistics as with tissue content data (acquired by conventional MRI), following which, the results require correction for multiple comparisons as with VBM (see Section 1.1.2.2.). Overall, in comparison to ROI methods, voxel-based approaches examining FA have the advantage of searching for abnormalities across the entire brain. Further, issues relating to the validity of investigator-determined ROIs and inter-rater variability are overcome (Abe et al., 2010; Snook et al., 2007). Additionally, using FA as an assessment measure has several advantages over other DT-MRI-derived parameters such as improved detail and signal-to-noise ratio and represents a robust anisotropy measure (Pierpaoli and Basser, 1996).

Thus far, the main structural and connectivity acquisition and analytical methods based on MRI have been examined whereby the experimental studies in this thesis based on conventional MRI will be analysed using whole-brain VBM of maps of tissue content while DT-MRI acquired data will be investigated using whole-brain voxel-based analyses of FA maps. However, to localise any regional differences in brain tissue, an understanding of cerebral neuroanatomy is required. Therefore, in the following sections, the various neuroanatomical divisions of the brain and (currently accepted) white matter tracts are defined with regard to location, boundaries and function.
1.2. Brain regions

The main aim of this section is to provide an overview of the gross neuroanatomical divisions of the brain and does not constitute a literature review of these brain regions as that would be beyond the scope of this thesis. Elaboration of the boundaries and functions of the various lobes as well as associated grey matter in cortical and subcortical structures is included. Furthermore, as knowledge of white matter tract anatomy is gradually being elucidated, descriptions of currently recognised white matter tract pathways and postulated functions will be provided in Section 1.3. This section serves as a neuroanatomical reference that will be used for guiding the understanding of any brain differences found in the following experimental chapters.

The brain is broadly divided into the cerebrum, brainstem and cerebellum. Each of these structures can be divided along the midline in the sagittal plane into left and right sides. The brainstem can be partitioned further into the medulla, midbrain and pons while the cerebrum is subdivided into five cortices/lobes (Fig. 1.5): frontal, temporal, parietal, occipital and insula (Williams and Worwick, 1980). Grey matter, white matter and subcortical grey matter structures are found within each of these lobes. In each cerebral hemisphere, grey matter cortical gyri are separated by sulci. White matter is found beneath the grey matter cortex of each hemisphere and is composed of axons of afferent and efferent white matter pathways that interconnect various areas within the ipsilateral or contralateral hemispheres. In the following subsections, an anatomical overview is provided of the various lobes of the brain together with functional descriptions of these structures.
Fig. 1.5 Overview of the various cortical regions of the brain as seen from its lateral aspect (adapted from http://www.netterimages.com)
1.2.1. Frontal lobe

The frontal lobe is the largest component of and comprises ¼ of the human brain. It is subdivided into medial, lateral, polar and orbital subdivisions. The frontal lobe is involved in a variety of functions encompassing motor, visual, cognitive, language and emotional domains. For instance, the primary motor cortex which is found in the most posterior part of the precentral gyrus controls movements of the contralateral side of the body. The left postero-inferior frontal cortex (also known as Broca’s area) controls expressive language function. The frontal lobe is also important for a range of cognitive functions such as planning, executive function, emotional regulation, attention and working memory. An area within the frontal lobe particularly involved in these cognitive functions is the prefrontal cortex (PFC) which corresponds to the area of the frontal lobe that lies in front of the motor and premotor areas and is subdivided into dorsolateral, dorsomedial, orbitolateral and orbitomedial regions (Howard et al., 2003). Other functions attributed to the frontal lobe include Theory of Mind, social and sexual behaviour, mood, personality, arousal, motivation, reasoning, problem solving, spontaneity, impulse control and judgement (Chayer and Freedman, 2001; Niedermeyer, 1998).

1.2.2. Temporal lobe

The temporal lobe is broadly divided into lateral and medial components whereby it consists of the superior, middle and inferior temporal gyri laterally while the medial surface of the temporal lobe contains important subcortical structures such as the
hippocampus, subiculum and amygdala and the perirhinal, entorhinal and parahippocampal cortices. The temporal lobe is involved in a variety of functions including memory, inner speech, balance, time perception, and language, auditory, visual and emotional processing (Hugdahl et al., 2009; Staiaman, 1998). The superior temporal gyrus includes a highly specialised area (within the lateral sulcus) where peripheral auditory signals are processed and is known as the primary auditory cortex (or Wernicke’s area). Adjacent areas to the primary auditory cortex in the temporal lobe are also involved in high-level auditory processing including speech. Additionally, the inferior part of the temporal cortices are particularly involved in high-level visual processing of complex stimuli such as faces and scenes which are subserved by the fusiform and parahippocampal gyri respectively while other functions include object perception and recognition. Other functions attributable to the temporal lobe encompass language functions such as comprehension and naming. A specialised system involved especially in emotional and memory processing is the limbic system which is derived from certain temporal lobe structures where further details relating to this system can be found later in this chapter under Section 1.2.8.

1.2.3. Parietal lobe

As the parietal lobe integrates information from various sensory modalities, it is not only important in spatial processing and proprioception but also navigation and the guidance of limb and eye movement. The most anterior part of the parietal lobe represents the primary somatosensory cortex which contains a map of sensory space
termed Penfield’s homunculus (Schott, 1993). The parietal cortex maps objects visually perceived into body coordinate positions by integrating information from the somatosensory cortex and the dorsal portion of the visual system. The non-dominant parietal lobe also integrates the contralateral side of the body with its environmental space thereby enabling spatial awareness, and is important for abilities such as drawing or dressing. However, body image disturbances have been postulated to arise from both non-dominant and dominant parietal lobe lesions (Trimble, 2007). Further, the parietal lobe is important for reading, phonological processing, arithmetic, finger recognition, left-right orientation and in language comprehension where some of these functions are achieved through integration with the other lobes.

1.2.4. Occipital lobe

The occipital lobe is the smallest lobe of the human cerebral cortex and is the most posterior part of the brain where it is located caudal to the parietal and temporal lobes. The occipital lobe is predominantly involved in visual processing (Bender et al., 1957) and the calcarine sulcus is the location of the primary visual cortex (also called striate cortex). After the retina is stimulated, nerve impulses pass through the lateral geniculate nucleus of the thalamus and retrolenticular limb of the internal capsule before projecting to the visual cortex via the optic radiations traversing the parietal and temporal lobes. Apart from vision, the occipital lobe is involved in colour recognition, movement and visuospatial processing. It is also involved in face
recognition and attentional inhibition of visual processing via interaction of the occipital and temporal lobes (Kanwisher et al., 1997; Slotnick et al., 2003).

1.2.5. Insula

The insula is located deep within the lateral sulcus and is hidden by frontal, temporal and parietal opercula (Fig. 1.5). The insula has extensive connections with the cerebral cortex, thalamus, limbic system, autonomic system and striatum (Augustine, 1985; 1996) and is thought to be involved in gustatory, olfactory, salivatory, visceral, affective, addictive, pain and vestibular processes (Naqvi et al., 2007; Soros et al., 2009; Wicker et al., 2003). It has also been postulated to be involved in networks mediating cognition, empathy, attention, salience and meditative states (Lutz et al., 2009; Menon and Uddin, 2010).

1.2.6. Cerebellum

The cerebellum is the main structure in the hindbrain and is divided rostro-caudally into the anterior lobe, posterior lobe and flocculonodular lobe. It is located dorsal to the pons and is connected to the pons via the superior, middle and inferior cerebellar peduncles (see Section 1.3.1.) which are white matter bundles composed of axons interconnecting the cerebellum with the rest of the brain. The cerebellum plays an important role in motor control although it does not itself initiate movement but instead contributes to the modulation of motor activity. Neural pathways
interconnect the cerebellum with motor and sensory systems for instance the motor
cortex and spinal cord. The cerebellum plays an integrative role in translating inputs
from these systems in order to fine-tune motor activity via precise coordination and
accurate timing of movements. More recently, the literature would suggest that the
cerebellum is also involved in higher order functions such as cognition, learning,
attention, memory, emotion, language and personality (Barrios and Guardia, 2001;
Schmahmann, 1997; Schmahmann and Sherman, 1998; Schmahmann and Pandya,
2008).

1.2.7. Major subcortical structures

The subcortical structures described in this section are the major grey matter
structures found below the cortex and exclude those described in other sections for
example, the medial temporal lobe structures. Although white matter is found
subcortically, descriptions of white matter anatomy will be provided in Section 1.3.
The descriptions here will cover the basal ganglia and diencephalon.

1.2.7.1. Basal ganglia

The basal ganglia are structures located at the base of the forebrain where the main
components are the caudate, putamen, globus pallidus, subthalamus and substantia
nigra (Fig. 1.6). The basal ganglia are extensively connected for instance with the
cerebral cortex and thalamus and are associated with a variety of functions. For
example, the basal ganglia are part of the extrapyramidal motor system and therefore exert a modulatory influence on a number of motor pathways where their inhibition permits activation of the motor system. The basal ganglia are also involved in non-motor functions including learning and decision-making, attention, motivation, memory, addiction and emotion processing (Herrero et al., 2002; Pennartz et al., 2009).

1.2.7.2. Diencephalon

The diencephalon is a region of the cerebrum which contains the epithalamus, subthalamus, hypothalamus and thalamus. These structures are found at the level of the temporal lobe and are especially well-protected given their location at the centre of the brain. The epithalamus includes the pineal gland and is involved in autonomic functions as well as sleep and wakefulness regulation. The subthalamus, which is a component of the basal ganglia circuitry, is located inferior to the thalamus and is interconnected with the brainstem and thalamus and is involved in the coordination of motor activity (Yasoshima et al., 2005). The thalamus constitutes a major relay centre subserving motor and sensory mechanisms which receives information from a variety of organs including the basal ganglia, cerebellum and all sensory pathways (with the exception of the olfactory tract). Apart from being a relay centre, the thalamus also integrates the impulses it receives and forwards these on to the cerebral cortex for further processing.
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Fig. 1.6 Coronal view of main basal ganglia structures (adapted from http://www.dana.org)
1.2.8. Limbic system

Currently, the limbic system is recognised to play an integral role in attention, memory and emotions (Sitoh and Tien, 1997); it consists of a collection of structures that are derived from several neuroanatomical regions rather than consisting of a distinct lobe (see Fig. 1.7). There has been much debate with regard to the structures comprising the limbic system and presently, it is proposed to consist of the following: olfactory cortex; hippocampus; laterobasal-cortical amygdala; the main parts of the cingulate and parahippocampal gyri; caudal orbital and medial PFC; part of the temporal polar cortex; and a large antero-ventral, agranular and dysgranular part of the insula (Heimer and Van Hoesen, 2006). As the hippocampus and amygdala are major constituents of the limbic system and are the only structures that have not been discussed in other sections of this chapter, they will be described in more detail below:

**Hippocampus**

The hippocampus (Greek for seahorse) is a club-like structure in the medial temporal lobe that is divided rostro-caudally into three parts: the head, body, and tail. The grey matter of the hippocampus is derived as an extension of the subiculum of the parahippocampal gyrus (Mark et al., 1993). The alveus and fimbria are white matter tracts that arise from the subiculum and hippocampus; these continue posteriorly as the fornix which serves as a major outflow pathway to the rest of the brain (see Section 1.3.4.). The hippocampal formation is significantly interconnected with the surrounding parahippocampal region and plays a central role in learning and memory through cortico-parahippocampal-hippocampal and parallel pathways (Witter et al.,
2000). Furthermore, the hippocampus and associated parahippocampal region is also important for spatial navigation (Maguire et al., 1998). Also, abnormalities in the hippocampus are regarded as highly epileptogenic (Gama et al., 2010) (see Section 1.6.).

**Amygdala**

The amygdala (Greek for almond) is located directly anterior to the head of the hippocampus and above the tip of the temporal horn of the lateral ventricle. The amygdala has a critical role in the modulation of memory and emotional and social processing (Amaral, 2003). For example, the amygdala contributes to the processing of memories of emotionally significant experiences (Roozendaal et al., 2009). Additionally, the amygdala is involved in emotional face recognition and is involved in interpreting stimuli that are particularly salient (e.g. threatening, rewarding or unpredictable stimuli) (Whalen, 2007).
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Fig. 1.7 Main components of the limbic system (adapted from http://www.netterimages.com)
1.3. White matter tracts

Previously, grey matter was considered the primary substrate for a variety of functions such as cognition and movement whereas very little was known about white matter anatomy and less so about its function (Schmahmann and Pandya, 2007a). However, the understanding of the anatomy, function and clinical importance of white matter has since improved through newer neuroimaging and neuropathological techniques and white matter is now recognised to be involved in neural circuits which subserve a variety of neurological, psychiatric, cognitive, emotional and behavioural functions (Kubicki et al., 2007; Mayer et al., 2010; Mukherjee et al., 2002; Neil et al., 2002). DT-MRI in particular has permitted the recent visualisation of white matter pathways in-vivo and continues to contribute to the emerging literature on the anatomy and role of white matter (Basser et al., 1994b; Catani et al., 2002; Schmahmann, 1997; Wakana et al., 2004).

Currently, white matter tracts in the brain are functionally subdivided into five categories (Wakana et al., 2004) which correspond to: a) tracts in the brainstem; b) projection fibres (cortex-brainstem, cortex-spinal cord, cortex-thalamus and thalamus-cortex connections); c) association fibres (cortex-cortex connections within the same hemisphere); d) limbic system tracts; and e) commissural fibres (interhemispheric connections). See Fig. 1.8 for localisation of some of these white matter bundles.
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Fig. 1.8 Horizontal sections of the brain with DT-MRI acquisition demonstrating white matter fasciculi at the level of the internal capsule and corpus callosum (adapted from (Wakana et al., 2004))
1.3.1. Tracts in the brainstem

White matter tracts in the brainstem encompass the superior, middle and inferior cerebellar peduncles and, the corticospinal tract and medial lemniscus.

**Superior cerebellar peduncle**

This is the main output (efferent) pathway from the dentate nucleus of the cerebellum and provides a route of communication between the deep nuclei of the cerebellum with the brainstem and thalamus.

**Middle cerebellar peduncle**

It is the largest of the three cerebellar peduncles and is composed almost entirely of afferent fibres originating within the pontine nuclei. The pontine nuclei and middle cerebellar peduncle are part of the extensive cortico-ponto-cerebellar network which recruits white matter pathways from the sensory and motor areas of the cerebral cortex.

**Inferior cerebellar peduncle**

This peduncle is composed of efferents from both the spinal cord and brainstem to the cerebellum. It is involved in integrating proprioceptive sensory input with motor vestibular functions such as posture maintenance and balance.

**Corticospinal tract**

The corticospinal tract is a large collection of axons that travel between the motor areas of the cerebral cortex and the spinal cord via the posterior limb of the internal
capsule (see Section 1.3.2.). The corticospinal tract is composed of upper motor neurones that control contralateral parts of the body.

**Medial lemniscus**

The medial lemniscus travels along the dorsal side of the midbrain and pons and is a pathway that carries sensory information to the thalamus from the gracile and cuneate nuclei of the medulla. It is part of the dorsal column-medial lemniscus-thalamus system which transmits sensory information such as fine touch, vibration and proprioception.

### 1.3.2. Projection fibres

These are white matter pathways that provide neural connections between the cerebral cortex and with lower parts of the brain and spinal cord. Projection fibre tracts contain afferent and efferent fibres that include the corticothalamic and corticofugal (corticoeffferent) fibres. These white matter projection fibres fan out to form the corona radiata (divided into anterior, superior and posterior regions) as they approach the cortex but converge to form the internal capsule in the medial aspect of the brain.

**Thalamic radiations**

The anterior, superior and posterior thalamic radiations are reciprocal projections from the thalamus to cortical brain regions (Mori et al., 2005). The anterior thalamic radiation projects from anterior and medial regions of the thalamus to the frontal
cortex. However, the superior thalamic radiation arises from the lateral region of the thalamus and projects to the postcentral gyrus of the parietal cortex. The posterior thalamic radiation originates from the posterior region of the thalamus and projects to the parieto-occipital cortices of the brain. The posterior thalamic radiation also includes the optic radiation which connects the lateral geniculate nucleus of the thalamus to the occipital lobe. All thalamic radiations converge into the internal capsule where the anterior and posterior limbs of the internal capsule contain the anterior and superior thalamic radiations respectively whereas the retrolenticular part of the internal capsule contains a mixture of the posterior thalamic radiation, optic radiation and association tracts.

*Corticofugal tracts*

The corticofugal white matter pathways are composed of the corticobulbar, corticopontine, corticoreticular and corticospinal tracts where the latter has been described previously (see Section 1.3.1.). The corticobulbar tract is a white matter pathway which connects the cerebral cortex to the brainstem and as the corticobulbar tract passes from the motor cortex to the brainstem, its fibres run through the internal capsule. The muscles of the head, neck and face are controlled by the corticobulbar system which recruits motor neurones within the brainstem motor nuclei. The corticopontine tract links the cortical areas of the various lobes i.e. frontal, temporal, parietal and occipital cortices with the pontine nuclei and the tract is involved in the planning and initiation of movement. Fronto-pontine fibres that originate in the frontal cortex descend to the pontine nuclei via the anterior limb of the internal capsule which is in contrast to the temporo-parieto-occipito-pontine fibres that travel lateral to the corticospinal fibres in the posterior limb of the internal capsule. The
corticoreticular tract connects the cortex to the reticular formation in the brainstem and consists of bilateral projections originating predominantly from the motor, premotor and somatosensory areas. It accompanies the corticospinal tract along its course and is involved in the regulation of the sleep/wake cycle and modulation of cardiac, pain and somatic motor systems.

**Internal capsule**

The internal capsule is a white matter collection that contains both ascending and descending axons from the cerebral cortex and medulla. The internal capsule separates the thalamus and caudate nucleus from the lentiform nucleus and contains axons from these subcortical nuclei as well as from projection fibres. The internal capsule is divided rostro-caudally into the anterior limb, genu, posterior limb and retrolenticular and sublenticular parts.

The anterior limb of the internal capsule contains fronto-pontine (corticofugal) fibres that project from the frontal cortex to the pons in the brainstem. It also contains the anterior thalamic radiation that connects the thalamus to the frontal lobes. Caudal to the anterior limb, the genu contains corticobulbar fibres that run between the cerebral cortex and brainstem. The posterior limb of the internal capsule contains the superior thalamic radiation and corticospinal and ascending sensory fibres (including the medial lemniscus and spinothalamic tract) and a small number of corticobulbar fibres. The retrolenticular part of the internal capsule subserves the optic system as it receives fibres from the lateral geniculate nucleus of the thalamus and more caudally, the retrolenticular part becomes the optic radiation. Some fibres (which carry auditory information) from the medial geniculate nucleus of the thalamus also
pass in the retrolenticular part but the majority are carried in the sublenticular part of
the internal capsule.

1.3.3. Association fibres

Association fibres are white matter bundles that interconnect ipsilateral brain regions
and include both long-range and short-range fibres. Short-range association fibres
encompass most of the cortical U-fibres. Most of the long-range association fibres
(presented below) penetrate the external capsule rather than the internal capsule
which is the case instead with the projection fibres (Wakana et al., 2004).

Superior longitudinal fasciculus

While this is the largest of the long-range association tracts and is also known as the
arcuate fasciculus (Klingler and Gloor, 1960), there is much debate presently as to
the course of this bundle, its subdivisions (thought to be up to four) as well as its
distinction from a separate arcuate fasciculus (Makris et al., 2005). Some of the
functions attributed to this fasciculus include: regulation of higher aspects of motor
behaviour; provision of a bidirectional pathway between the PFC and parietal lobe;
involvement in praxis, associative tasks and visuospatial perception; and an
important role in language/speech by linking Broca's and Wernicke's language areas
(Catani and Mesulam, 2008; Heilman and Watson, 2008).

Inferior longitudinal fasciculus
This fasciculus is shorter than its superior counterpart and provides for a major connection between the temporal and occipital lobes (Dejerine, 1895). It is involved in a number of visual tasks including face recognition, reading and visual perception and visual memory (Catani and Thiebaut de Schotten, 2008).

**Superior fronto-occipital fasciculus**

This fibre bundle is also known as the subcallosal fasciculus and is thought to connect temporo-occipital regions with the frontal cortex and insula but has historically been poorly described (Crosby et al., 1962; Schmahmann and Pandya, 2007b). The fasciculus plays a role in the awareness of and use of visual information for the purpose of guiding movements and also in the spatial aspects of cognitive processing (Rizzolatti et al., 1990; Rizzolatti and Matelli, 2003).

**Inferior fronto-occipital fasciculus**

This is a bow-tie shaped white matter bundle that connects the inferolateral and dorsolateral frontal cortex with the temporal and occipital cortices (Crosby et al., 1962; Wakana et al., 2004). It also provides connections between the auditory and visual association areas and the PFC (Kier et al., 2004; Petrides and Pandya, 1988).

**Uncinate fasciculus**

The uncinate fasciculus connects the anterior temporal lobe with the polar and orbital frontal cortex (Catani et al., 2002). The uncinate fasciculus represents the most prominent connection between the frontal and temporal lobes (Kubicki et al., 2002a) and is thought to be involved in language, emotion processing and memory (Catani and Mesulam, 2008; Gaffan and Wilson, 2008).
*External capsule*

This capsule is a thin layer of white matter that separates the grey matter of the claustrum from the putamen and it is penetrated by most of the long-range cortico-cortical white matter association fibres.

**1.3.4. Limbic system tracts**

The limbic system tracts are major association fibres that interconnect the constituent structures of the limbic system (Section 1.2.8. and Fig. 1.7) and also with other regions of the brain.

*Cingulum*

The cingulum (Latin for girdle) consists of fibres that originate from the cingulate gyrus, thalamus and cortical association areas (Burgel et al., 2006; Nezamzadeh et al., 2009). It is the most prominent white matter fibre tract in the limbic system and provides a major pathway between the anterior thalamus and hippocampus (Burgel et al., 2006). The cingulum is an important white matter structure for cognitive, executive and emotional processing (Ardekani et al., 2003; Matthews et al., 2008; Rudrauf et al., 2008).

*Fornix*

The fornix (Latin for vault or arch) originates in the hypothalamic area and represents the main efferent pathway of the hippocampus and provides connections
between the hippocampus and hypothalamus and septal area. The fornix contributes to memory functions (Gaffan and Wilson, 2008; Ross, 2008).

**Stria terminalis**

The stria terminalis is a major outflow pathway of the amygdala and provides for a network of afferent and efferent fibres that connect the hypothalamus, amygdala and septal area (Mori et al., 2005).

1.3.5. **Commissural fibres**

The commissural fibres are white matter bundles that cross both cerebral hemispheres.

**Anterior commissure**

It is composed of anterior and posterior fibres where the former connect the amygdala with the temporal pole while the latter principally connects the inferior temporo-occipital cortices (Catani et al., 2002; Di Virgilio et al., 1999). The anterior commissure is often used as an important landmark on sagittal sections of conventional MR images in conjunction with the posterior commissure.

**Posterior commissure**

Also known as the epithalamic commissure, the posterior commissure interconnects the pretectal nuclei and mediates the consensual pupillary light reflex.
Corpus callosum

The corpus callosum is the largest white matter bundle in the brain and represents an important pathway for interhemispheric connectivity. It is derived from white matter fibres that originate from all the lobes of each hemisphere and is subdivided in the midline into anterior (rostrum and genu), middle (body) and posterior (splenium) portions (Catani et al., 2002). Most of the white matter fibres of the corpus callosum interconnect homologous cortical areas at approximately mirror-image sites; however, in a minority, these connections end in asymmetrical regions (Huang et al., 2005; Nolte, 1999). The corpus callosum plays a fundamental role in learning, attention and cognition and relaying sensory-motor information between homologous areas in the cerebral hemispheres as well as supporting functional integration (Doron and Gazzaniga, 2008; Glickstein and Berlucchi, 2008; Huang et al., 2005; Zaidel and Iacoboni, 2003).

Overall, this section has provided a summary of distinct brain regions as well as the white matter fibres linking them. While the understanding of white matter anatomy and function has improved, it is still an area that requires further investigation and clarification. In the following sections, the literature on a variety of psychiatric disorder is examined where those that have been relatively understudied warrant further investigation through the MRI techniques described previously in the thesis.

1.4. Understanding the neurobiology of psychiatric disorders using neuroimaging
Currently, psychiatric disorders are usually classified according to two major international systems: the International Classification of Diseases (ICD-10) and Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) respectively (American Psychiatric Association, 2000; World Health Organization, 1993). However, criteria for diagnoses in these classification systems are largely dependent on behavioural manifestations rather than underlying aetiology or pathophysiology (Charney et al., 2002; Hasler et al., 2004; Hasler et al., 2006). As psychiatric classification displays much heterogeneity (Chakravarti and Little, 2003; Lewis, 2002), there is a great need for research in the clinical neurosciences to characterise key underlying biological mechanisms. Methodologies such as genetic study, molecular and cellular biology, animal models, pharmacological studies, and brain imaging may better ascertain such aetiology which may then be translated to better diagnosis and treatment of a disorder (Abou-Saleh, 2006; Einat et al., 2003; Phillips, 2007).

With the application of biological methods to the study of psychiatric disorder, there is a growing move towards describing endophenotypes which are defined as measurable components unseen by the unaided eye along the pathway between disease and distal genotype (Gottesman and Gould, 2003). Other terms that are synonymous with an endophenotype include biological marker, intermediate phenotype, subclinical trait and vulnerability marker where an endophenotype may be represented by abnormal neuroanatomy, neurophysiology, biochemistry, endocrinology, cognition, or neuropsychology. As the origins of psychiatric disorder are complex and arise primarily from dysfunction in brain tissue, the identification of
neural endophenotypes are only gradually emerging (Stahl and Niculescu, 2002). Accordingly, the focus of this thesis is on establishing potential endophenotypes in the form of abnormal neuroanatomy through the application of MRI to psychiatric disorders that have been relatively understudied.

While it is possible to study psychiatric disorder through neuroimaging, there is however a wide spectrum of psychiatric disorder. In ICD-10 and DSM-IV, psychiatric disorders are classified according to nosological categories where the categories that have received much neuroimaging attention include affective, addictive, psychotic, anxiety, neurodevelopmental and neurodegenerative disorders (Capote, 2009; Fleck et al., 2008; Malhi and Lagopoulos, 2008; Sava and Yurgelun-Todd, 2008; White et al., 2008; Williams, 2008). For example, neurobiological abnormalities have been consistently reported in affective disorders (including depression and bipolar affective disorder) and similarly with psychotic disorders and in particular schizophrenia. Anxiety disorders encompassing post-traumatic stress disorder and obsessive-compulsive disorder are other areas that have been investigated significantly. Finally, neurodevelopmental disorders such as attention deficit hyperactivity disorder (ADD/ADHD), and neurodegenerative disorders such as mild cognitive impairment and Alzheimer’s disease have also received wide research attention.

However, neural correlates are not well established in many other psychiatric disorders due to the lack of extensive study, inconsistencies in the reported literature or as a result of the heterogeneity in the conditions investigated which may have arisen from the phenomenological classifications used in psychiatry. For instance,
individuals with an intellectual disability/mental retardation are at high-risk of developing psychiatric impairment but the literature on comorbid psychiatric disorders and in particular, schizophrenia spectrum disorders is limited (Hemmings, 2006). Among the intellectual disabilities, 22q11 deletion syndrome (22q11DS) is associated with approximately a 30-fold increase in rates of psychosis (Murphy et al., 1999; Murphy and Owen, 2001) which confers a risk of developing schizophrenia only second to that of being an offspring to parents with schizophrenia or being the monozygotic co-twin of a proband with schizophrenia. The very high prevalence of psychiatric disorders in people with 22q11DS is likely to be caused by haploinsufficiency of one or more genes deleted on the chromosome 22q11.2 region and subsequent differences in brain maturation and neurotransmitter systems. Thus, 22q11DS provides a unique neurobiological template for understanding the evolution of psychotic disorders not only in those with an intellectual disability but also individuals with psychosis in the general population (Murphy and Owen, 2001).

Similarly, the understanding of psychiatric disorder comorbid with physical disorder is currently very poor. Among the category of organic disorders, epilepsy is associated with significant psychiatric comorbidity (de Boer et al., 2008) where a particular subtype, temporal lobe epilepsy (TLE) predisposes to much higher rates of psychosis relative to the general population (Gaitatzis et al., 2004). Although advancements have been made in understanding the neurological mechanisms leading to seizures, however, the underlying neurobiology of psychosis seen in TLE is not well understood (Diehl, 1989; Lautenschlager and Forstl, 2001). Analogous to 22q11DS, understanding the causative mechanisms for psychosis seen in TLE may not only benefit those with epilepsy but also helps to characterise pathways that
cause psychosis in the general population and establish any potential shared
aetiological mechanisms between these disorders.

Within the category of personality disorders, antisocial personality disorder (ASPD)
has received much clinical and judicial attention. It is characterised by a pervasive
pattern of disregard for, and violation of, the rights of others that begins in childhood
or early adolescence and continues into adulthood. It results in significant
interpersonal difficulties and poses significant burden to clinicians, justice system
and wider society. However, there are only a limited number of studies that have
examined the structural brain deficits and even less so, the brain white matter
connectivity abnormalities in people with ASPD where the neurobiological
determinants of ASPD are currently not well-established (New et al., 2008; Nunes et
al., 2009; Wahlund and Kristiansson, 2009). Identification of these neural correlates
may aid with early diagnosis and intervention and indeed potentially lessen societal
burden.

Finally, in the neurodevelopmental disorders category, there is currently no
established method of neuroimaging that aids either with the diagnosis or treatment
of autism spectrum disorders (including Asperger syndrome). Autism spectrum
disorder is characterised by significant difficulties in social interaction,
communication, and unusual or stereotyped routines and behaviour. People with
Asperger syndrome typically do not have delay in the acquisition of language but
still show the other characteristic autistic impairments. Although autism spectrum
disorder has a significant biological basis and there is increasing evidence from in-
vivo neuroimaging studies that specific brain regions are implicated (Levy et al.,
2009), however, little is known about the underlying neuroanatomical connectivity between these brain regions and Asperger syndrome has yet to be specifically investigated. Therefore research is required to unravel the heterogeneity of autism spectrum disorders and may indeed provide future tools that better inform diagnosis and treatment (Lainhart, 2006).

In summary, classification of psychiatric disorders internationally is based on phenomenological descriptions and thus dependent on behavioural criteria rather than biological aetiology. While the investigation of psychiatric disorder using neurobiological techniques is improving, there are still many disorders that have yet to be assessed. In this thesis, I will therefore examine the neurobiology of the following psychiatric disorders using neuroimaging techniques based on MRI and voxel-based methods as they have to date been understudied: 1) psychiatric disorder associated with 22q11DS; 2) psychosis associated with TLE; 3) ASPD; and 4) Asperger syndrome. The following sections will cover a literature review of each of these disorders with specific focus on epidemiology, causation, diagnostic criteria, comorbid behavioural and psychiatric disorder and previous neuroimaging findings.

1.5. 22q11 deletion syndrome (22q11DS)

22q11DS is the most common human genetic deletion syndrome and is associated with deletions in chromosome 22 (Williams, 2011). 22q11DS was once considered a rare congenital disorder but since the advent of molecular genetics, this view has now been countered. In 1992, a major breakthrough occurred in the study of
disorders related to chromosome 22 when deletions were specifically localised to the long arm of chromosome 22 (22q11) (Scambler et al., 1992). Subsequently, several reports followed which confirmed the microdeletion in chromosome 22 (Driscoll et al., 1992; Kelly et al., 1993). Tremendous interest in the syndrome still continues today as it is a complex disorder that affects essentially every organ system in the body and psychiatric disturbance is prominent.

Individuals with clinical symptoms of 22q11DS were described some 50 years ago by Sedlackova (Sedlackova, 1955) in Czechoslovakia and later by Strong (Strong, 1968). However, the earliest categorisation of the disorder as a syndrome did not occur until the 1970s (Shprintzen et al., 1978) and it was not until the early 1990s that research interest in the disorder really emerged. It is possible that 22q11DS was not formally categorised as a distinct syndrome even earlier because children with the disorder experienced multiple physical complications such as congenital heart defects that resulted in early death. Also, a syndrome is defined as a condition with multiple anomalies, all of which originate from a single cause and it was not until 1992 that the genetic deletion was identified at the 22q11.2 band. Further, individuals with 22q11DS often presented to a variety of therapeutic disciplines with each focusing on their own explicit area of expertise rather than the integrated approach that is currently practiced.

Though the syndrome is widely known as 22q11DS, it is also known as Velo-cardiofacial syndrome (VCFS), Sedlackova syndrome, DiGeorge syndrome, Shprintzen syndrome, Cayler syndrome, Takao syndrome and Conotruncal anomaly face syndrome amongst others. These nosologic labels represent not only the extensive
variety of academic disciplines involved in the study of VCFS but also the possibility that competing research teams have each advocated the use of their preferred titles. As some of these labels have been provided by specialists in their own circumscribed field of study, they may not be entirely reflective of the full spectrum of the syndrome. Consequently, researchers and clinicians may have mistakenly believed that these labels represent discrete syndromes that are each caused by the same underlying deletion of chromosome 22 (Robin and Shprintzen, 2005). For the rest of the thesis, the syndrome will be referred to by its current description of 22q11DS.

1.5.1. Epidemiology

Due to broad phenotypic expression in 22q11DS, it was difficult to precisely quantify its incidence and prevalence rates prior to genetic study of the disorder. However, since genetic testing has become widely available, the incidence of 22q11DS has been estimated to be between 1:4000-7000 live births (Botto et al., 2003; Oskarsdottir et al., 2004; Scambler, 2000). These figures should be regarded as the absolute minimum as individuals with more subtle forms of 22q11DS may only be diagnosed at a later stage in life. Those with classical forms of the disorder, such as palatal defects and congenital heart anomalies, are typically detected early while those with a milder phenotype may only be diagnosed when they present in late childhood with behavioural, learning or psychiatric disturbance, usually between seven and nine years of age. A further factor that determines the diagnosis of
22q11DS is clinician experience in developmental disorders. Undetected pregnancy losses or stillbirths may account for additional underreporting of the disorder.

Although 22q11DS appears to affect both genders equally (Botto et al., 2003), it is likely that the population prevalence of 22q11DS varies with place of birth (Shprintzen, 2008). This is a consequence of the high frequency of severe and life-threatening congenital heart defects associated with 22q11DS and mortality rates may be higher during the neonatal period especially for those born in areas where there is poorer access to specialist surgical care or neonatal intensive care units.

1.5.2. Causation

While the majority of 22q11DS cases are associated with an interstitial deletion of chromosome 22q11 (Carey et al., 1992), however, other genetic abnormalities involving chromosome 22q11, such as balanced translocations, terminal deletions, non-random rearrangements and mosaicism have been reported in a minority to contribute to the disorder. While the majority of deletions occur de novo, 5-10% of 22q11DS probands show an autosomal dominant pattern of inheritance for the deletion (Ryan et al., 1997; Shprintzen et al., 1981). There are also rare instances when individuals may possess the typical 22q11DS phenotype but lack chromosome 22 deletions.

22q11DS is regarded as a gene haploinsufficiency syndrome as a result of the deletion being carried in only one arm of chromosome 22 (Lindsay, 2001). Of the
22q11DS cases with chromosome 22 deletion, 90% have a 3Mb interstitial deletion, known as the typically deleted region (TDR), while the remainder has a smaller or 'nested' deletion (1.5-2Mb). Though chromosome 22 is regarded as one of the smallest autosomes in the human genome, the long arm of chromosome 22 contains a large number of genes; however, no specific gene has been identified as the causative mechanism that may fully explain all the features of the disorder (Scambler, 2000).

The genes in the TDR are thought to play a critical role in neural crest development and migration and therefore in the formation of the third and fourth pharyngeal arches/pouches and cardiac outflow tract. Thus the structures predominantly affected in 22q11DS are to some extent derived from the branchial arch/pharyngeal pouch structures e.g. the thymus gland, parathyroids and face. The chromosome 22q11.2 locus is also rich in genes including those that are involved in neurotransmission and neurodevelopment. As the large majority of individuals with 22q11DS have genes deleted in the TDR (approximately 60 genes), the process of determining the specific factors involved in the causation of both physical and psychiatric/behavioural disorders is currently underway.

One of the deleted genes mapped to the chromosome 22q11 region codes for catechol-O-methyltransferase (COMT), an enzyme that degrades dopamine (Grossman et al., 1992). Additionally, COMT appears to be a candidate gene accounting for the higher levels of psychiatric morbidity seen in 22q11DS (Craddock et al., 2006; Dunham et al., 1992). COMT activity attains maximal levels in early adulthood especially in the PFC region (see Section 1.2.1.) and COMT is also
thought to modulate brain development and function (Hoglinger et al., 2004; Zinkstok et al., 2006). COMT may influence white matter integrity through the regulation of dopamine levels, which in turn can further modulate the proliferation and differentiation of oligodendrocytes and thus affect the formation of myelin (Bongarzone et al., 1998).

The COMT gene undergoes a naturally occurring polymorphism which has been reported to affect dopamine regulation (Akil et al., 2003); the polymorphism leads to an amino acid substitution (Valine[Val] to Methionine[Met]) and results in decreased thermostability and variable enzymatic activity. The Met/Met variant of COMT displays approximately 40% less enzymatic activity than Val/Val (Chen et al., 2004). As a consequence of chromosomal deletions, 22q11DS individuals possess only one working copy of the COMT gene (i.e. they are haploinsufficient) which results in either a high activity Valine (Val-COMT) or low activity Methionine (Met-COMT) isoform of the COMT enzyme. Disrupted dopaminergic modulation and neurotransmission may occur as a result of haploinsufficiency and has indeed been reported in adults with 22q11DS without a psychiatric history (Boot et al., 2008) which may help explain their vulnerability for the development of psychiatric disorders. More recently, in a study assessing non-deleted adults with intellectual disability using DT-MRI, COMT Val158Met polymorphism was found to affect the association between intelligence quotient (IQ) and white matter architecture (Li et al., 2009). The authors showed that FA values in the PFC and hippocampus were associated with IQ whereby subjects who were homozygous for Val showed steeper slopes for regression of FA value on IQ than Met heterozygotes. Additionally, the high activity Val allele has been associated with lower full scale IQ (FSIQ) and
verbal IQ scores than the low activity Met allele in 22q11DS children (Shashi et al., 2006) while the Val allele has been found to show a greater decline in verbal IQ from childhood to adulthood than the Met allele in 22q11DS (Gotheff et al., 2005).

Another gene deleted in the 22q11 domain is *TBX1*, a member of the T-box gene family of transcription factors with more than 20 genes identified in humans (Packham and Brook, 2003). It is recognised to contribute to regulation of developmental processes and has a role in modulating defects arising from the pharyngeal apparatus (Aggarwal and Morrow, 2008; Stoller and Epstein, 2005). A further gene deleted in the 22q11.2 region is proline dehydrogenase (PRODH), a mitochondrial membrane enzyme that catalyses the first step in the proline degradation pathway (Bender et al., 2005). Proline is a non-essential amino-acid and may have a modulatory role in both glutaminergic and acetylcholine activity (Delwing et al., 2003) and hyperprolinemia has not only been documented in individuals with 22q11DS (McDermid and Morrow, 2002) but also some individuals with schizophrenia (Liu et al., 2002).

The very high prevalence of psychiatric disorders in 22q11DS is likely to be caused by haploinsufficiency as a result of deletions at 22q11.2 of one or more genes and subsequent differences in brain structure, cerebral maturation and neurotransmitter systems. Given that 22q11DS is a genetic disorder with a high prevalence of psychiatric disorders, identification of susceptibility genes that contribute to brain maturation and also the development of psychiatric impairment in 22q11DS may be relevant to the non-deleted general population (Murphy and Owen, 2001; Prasad et al., 2008).
1.5.3. Diagnostic criteria

22q11DS has an extremely broad phenotypic spectrum with more than 180 clinical characteristics that encompass both physical and behavioural attributes (Robin and Shprintzen, 2005; Shprintzen et al., 2005). Diagnosis through clinical criteria alone has proven unreliable as no single clinical feature occurs in all 22q11DS cases and there are no reported cases of the syndrome that have all or even most of the clinical findings. Therefore, as the syndrome shows marked variability in phenotypic expression, recent advances in molecular genetics have permitted a more robust identification of 22q11DS probands.

Given that 22q11DS is associated with a microdeletion of chromosome 22 at the q11.2 band, it is possible to detect this genetic deletion through a variety of cytogenetic laboratory techniques. Karyotyping is a method that assesses the physical structure of all chromosomes and has the potential to detect large chromosomal rearrangements or deletions. However, the majority of deletions that cause 22q11DS are too small to be assessed by karyotyping alone. Therefore, fluorescence in situ hybridisation (FISH) or multiplex ligation-dependent probe amplification (MLPA) may be better suited for accurate diagnosis. Although FISH is most commonly used for diagnosis (Robin and Shprintzen, 2005), chromosomal microarray analysis is gradually replacing FISH as the first line diagnostic tool for genomic disorders in general.
FISH directly assesses the area on chromosome 22 that is related to 22q11DS i.e. the 3Mb TDR. With FISH, chromosome preparations are obtained from peripheral blood samples that have been denatured to allow hybridisation of a probe specific to the TDR. Probes are stained with a dye and under proper laboratory conditions these fluorescent probes will bind to the corresponding area of chromosome 22q11. For people having two normal (non-deleted) chromosome 22s, two stained probes appear with fluorescence microscopy. However, if there is a deletion on one of the chromosome 22s, there is lack of a substrate for the probe to adhere to and therefore only one probe appears (which is the case in 22q11DS). The decision to perform genetic testing for the diagnosis of 22q11DS depends on clinical suspicion and the degree of clinical experience in developmental disorders. When major congenital heart anomalies are present, especially in the form of conotruncal anomalies and ventricular septal defects (VSDs), the likelihood of diagnosing 22q11DS is much higher. However, the large majority of individuals with 22q11DS (90%) have de novo mutations where neither parent is affected.

Though 22q11DS shows broad phenotypic expression, some clinicians adopt a minimal diagnostic criteria or a probability approach to diagnosing genetic syndromes (Shprintzen, 2008). If this approach is employed in the absence of genetic confirmation, it is possible to diagnose someone who has cleft palate, intellectual disability, congenital heart defects, immunodeficiency, hypotonia, developmental delay, and small ears with overfurred helices with 22q11DS while these clinical criteria may be equally applicable to trisomy 21/Down syndrome. Some patients with 22q11DS may also be incorrectly diagnosed as having the CHARGE syndrome (i.e. Coloboma of the eye, Heart defects, Atresia of the nasal choanae, Retardation of
growth and/or development, Genital and/or urinary abnormalities, and Ear
abnormalities and deafness). Although CHARGE is related to a mutation or deletion
in the chromodomain helicase DNA-binding protein-7 (CHD7) gene located on
chromosome 8 (Vissers et al., 2004) and genetic testing is available for this disorder,
it is still a largely clinical diagnosis as only 60% of patients tested have the CHD7
mutation (Lalani et al., 2006).

A further differential diagnosis to consider is another chromosome 22q deletion
syndrome i.e. the 22q13.3 syndrome (Phelan-McDermid syndrome or deletion 22q13
syndrome). This syndrome is a chromosome microdeletion syndrome also on the
long arm of chromosome 22 but in the 13.3 band. It is characterised by neonatal
hypotonia, global developmental delay, normal to accelerated growth, absent to
severely delayed speech and minor dysmorphic features. Although this syndrome is
not routinely tested for, it can be confirmed via FISH or array comparative genomic
hybridisation and should be considered in all cases of hypotonia of unknown
aetiology and in individuals with absent speech (Phelan, 2008). Partial monosomy of
chromosome 10p is a rare genetic disorder where a significant proportion of
individuals with the disorder demonstrate features of 22q11DS. Its main
characteristics are hypoparathyroidism, deafness and renal dysplasia and is also
known as the HDR syndrome (Lichtner et al., 2000). However, those with HDR lack
the cardiac, palatal and T-cell abnormalities that are present in 22q11DS.

An environmental disorder which causes impairment in neural crest development
and branchial arch abnormalities is Isotretinoin treatment for acne. As a result of
increased use of Isotretinoin, there has been a concomitant increase of associated
birth defects with Isotretinoin embryopathy producing a phenocopy of 22q11DS (Aggarwal and Morrow, 2008; Coberly et al., 1996) and may be related to downregulation of Tbx1 (Zhang et al., 2005).

1.5.4. Behavioural and psychiatric disorder

While the variety of psychiatric presentations in 22q11DS is heterogeneous, high rates of psychiatric morbidity have been reported ever since it was described as a genetic disorder (Shprintzen et al., 1992). Early studies of children with 22q11DS reported a characteristic personality with poor social interaction, both quantitatively and qualitatively, that was associated with a bland affect with minimal facial expression. It was also reported that children often exhibited extremes of behaviour, notably behaviour that was disinhibited/impulsive or serious/shy (Golding-Kushner et al., 1985; Swillen et al., 1997). Additionally, parents of 22q11DS children up to the age of three years have often reported a variety of somatic complaints presenting in their children e.g. eating difficulties or withdrawn behaviour. This social withdrawal often persists into adolescence and is compounded by attentional deficits and poor social skills which may be accounted by impaired communication abilities.

While only a limited number of studies have assessed the psychiatric profile in 22q11DS children, these have reported high rates of comorbid psychiatric disorder (Arnold et al., 2001; Baker and Skuse, 2005; Feinstein et al., 2002). The most prevalent of which are mood disruptions, attention deficits and psychotic phenomena that appear to be independent of intellectual impairment (Baker and Skuse, 2005).
ADD/ADHD is common, with rates of between 35-46% in children and young adults (Feinstein et al., 2002; Gothelf et al., 2004a; Niklasson et al., 2001) and is perhaps the most common psychiatric disorder in 22q11DS (Antshel et al., 2006; Zagursky et al., 2006). This is in marked contrast to an estimated prevalence of between 3–7% for non-deleted school-aged children (Polanczyk et al., 2007).

Rates of psychotic disorders such as schizophrenia and schizotypy are significantly increased at 20-30% in 22q11DS, a rate approximately 25 times that in the general population (Murphy et al., 1999; Pulver et al., 1994). Deletion of chromosome 22q11.2 is the third highest risk factor for the development of schizophrenia, with only a greater risk conferred by being the child of two parents with schizophrenia or the monozygotic co-twin of an affected individual. Schizotypy is significantly increased in both 22q11DS children and adults (Baker and Skuse, 2005; Murphy et al., 1999) whereby schizotypy in 22q11DS and the non-deleted population is considered a personality disorder with personality traits and subclinical psychopathological features that are qualitatively similar to symptoms of a psychotic illness (Baker and Skuse, 2005). Of note, when schizotypy is present in childhood in both deleted and non-deleted populations, it confers greater susceptibility to the development of psychosis in later life (Chapman et al., 1994; Poulton et al., 2000; Siever and Davis, 2004). Recently, reports have suggested that the development of psychotic disorders in 22q11DS is a gradual process with an initial presentation of subthreshold psychotic symptoms in childhood (Gothelf et al., 2007) and that 22q11DS children often display more serious psychiatric symptoms as they go through adolescence compared to children with idiopathic learning disability and
thus 22q11DS represents an important group to monitor not only during their early life but throughout their life-span.

While higher rates of autism spectrum disorder in 22q11DS have also been reported at between 14-50% (Fine et al., 2005; Niklasson et al., 2001; Vorstman et al., 2006), Vorstman and colleagues suggest that autistic and psychotic disorders are major features of the behavioural phenotype seen in children with 22q11DS. The same authors also suggest that the autistic symptoms found in their study may be a reflection of the neurodevelopmental abnormalities in 22q11DS individuals with schizophrenia where autistic symptoms may represent prodromal features of psychosis rather than exclusively an autism spectrum disorder.

Additionally, mood disorder is common in 22q11DS children and adults where rates of between 12-47% have been reported in the literature (Arnold et al., 2001; Carlson et al., 1997; Murphy et al., 1999; Papolos et al., 1996). Although an increased rate of bipolar affective disorder (BPAD) was initially reported (Papolos et al., 1996), subsequent studies have described an unstable mood disorder instead of BPAD (Vogels et al., 2002). Obsessive-compulsive disorder, have also been described in a number of studies, with rates ranging from 8-33% (Gothelf et al., 2004b; Papolos et al., 1996). Apart from genetic predisposition, pubertal changes and increased social demands have been further suggested to place 22q11DS individuals at a heightened risk of developing anxiety and depressive disorders (Swillen et al., 1999).

1.5.5. Previous neuroimaging findings
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Early neuroimaging studies in subjects with 22q11DS were mostly qualitative and differences reported include an increased incidence of midline abnormalities such as white matter hyperintensities and septum pellucidum defects (Mitnick et al., 1994; van Amelsvoort et al., 2001) and severe cerebral malformation (Bolland et al., 2000; Kraynack et al., 1999). Cortical malformations manifesting with polymicrogyria have been found (Robin et al., 2006) which are characterised by a thick cortex in association with shallow sulci usually caused by ischemic injury during a critical period of embryonic brain maturation (Barkovich et al., 1995). Qualitative studies were an important first step that subsequently led to quantitative studies that examined both gross anatomical changes and subtle cerebral anatomy differences.

Quantitative studies have found a significant reduction in total brain volume in children and adolescents with 22q11DS relative to normally developing subjects and of the order of 8.5-11%. The volume of posterior brain structures in particular, such as the cerebellum, temporal and parietal lobes, appear to be the main structures affected with reduction being largely accounted for by a decrease in white matter volume, with relatively preserved or enlarged frontal lobe tissue (Bish et al., 2006; Eliez et al., 2000; Kates et al., 2001; van Amelsvoort et al., 2001; van Amelsvoort et al., 2004). ROI studies have also postulated a rostro-caudal gradient in volume reduction whereby anterior regions are relatively preserved/enlarged while the structures in more posterior regions undergo volumetric reduction. Several structures exhibit such a gradient and include both white matter and grey matter areas such as the caudate nucleus (Campbell et al., 2006; Eliez et al., 2002; Kates et al., 2004),
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corpus callosum (Antshel et al., 2005; Machado et al., 2007), thalamus (Bish et al., 2004) and fusiform gyrus (Glaser et al., 2007).

In a comparison of 22q11DS children to healthy controls using a combination of automated VBM and manual (hand tracing) MRI analytical techniques (Campbell et al., 2006), specific vulnerability of the cerebellar-cortical and fronto-striatal networks was reported. Campbell and colleagues (2008) also recently compared the brain anatomy of children with 22q11DS to age-, gender-, and FSIQ-matched children with Williams syndrome (Campbell et al., 2008) where despite similar overall brain volumes, there were significant differences in regional brain anatomy in 22q11DS as reflected by increases in striatal volume as well as reduction in cerebellar volume. The authors suggested that these regional differences may be specific to 22q11DS.

With regard to neuroanatomical abnormalities associated with psychotic symptoms in 22q11DS, bilateral reduction in cingulate gyrus grey matter volume particularly affecting the anterior cingulate gyrus has been reported (Dufour et al., 2008). Additionally, volume reduction in whole-brain measures of both white and grey matter volume has been reported in people with 22q11DS and comorbid schizophrenia compared to those with 22q11DS without schizophrenia as well as healthy controls; volume reduction occurred to a greater extent in white matter and was accompanied by concomitant increases in total and sulcal cerebrospinal fluid volume (van Amelsvoort et al., 2004). In the same study, both 22q11DS groups (with and without associated schizophrenia) had reduced cerebellar volume compared with healthy controls and the authors concluded that within 22q11DS,
schizophrenia is associated with generalised differences in brain anatomy, but white matter anatomy may be particularly implicated. However, there are only limited studies examining white matter in 22q11DS using DT-MRI and none have investigated psychotic disorders in the syndrome using this modality.

Given increasing evidence that people with 22q11DS have regionally specific differences in brain anatomy (and perhaps especially affecting white matter) and that supra-regional brain systems often share common developmental influences (Cheverud, 1984), this suggests that people with 22q11DS may have differences in both brain connectivity and in the microstructure of white matter (Kiehl et al., 2008).

In the first DT-MRI study of 22q11DS (Barnea-Goraly et al., 2003), significantly reduced FA of white matter was reported in frontal, parietal and temporal regions of the 22q11DS subject group which included 19 children, adolescents and adults with the disorder. Additionally, FA deficits were also reported in white matter tracts connecting the frontal and temporal lobes of the same group. This study was a valuable first step. However, both adults and children were combined as one group, and most of the cerebellum and brainstem was excluded from analysis.

A subsequent DT-MRI study confined to 18 children and adolescents with 22q11DS (Simon et al., 2005) reported decreased FA in the corpus callosum of 22q11DS subjects but increased FA in the cingulum and right inferior parietal lobule of the same group. The authors postulated a posteriorly displaced corpus callosum in young people with 22q11DS to account for their findings; however, a healthy paediatric brain template was used for spatial normalisation in this study which is a potential cause of misregistration.
In summary, while there is mounting evidence that people with 22q11DS have significant differences in the development of specific brain regions, however, impaired connectivity between these brain regions may also have a significant contribution to the evolution of psychiatric deficits seen in 22q11DS. However, only two previous DT-MRI studies have directly examined the microstructural integrity or connectivity of white matter networks in 22q11DS and both these studies have been associated with methodological issues. Further, there have been no prior studies using this modality to investigate comorbid psychotic disorders such as schizophrenia or schizotypy which are frequently associated with 22q11DS. Finally, although 22q11DS demonstrates haploinsufficiency of genes in the chromosome 22q11.2 region, there have been no prior studies on the effect of COMT polymorphism on white matter measures of FA in people with the syndrome.

1.6. Temporal lobe epilepsy (TLE)

Epilepsy and seizures have long been described since antiquity when they were regarded as a form of religious experience. People used to believe that epileptic seizures were potentially a manifestation of demonic attacks or that people with epilepsy experienced visions sent by the gods. The understanding of epilepsy has since improved and there is recognition currently of a variety of seizure disorders. Seizures are defined as time-limited paroxysmal events that result from abnormal, involuntary, rhythmic neuronal discharges in the central nervous system while
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epilepsy is characterised by spontaneous recurrence of unprovoked seizures (Shneker and Fountain, 2003).

Seizures are also known by other terms such as fits, convulsions, attacks, spells and ictus but seizure is the preferred term presently. Epilepsy is classified into several syndromes of which there are over 40 types and fundamentally, epilepsy is divided according to location of the lesion (localised or generalised) and known or suspected cause (idiopathic, symptomatic, or cryptogenic) (Commission on Classification and Terminology of the International League Against Epilepsy, 1989). It is recognised that currently, there is a growing need to expand the description of epilepsy (Engel, 2001) and there have been proposals for classification according to these five axes: 1) aetiology (association with an epilepsy syndrome or association with genetic defects or pathologic substrates); 2) seizure semiology/ictal phenomenology (observable manifestations of the seizures); 3) seizure type (localisation within the brain and precipitating stimuli); 4) as a part of discrete, identifiable epilepsy syndromes; and 5) impairment.

Of the various forms of epilepsy, TLE is of particular importance to psychiatry given the propensity for psychiatric impairment and in particular depressive, anxiety and psychotic disorders (Gaitatzis et al., 2004). TLE is an epileptic disorder described as arising from epileptogenic mechanisms in the temporal lobe and was initially recognised in 1881 by John Hughlings Jackson, who described ‘uncinate fits’ arising from the uncal part of the temporal lobe that was associated with a ‘dreamy state.’ TLE is now recognised to be frequently related to underlying structural brain lesions especially in the medial temporal lobe and is classified as symptomatic localisation-
related epilepsy. Indeed, when TLE is associated with structural lesions, it is known as symptomatic TLE; however, when recurrent seizures arise from the temporal lobe in the absence of lesions, the seizure disorder is referred to as cryptogenic (Commission on Classification and Terminology of the International League Against Epilepsy, 1989). Localisation-related epilepsies are also known as partial or focal epilepsies where TLE is particularly well-described and is the most common form of such an epileptic disorder in adults (Wiebe, 2000). TLE is usually further subdivided into seizures arising from the medial/mesial temporal lobe or laterally in the neocortex (Pascual, 2007). Mesial TLE is characterised by abnormalities in the medial temporal lobe structures and particularly hippocampal sclerosis (Engel et al., 1997). Further, while TLE is the most refractory epileptic disorder to medical treatment, it however also represents a syndrome whereby underlying structural lesions are surgically remediable (Engel, 1996; 1998).

1.6.1. Epidemiology

Epilepsy is regarded as one of the most common and debilitating neurological disorders with a prevalence of 5-10 per 1000 population (MacDonald et al., 2000) while a significant proportion of the population (≈10%) will have one or more seizures at some point during their life (Hauser et al., 1996). Although many antiepileptic drugs are readily available in developed nations, however, 30-40% of epileptic patients continue to have seizures that are not adequately controlled by pharmacological intervention (Kwan and Brodie, 2000).
There are significant direct and indirect costs related to epilepsy morbidity where it’s
global burden among the disorders of the nervous system ranks just after affective
disorders, alcohol dependence syndrome and dementia (Begley et al., 1994). Also,
there are significant psychiatric, psychological, legal and social sequelae which
contribute to an increased burden in epilepsy (de Boer et al., 2008) which according
to the World Health Organization, accounts for approximately 1% of the global
burden of disease, as measured by disability-adjusted life years (DALYs). This is
comparable to the global burden of disease attributable to some neoplasms e.g. lung
and breast carcinoma. Further, epileptic disorders are frequently associated with
social stigma which may contribute to impaired educational and employment
attainment. Additionally, in most jurisdictions, those with epilepsy may be legally
precluded from activities that pose a threat to themselves or others such as driving
while in less developed societies, people with epilepsy may also be excluded from
marriage which may further disrupt their social integration (de Boer et al., 2008).

Although the estimated rates for epilepsy are reported as significant, the true
prevalence is very likely to be much higher. For example, while approximately half
of all forms of epilepsy correspond to partial epilepsy and partial epilepsy is most
often of temporal lobe origin, however, the true prevalence of TLE is currently
unclear as not all cases of presumed TLE are confirmed by video-EEG investigation
or interictal EEG findings and consequently, many cases of TLE are classified by
clinical history. Additionally, although TLE affects the genders equally, it is more
prevalent in female patients as they may experience catamenial worsening of their
epilepsy (an increase in seizure activity during the menstrual period). Further,
although TLE occurs in all age groups, it is especially underrecognised in the elderly
population where it may present as an acute confusional state or may be associated with memory lapses instead of the typical physical manifestations seen in epilepsy of younger populations.

There is also significant burden in epilepsy which occurs as a result of progressive features of the epileptic disorder such as increasing seizure frequency or cognitive impairment (Collaborative Group for the Study of Epilepsy, 1992). Further, these progressive features are especially common in patients with localisation-related epilepsy of temporal-lobe origin (Pitkanen and Sutula, 2002) as the temporal lobe is regarded as the most epileptogenic region of the brain and lesions such as hippocampal sclerosis in particular predispose to the development of epilepsy. Additionally, epilepsy may lead to significant psychological and psychiatric comorbidity which is summarised in Section 1.6.4.

1.6.2. Causation

TLE is associated with gross structural abnormalities affecting the temporal lobe such as hippocampal sclerosis, cortical dysgenesis, tumours and vascular malformations. Some of these abnormalities are thought to arise through genetic mechanisms or potential environmental insults. While such insults include birth injury, febrile convulsions, status epilepticus, stroke, head trauma or central nervous system infection, when associated with underlying genetic vulnerability, these may lead to acquired changes in brain anatomy (Lewis, 2005). Neurological insults are postulated to trigger a biological cascade in the brain during a latent period termed
epileptogenesis, which subsequently leads to the occurrence of spontaneous seizures and to the development of an epileptic disorder (Pitkanen and Sutula, 2002).

Pitkanen and Sutula (2002) describe a series of molecular, cellular and brain network changes during epileptogenesis that produce sustained alterations in neuronal circuitry. These processes result in aberrant reorganisation of neurones that is characterised by neuronal loss (acute or delayed) and abnormal neoneurogenesis, gliosis, plasticity (axonal or dendritic), inflammation and molecular reorganisation. The literature would suggest that although an early insult may cause asymmetric temporal lobe damage that is present at the onset of epilepsy, generalised seizures may further induce neuronal damage that progresses over the course of the disorder (Bernasconi et al., 2002).

Additionally, TLE demonstrates a genetic component which is shared with febrile seizures (Baulac et al., 2004; Hamati-Haddad and Abou-Khalil, 1998) and familial TLE syndromes have been reported (Berkovic et al., 1996; Cendes et al., 1998; Hedera et al., 2007). While most familial TLE is reported to originate in the mesial temporal lobe, genetic mutations are also seen in autosomal dominant partial epilepsy (Kalachikov et al., 2002; Ottman et al., 1995) which has been associated with auditory hallucinations that are postulated to arise from seizure activity in the lateral temporal lobe (Winawer et al., 2000) where this disorder is also known as autosomal dominant partial epilepsy with auditory features (ADPEAF). However, there appears to be clinical and genetic heterogeneity in epileptic disorders (Cavalleri et al., 2005; Santos et al., 2002) which is likely to be accounted for by a complex
mode of inheritance with potential genes identified only accounting for a small proportion of families and sporadic cases (Gutierrez-Delícado and Serratosa, 2004).

### 1.6.3. Diagnostic criteria

The evaluation of seizures and the diagnosis of epilepsy is often based on clinical judgement and dependent on answering the following (Shneker and Fountain, 2003):
1) is this a seizure or a different type of paroxysmal event; 2) is this a provoked or unprovoked seizure; and 3) what is the probability of recurrence of seizures (so as to establish a diagnosis of epilepsy)? However, a more robust evaluation of TLE involves examination of a combination of seizure semiology, MRI and EEG data which are taken into consideration prior to establishing a diagnosis. Nevertheless, the presence of normal MRI or EEG investigations does not exclude the possibility of an epileptic disorder. Assessment of cerebrospinal fluid via lumbar puncture is not routinely conducted in the assessment of TLE but rather when central nervous system infection is suspected.

**Seizure semiology**

The seizure semiology in TLE can be broadly divided into three seizure patterns (Commission on Classification and Terminology of the International League Against Epilepsy, 1989): simple partial seizures, complex partial seizures and secondarily generalised tonic-clonic seizures. Each of these seizure types will be described in more detail next.
Simple partial seizures usually involve small regions of the temporal lobe such as the medial temporal lobe structures e.g. hippocampus or amygdala. As there is no impairment of consciousness with simple partial seizures, consequently, the seizures may be experienced as abnormal perceptions potentially involving a variety of sensory systems e.g. olfactory, auditory, visual, tactile or gustatory. Additionally, memories may be altered in the form of déjà vu (a feeling of familiarity), jamais vu (a feeling of unfamiliarity), recall of a set of memories, or amnesia. Feelings of depersonalisation or derealisation may also occur and can be associated with dysphoric, euphoric or other emotional experiences. Simple partial seizures are often brief and last less than 60 seconds.

Complex partial seizures are seizures which impair consciousness and these usually commence with a simple partial seizure and subsequently, seizure activity extends to a larger portion of the temporal lobe which then results in impaired consciousness. People experiencing a complex partial seizure may be observed to display unusual speech or unusual behaviours which include staring, automatic movements of the limbs or mouth (automatisms) or impaired ability to respond to external cues. Complex partial seizures last longer than simple partial seizures and are usually less than two minutes duration.

The final type of seizure pattern to consider is the secondarily generalized seizure which begins in the temporal lobe but then spreads to the rest of the brain. This form of seizure usually begins with either a simple or complex partial seizure. Subsequently, the trunk, arms and legs stiffen in either a flexed or extended position which is then followed by coarse, rapid and rhythmic jerking of these muscles.
Secondarily generalised tonic-clonic seizures usually last the longest and are typically less than three minutes in duration.

Following each of these seizure patterns is the postictal state, which reflects a period of recovery in neurological function. The degree and length of impairment in the postictal state directly correlates with the severity of the three seizure types described above. The postictal state in the case of complex partial and secondarily generalised tonic-clonic seizures often lasts much longer than the seizure itself and as the temporal lobe is significantly involved in short-term memory processing, there is usually amnesia for the seizure event.

**MRI**

The ability to identify lesions contributing to the development of TLE has improved significantly since the use of MRI methodology which continues to be refined (Mohamed and Luders, 2000). It has enabled the *in-vivo* identification of major structural lesions in the temporal lobe such as hippocampal sclerosis, brain tumours, and malformations of cortical or vascular origin. Further, the superiority of MRI over CT in terms of sensitivity and specificity is well established and MRI is the gold standard neuroimaging technique in both children and adults for identifying the aetiology of epilepsy (Brooks et al., 1990; McLachlan et al., 1985; Mohamed and Luders, 2000). MRI is also used presurgically to aid in the planning of and stereotactic removal of abnormal tissue in the temporal lobe and also for the placement of stereotactic intracerebral monitoring electrodes for electrophysiological assessment (Duncan, 1997; Kelly et al., 1987; Pillay et al., 1992). Additionally, MRI is not only used qualitatively to assess gross neuroanatomical changes in the
temporal lobe but is also used to quantify more subtle differences for instance, atrophic volume changes in the medial temporal lobe structures (Van Paesschen et al., 1997).

**EEG**

When TLE is classified as symptomatic, there is evidence of a structural or electrophysiological abnormality in the temporal lobe. EEG is able to identify seizures arising from the temporal lobe where gross structural lesions are absent but where electrophysiological abnormalities may be occurring. While EEG enables the identification of focal spikes arising in the temporal region during a seizure event, it is also used to detect interictal abnormalities (Shneker and Fountain, 2003).

However, in the context of a normal EEG but suspected epilepsy, sleep or sleep-deprived EEG may be applied as certain types of seizure are associated with phases of the sleep-wake cycle. Additionally, stimuli that provoke a seizure may be attempted during an EEG recording such as photic-stimulation so as to facilitate diagnosis.

To enable the correlation between observable seizure phenomena with EEG activity, synchronised video-EEG telemetry has been recently developed and is particularly useful in confirming the presence of a seizure disorder or classifying seizure type. Further, it may be used in presurgical evaluation for localisation of abnormal neuronal tissue and it may also be used to confirm a paroxysmal event such as non-epileptic seizures when there is suspicion that it may be accounting for apparent seizure activity (Cascino, 2002).
1.6.4. Behavioural and psychiatric disorder

While epilepsy in general contributes to social, cognitive, behavioural and psychiatric impairment (de Boer et al., 2008; Kwan and Brodie, 2001; Torta and Keller, 1999), higher rates of psychopathology have been reported especially in individuals with TLE compared to those with generalised epilepsy, people with chronic non-neurological disorders and the general population (Adams et al., 2008; Edeh and Toone, 1987; Torta and Keller, 1999). Indeed, the lifetime risk of psychiatric disorder in TLE is significant and estimated to be as high as 60% (Edeh and Toone, 1987; Swinkels et al., 2005). The high prevalence of psychopathology in TLE is conceptually thought to arise from an interaction of several variables (Hermann and Whitman, 1984) which are divided into: 1) brain-related factors; 2) nonbrain-related factors; and 3) treatment-related factors.

An alternative but related conceptualisation (Torta and Keller, 1999) to account for higher psychiatric comorbidity considers the interplay between: 1) clinical features of TLE (e.g. age at onset of epilepsy, duration of disorder, type and frequency of seizures); 2) psychosocial factors (e.g. chronicity of epilepsy, low socioeconomic status, low educational attainment, negative cultural bias towards epilepsy); and 3) biological mechanisms (e.g. emotional and cognitive side effects of antiepileptic medication). This classification of factors bears resemblance to Engel’s biological-psychological-social model of aetiological factors (Adler, 2009).
Although psychiatric disorders such as anxiety and depressive disorders are common in all forms of epilepsy including TLE (Hermann et al., 2000; Lehrner et al., 1999; Tellez-Zenteno et al., 2007), patients with unilateral left TLE may additionally demonstrate personality and behavioural disturbance characterised by increased levels of emotional dependency, nervousness and perseveration but a reduction in drive and externally judged composedness (Feddersen et al., 2005). However, the relationship between laterality of seizure foci and psychiatric disturbance has not been consistently demonstrated in the literature (Kalinin and Polyanskiy, 2009).

Further, given that psychosis is particularly overrepresented in TLE (Shukla et al., 1979; Taylor, 1972; Umbricht et al., 1995) and that TLE and associated psychosis (TLE+psychosis) is one of the psychiatric disorders that will be examined in this thesis, there will be particular emphasis on TLE+psychosis in the present section.

Indeed, the relationship between the temporal lobe and psychosis has long been observed (Flor-Henry, 1969; Gibbs et al., 1948; Slater et al., 1963) and the prevalence of psychosis in TLE is substantial (2-7%) which is several times greater than that seen in the general population (Bredkjaer et al., 1998; Gaitatzis et al., 2004; Kendler et al., 1996; Mendez et al., 1993; Torta and Keller, 1999). Additionally, some forms of TLE+psychosis have been noted to closely resemble schizophrenia with regard to symptomatology, course, antipsychotic response as well as brain morphometric changes (Barr et al., 1997; Flor-Henry, 1969; Toone, 2000). While TLE+psychosis has been postulated to arise from abnormalities in foetal brain development, and to represent a model or 'mock-up' of schizophrenia (Roberts et al., 1990), it is also recognised that these conditions possibly share common genetic or environmental causes (Qin et al., 2005) and recently, the mean interval between the
onset of epilepsy and that of psychosis has been reported to be approximately 14.4 years (Adachi et al., 2010).

The psychosis seen in TLE is usually divided according to its relationship with seizure activity i.e. ictal, postictal or interictal where they correspond to psychosis developing during, after a seizure or in-between seizures respectively (Logsdail and Toone, 1988). Additionally, there may be other forms of psychosis such as psychosis related to antiepileptic treatment, ‘forced normalization’ or ‘alternative psychosis’ and de novo psychosis following epilepsy surgery (Nadkarni et al., 2007). Although postictal and interictal psychoses have varying time courses (the former tends to occur within a week of seizure activity after a lucid period and recognised to be short-lived), they share common features such as a high prevalence of complex partial seizures, bitemporal seizure foci and clustering of multiple seizures (Umbricht et al., 1995). It is also possible for postictal psychoses to progress to an interictal form (Tarulli et al., 2001).

Psychosis and other psychiatric impairment seen in TLE are postulated to arise from dysfunction in the limbic system (Section 1.2.8.) with more prominent psychiatric disturbance expected in individuals with an epileptic focus in this region of the brain (Swinkels et al., 2005). Also, as TLE is also often refractory to medical treatment, this further exacerbates psychiatric comorbidity seen in the disorder.

1.6.5. Previous neuroimaging findings
While TLE has been associated with a variety of environmental insults that when coupled with genetic predisposition lead to changes in brain anatomy (Lewis, 2005), up until the 1980s, such differences were assessed through post-mortem neuropathological studies. However, MRI is the most frequently used modality in the assessment of TLE currently as such changes are readily imaged \textit{in-vivo} and have been assessed by gross qualitative examination of scans or quantified more accurately by manual and computerised statistical methods. These have enabled the \textit{in-vivo} identification of temporal lobe abnormalities and centres worldwide are increasingly adopting epilepsy surgery programmes so as to resect some of the discrete brain abnormalities and therefore have implications for improved seizure control. However, although TLE is particularly associated with abnormalities in the temporal lobe and where most studies have focused on assessment of the hippocampus, newer MRI studies are starting to provide \textit{in-vivo} evidence of abnormalities extending beyond the boundaries of the medial temporal lobe (Bernasconi et al., 2004; Lin et al., 2007; Mueller et al., 2004).

Indeed, grey matter pathology has been reported in areas such as the limbic system and thalamus (Keller et al., 2002b; Natsume et al., 2003) while extralimbic abnormalities have been found in patients with pharmacologically intractable TLE especially in frontal brain regions (Bernasconi et al., 2004). While white matter reduction has also been found in TLE and is usually ipsilateral to seizure foci and predominantly involves temporal lobe abnormalities e.g. temporo-polar, entorhinal, and perirhinal cortices (Bernasconi et al., 2003; Bernasconi et al., 2004), however, a recent DT-MRI study examining white matter integrity in TLE reported reduced FA in not only the anterior and mesial temporal lobes but also (relative to the side of
seizure onset) in the ipsilateral cerebellum, as well as the contralateral fronto-parietal lobe (Riley et al., 2010). Consequently, the pattern of structural changes seen in TLE has been suggested to arise from widespread disconnectivity between several networks e.g. fronto-limbic and temporo-cerebellar pathways.

For example, it has been postulated that prefrontal atrophy seen in TLE may be due to excitotoxic epileptiform discharges from a reciprocally connected pathological hippocampus, and as a result, may contribute to cognitive deficits such as executive dysfunction in those with TLE (Keller et al., 2002a; Keller et al., 2002b). Similarly, cerebellar atrophy has also been reported (Keller et al., 2002b) where the cerebellum may also be vulnerable to such excitotoxic damage due to its connectivity with a pathological hippocampus (Sandok et al., 2000). While there have been several neuroimaging studies assessing TLE without psychiatric comorbidity, however, there have only been a limited number of MRI studies that have assessed regional volumetric differences in subjects specifically with TLE+psychosis.

Three previous conventional MRI studies used manual ROI volumetry to compare subjects with TLE+psychosis versus those with TLE only where findings across these studies have overall been inconsistent. Findings have included volume reduction in the temporal, frontal and parietal cortices; and in the superior temporal gyrus and left hippocampus as well as bilateral enlargement of the amygdala (Marchetti et al., 2003; Marsh et al., 2001; Tebartz Van Elst et al., 2002). A subsequent study attempted to overcome the difficulties of reproducibility introduced by manual volumetry by using automated whole-brain VBM (Rusch et al., 2004). While this study limited assessment to only grey matter and observed no cortical
differences between groups, the authors suggested that TLE+psychosis may be a
distinct entity to schizophrenia as their findings were not consistent with the
neuroimaging literature in schizophrenia (Gur et al., 2007; Wright et al., 2000).

In summary, there have only been limited MRI reports in the study of
TLE+psychosis where the majority of past studies have been based on manual ROI
measurements. Additionally, there has been only one previous VBM study of
conventional MRI data, however, the authors confined assessment to only grey
matter. So far, the reported neuroanatomical literature is inconsistent in
TLE+psychosis and forms an area that requires much investigation not only in the
field of psychiatry but also in the overlapping field of neurology. Therefore, as
examination of both grey and white matter compartments based on automated VBM
has never been completed previously, it represents a novel and important area of
study. Through such investigation, identification of abnormal regions contributing to
the psychosis seen in TLE may also be relevant to the general population as these
same regions may be driving psychosis in both populations.

1.7. Antisocial personality disorder (ASPD)

Although the modern definition of psychopathy has changed little since Hervey
Cleckley published *The Mask of Sanity* in which he described the psychopath as a
charming, callous, superficial individual, lacking conscience and genuine emotion
(Cleckley, 1941), the concept of psychopathy has been related to Theophrastus, a
student of Aristotle in ancient Greece, in his description of *The Unscrupulous Man*
Chapter 1: The application of voxel-based methods to magnetic resonance imaging in the study of psychiatric disorder

(Millon and Davis, 1996). Notable works in the study of psychopathy in the last two centuries have also been documented by Philippe Pinel who initially recognised psychopathy as a specific disorder in 1801 (*la folie raisonnante and manie sans delire*) and James Prichard who described the concept of ‘moral insanity’ to account for irresponsible and socially deviant behaviour that was not associated with known forms of mental disorder at the time (Pichot, 1978; Prichard, 1835). Henry Maudsley later described psychopathic individuals as ‘moral imbeciles’ that may be less sensitive to the rehabilitation effects of the criminal justice system (Maudsley, 1895).

The term psychopath was first used by the German psychiatrist Emil Kraepelin in 1915 while the first scientific description of acquired impairment of social behaviour in association with neurobiological aetiology (frontal lobe damage) was described in the 19th century following the case of Phineas Gage (Harlow, 1993; 1869). Other authors have attempted to establish the aetiology of psychopathy and some such as Cesare Lombroso have suggested that those with the disorder are perhaps ‘born criminals’ (Gibson, 2002; Hamilton, 2008).

Although there is much overlap between frontal lobe syndrome, ASPD and with the syndrome of psychopathy, the latter two pose significant burden to the judicial system, clinicians and wider society (Coid, 2003; Sourander et al., 2007) through interpersonal and behavioural disruption (Kirkman, 2008). While psychopathy is regarded as a particularly severe subtype of ASPD (Coid and Ullrich, 2010; Dolan and Doyle, 2007), however, the emotional dysfunction at the core of Cleckley’s description of the psychopath is not considered essential for a diagnosis of ASPD as the behavioural manifestations are regarded as sufficient (Swanson et al., 1994).
Little is known about the underlying neurobiology of these disorders as only a few studies have examined the structural, functional or connectivity brain abnormalities in people with psychopathy and ASPD.

1.7.1. Epidemiology

While ASPD has a prevalence rate of 2-3% among community samples, rates in certain settings may be as high as 60% for instance in male prisoners (Hare, 1983; Moran, 1999). Indeed, there appears to be a gender imbalance where ASPD is 4-5 times more common in males relative to females in the community where it tends to affect those who are younger (between 25-44 years of age) and with poorer educational attainment (Coid, 2003; Kessler et al., 1994; Moran, 1999). ASPD is significantly associated with medical, psychiatric and social comorbidities such as substance misuse (Moran, 1999; Singleton et al., 1998) while epidemiological studies have also shown increased rates of violence, self-harm and a chronic course with ASPD (Fountoulakis et al., 2008; Ruiz et al., 2008). Although lifetime prevalence rates have been reported to decline with increasing age (Paris, 1997), however, the disorder carries an increased risk of death through unnatural causes in younger individuals which may account for reduced prevalence rates in older populations (Coid, 2003; Moran, 1999). Other settings where rates of ASPD are increased include the emergency department where a prevalence rate of 16% was found (Robins et al., 1977) and similarly in adults with ASPD who had been seen during their childhood at child guidance clinics where rates of 25% for men and 12% for women were reported (Robins, 1966). Although there have only been a few
studies in the general practice environment, these have similarly reported high rates of 5-11% (Barry et al., 1997; Moran, 1999; Smith et al., 1991).

Prevalence rates in ASPD also appear to demonstrate geographical variation. Internationally, a high rate of 11% in clinic populations in Iran have been reported versus 1.85% in Lebanon (Smith, 1978) while it has been suggested to be 0% in Japan; although these samples have used different diagnostic criteria, there may be protective culture-specific factors to account for reduced rates in certain countries such as Japan (Sato and Takeichi, 1993). However, in most countries, ASPD is associated with homelessness and inner-city populations where rates are inversely proportional with rural environments (Coid, 2003). Overall, the most common environment where ASPD is encountered is within the criminal justice system for example prisons where the prevalence is estimated to be as high as 40-60% among male sentenced prisoners.

Although the vast majority of offenders met criteria for ASPD in a previous report, only 15-25% of the same sample met criteria for psychopathy measured using the Psychopathy Checklist - Revised (Hare et al., 2000; Hart and Hare, 1997). There is currently much debate with regard to separating the diagnostic constructs of ASPD, psychopathy, sociopathy and dissocial personality into distinct diagnostic entities (American Psychiatric Association, 2000; Kirkman, 2008; Ogloff, 2006; World Health Organization, 1993); however, these terms continue to be used in much research interchangeably and synonymously (Fitzgerald and Demakis, 2007). The features of these disorders are explored in more detail later in Section 1.7.3.
1.7.2. Causation

Although data on the natural history of ASPD is currently lacking, it appears to be associated with conduct disorder that develops during childhood and adolescence (Robins, 1966; Robins and Regier, 1991). Conduct disorder not only significantly contributes to the subsequent development of ASPD in adults (Lahey et al., 2005) but also results in an increased risk of psychiatric disturbance such as substance misuse, anxiety disorders and other personality disorders including borderline personality disorder (Moran, 1999). Conduct disorder is however a heterogeneous construct manifesting with aggression to people and animals, destruction of property, serious violations of rules and, deceitfulness, lying or stealing (Blair et al., 2006; Olsson, 2009). ASPD is also more likely to be diagnosed when ADHD is present (Bloomingdale and Bloomingdale, 1988); however, when conduct disorder and ADHD coexist, children have been reported to demonstrate even more severe and enduring antisocial behaviours (Loeber et al., 1990; Moffitt, 1990).

ASPD has also long been reported to demonstrate familial aggregation where biological parents and siblings fulfil criteria for the disorder (Cloninger et al., 1978). Additionally, high rates have been described in adopted away offspring of biological parents with ASPD suggesting a genetic basis for the disorder (Crowe, 1974; Slater and Cowie, 1971; Viding et al., 2005). However, studies since then have found a significant interplay between genes and environment as contributing to development of the disorder (Cloninger et al., 1982; Moffitt, 1987; 2005).
With regard to environmental triggers, abused and neglected children demonstrate increased rates of aggression and impulsivity as well as ASPD later on in life (Farrington and Loeber, 2000; Luntz and Widom, 1994; Widom, 1989). Also, Farrington, through a variety of rating scales to assess antisocial personality, postulated several predictor variables that encompass individual and environmental domains to contribute to future antisocial behaviours that may result in delinquency and criminal offending; these include family deviance, poor parenting skills, socioeconomic deprivation, schooling difficulties, attentional and impulsivity deficits, and antisocial behaviour during childhood (Farrington, 1990). Other factors on an individual level that may predispose to ASPD relate to underlying childhood temperaments such as aggression (Miller et al., 1999; Moran, 1999; Schwab-Stone et al., 1999) and undercontrolled children who display ‘impulsive and irritable’ traits (Caspi et al., 1996).

While ASPD demonstrates a spectrum of severity with psychopathy being a particularly severe subtype, psychopathy has been suggested to be divided into primary and secondary forms. Primary psychopathy is associated with significant biological aetiology whereas the secondary form is determined predominantly by environmental influences where it has also been described as sociopathy (Lykken, 1995; Mealey, 1995). Unlike sociopaths, psychopaths constitute a relatively stable portion of any population and can be from any sociocultural background whereas the former tend to arise from deprived milieu for instance as a result of poverty, neglect, abuse and violence (Walsh and Wu, 2008). Overall, the development of ASPD and psychopathy is complex and likely to be determined by an interaction of genetic and
environmental factors, as with many other psychiatric disorders, (Caspi et al., 2002; Mann, 1997).

1.7.3. Diagnostic criteria

It is currently recognised that there is much overlap between frontal lobe syndrome and ‘functional’ or ‘non-organic’ personality disorders, particularly ASPD and psychopathy (Damasio, 2000). The Psychopathy Checklist (PCL, Hare, 1980) and the later Psychopathy Checklist - Revised (PCL-R, Hare, 1991) were designed to operationalise Cleckley’s concept of psychopathy as a basis for diagnosing the disorder. The PCL-R consists of 20 items characterised broadly by two dimensions: Factor 1 items are primarily interpersonal or emotional traits such as remorselessness, deception, shallow affect and callousness, whereas Factor 2 items assess behavioural symptoms such as violence, criminality, and dysfunctional lifestyle. For a diagnosis of psychopathy, attributes from both of these factors need to be present. While PCL-R scores ≥ 30 have traditionally been used to classify an individual as having psychopathy (Hare, 2003), more recent studies have argued for a score of ≥ 25 as sufficient for diagnosis (Edens and Petrila, 2006; Edens et al., 2010; Rutherford et al., 1999).

While the related construct of ASPD in DSM-IV (American Psychiatric Association, 2000) includes several traits present in psychopathy (e.g. lack of guilt/remorse, and impulsivity), diagnostic criteria can be met based entirely on antisocial behaviours.
(e.g. violation of social norms, irresponsibility, and criminality). Hence, the emotional deficits fundamental to psychopathy are not necessary for a diagnosis of ASPD, even if these are present in some cases (Arrigo and Shipley, 2001; Walsh and Wu, 2008). Although most adult psychopathic offenders meet criteria for ASPD, only approximately one third of those with ASPD are psychopathic suggesting that these are non-equivalent diagnoses (Hart and Hare, 1997). While psychopathy has been regarded as a particularly severe subtype of ASPD (Dolan and Doyle, 2007), psychopathy and ASPD are however distinguished from behaviours secondary to frontal lobe lesions by high levels of both reactive (elicited by frustration) and instrumental (goal-directed) violence (Blair, 2001; Glenn and Raine, 2009).

While psychopathy is not classified within ICD-10 or DSM-IV, it remains an important construct for understanding the emotional and behavioural disturbances that underpin ASPD. Also, while the PCL and the later PCL-R represent the ‘gold standard’ for formally diagnosing psychopathy (see Table 1.1 for test items), a summary of the criteria for diagnosing ASPD, dissocial personality disorder and psychopathy can be found in Table 1.2 where the description of ASPD in DSM-IV and dissocial personality disorder in ICD-10 include several behavioural and emotional traits reflecting psychopathy (e.g. lack of guilt/remorse, and impulsivity).
### Table 1.1 Test-item domains on the Psychopathy Checklist - Revised (adapted from (Walsh and Wu, 2008))

<table>
<thead>
<tr>
<th>Insensitivity to other’s feelings/missing empathy</th>
<th>Self-absorbed</th>
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<tbody>
<tr>
<td>Lack of emotional depth or conscience</td>
<td>Grandiose sense of self-worth</td>
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<tr>
<td>Extensive history of pathological lying and deception</td>
<td>Relatively fearless</td>
</tr>
<tr>
<td>Impulsiveness and unreliability</td>
<td>Good intelligence</td>
</tr>
<tr>
<td>Tendency to blame others whenever things go wrong</td>
<td>Superficial charm/charismatic</td>
</tr>
<tr>
<td>Failure to profit from adverse experiences</td>
<td>Manipulates others</td>
</tr>
<tr>
<td>A parasitic lifestyle</td>
<td>Lack of long-term life goals</td>
</tr>
<tr>
<td>Incapacity for love and other emotional relationships</td>
<td>Promiscuous sex life</td>
</tr>
<tr>
<td>High need for stimulation</td>
<td>Prone to boredom</td>
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Table 1.2 Criteria for diagnosis of ASPD, psychopathy and dissocial personality disorder (adapted from (Loeber et al., 2003))

<table>
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<tr>
<td><strong>DSM-III-R (American Psychiatric Association, 1987)</strong></td>
<td><strong>A. Current age at least 18</strong></td>
</tr>
<tr>
<td><strong>A. Current age at least 18</strong></td>
<td><strong>B. Evidence of conduct disorder onset before age 15</strong></td>
</tr>
<tr>
<td><strong>B. Evidence of conduct disorder before age 15</strong></td>
<td><strong>C. A pervasive pattern of disregard for and violation of the rights of others as indicated by at least three of the following:</strong></td>
</tr>
<tr>
<td><strong>C. A pattern of irresponsible and antisocial behaviour since age 15, as indicated by at least four of the following:</strong></td>
<td><strong>1. Failure to conform to social norms with respect to lawful behaviour</strong></td>
</tr>
<tr>
<td><strong>1. Unable to sustain consistent work behaviour</strong></td>
<td><strong>2. Deceitfulness</strong></td>
</tr>
<tr>
<td><strong>2. Fails to conform to social norms with respect to lawful behaviour</strong></td>
<td><strong>3. Impulsivity or failure to plan ahead</strong></td>
</tr>
<tr>
<td><strong>3. Is irritable and aggressive</strong></td>
<td><strong>4. Irritability and aggressiveness</strong></td>
</tr>
<tr>
<td><strong>4. Fails to honor financial obligations</strong></td>
<td><strong>5. Reckless disregard for safety of self or others</strong></td>
</tr>
<tr>
<td><strong>5. Fails to plan ahead or is impulsive</strong></td>
<td><strong>6. Consistent irresponsibility as indicated by repeated failure to sustain consistent work behaviour or honour financial obligations</strong></td>
</tr>
<tr>
<td><strong>6. Has no regard for the truth</strong></td>
<td><strong>7. Lacks remorse</strong></td>
</tr>
<tr>
<td><strong>7. Is reckless regarding his or her own or others’ personal safety</strong></td>
<td><strong>8. Impulsive</strong></td>
</tr>
<tr>
<td><strong>8. If parent, lacks ability to function as a responsible parent</strong></td>
<td><strong>9. Poor behavioural controls</strong></td>
</tr>
<tr>
<td><strong>9. Has never sustained a totally monogamous relationship for more than one year</strong></td>
<td><strong>10. Irresponsible</strong></td>
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<tr>
<td><strong>10. Lacks remorse</strong></td>
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<table>
<thead>
<tr>
<th>Criteria for Psychopathic Personality Disorder (Hare, Hart and Harpur, 1991)</th>
<th><strong>Factor 2: chronically unable and antisocial lifestyle</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Factor 1: callous, selfish, remorseless use of others</strong></td>
<td><strong>Factor 2: chronically unable and antisocial lifestyle</strong></td>
</tr>
<tr>
<td><strong>1. Glib and superficial</strong></td>
<td><strong>Factor 2: chronically unable and antisocial lifestyle</strong></td>
</tr>
<tr>
<td><strong>2. Inflated and arrogant self-appraisal</strong></td>
<td><strong>6. Early behaviour problems</strong></td>
</tr>
<tr>
<td><strong>3. Lacks remorse</strong></td>
<td><strong>7. Adult antisocial behaviour</strong></td>
</tr>
<tr>
<td><strong>4. Lacks empathy</strong></td>
<td><strong>8. Impulsive</strong></td>
</tr>
<tr>
<td><strong>5. Deceitful and manipulative</strong></td>
<td><strong>9. Poor behavioural controls</strong></td>
</tr>
<tr>
<td><strong>Criteria for Dissocial Personality Disorder according to ICD-10 (World Health Organization, 1992)</strong></td>
<td><strong>10. Irresponsible</strong></td>
</tr>
<tr>
<td><strong>Dissocial personality disorder is defined as ‘usually coming to the attention of gross disparity between behaviour and the prevailing social norms’. It is characterised by:</strong></td>
<td><strong>Dissocial personality disorder is defined as ‘usually coming to the attention of gross disparity between behaviour and the prevailing social norms’. It is characterised by:</strong></td>
</tr>
<tr>
<td><strong>1. Callous unconcern for the feelings of others</strong></td>
<td><strong>1. Callous unconcern for the feelings of others</strong></td>
</tr>
<tr>
<td><strong>2. Gross and persistent attitude of irresponsibility and disregard for social norms, rules and obligations</strong></td>
<td><strong>2. Gross and persistent attitude of irresponsibility and disregard for social norms, rules and obligations</strong></td>
</tr>
<tr>
<td><strong>3. Incapacity to maintain enduring relationships, though having no difficulty establishing them</strong></td>
<td><strong>3. Incapacity to maintain enduring relationships, though having no difficulty establishing them</strong></td>
</tr>
<tr>
<td><strong>4. Very low tolerance to frustration and a low threshold for discharge of aggression, including violence</strong></td>
<td><strong>4. Very low tolerance to frustration and a low threshold for discharge of aggression, including violence</strong></td>
</tr>
<tr>
<td><strong>5. Incapacity to experience guilt and to profit from experience, particularly punishment</strong></td>
<td><strong>5. Incapacity to experience guilt and to profit from experience, particularly punishment</strong></td>
</tr>
<tr>
<td><strong>6. Marked proneness to blame others, or to offer plausible rationalisations, for the behaviour that has brought the patient into conflict with society.</strong></td>
<td><strong>6. Marked proneness to blame others, or to offer plausible rationalisations, for the behaviour that has brought the patient into conflict with society.</strong></td>
</tr>
<tr>
<td>Conduct disorder during childhood and adolescence, though not invariably present, may further support the diagnosis.</td>
<td><strong>Conduct disorder during childhood and adolescence, though not invariably present, may further support the diagnosis.</strong></td>
</tr>
</tbody>
</table>
1.7.4. Behavioural and psychiatric disorder

ASPD is associated with significant psychiatric and behavioural disturbance which leads to impairment in interpersonal, social and occupational functioning (Moran, 1999; Reich and Vasile, 1993). The abnormal behaviour patterns often appear during childhood or adolescence and continue into adulthood where they may remain enduring. ASPD demonstrates 5-7 times more antisocial and conduct disorder symptoms than those without the personality disorder (Swanson et al., 1994). Table 1.2 lists the typical behavioural features associated with the disorder.

ASPD is often comorbid with other personality disorders and may be diagnosed especially alongside other ‘Cluster B’ personality disorders such as borderline and narcissistic personality disorders or, paranoid personality disorder which belongs to Cluster A personality disorders (American Psychiatric Association, 2000; Coid, 2003; World Health Organization, 1993). Paranoid personality disorder is often found in conjunction with ASPD in criminal populations and is characterised by pervasive mistrust and suspiciousness whereas people with borderline personality disorder experience unstable moods, self-image and identity which may be associated with impulsivity, recurrent self-harm and difficulty in interpersonal relationships (Singleton et al., 1998). Narcissistic personality disorder is manifested by arrogance, grandiosity, lack of empathy, interpersonal exploitation, a sense of self-importance and self-entitlement, envy of others and the belief that others envy them (American Psychiatric Association, 2000). While there are often overlaps between the various personality disorders where traits may be present in association
with ASPD, full criteria for achieving diagnoses of other personality disorders may not be reached.

ASPD is also frequently associated with substance use disorders where the lifetime prevalence has been estimated to be as high as 80% (Kessler et al., 1996) and when both coexist, is related to poorer outcome (Regier et al., 1990; Rounsaville et al., 1991). Substances that are commonly abused include opiates, alcohol, cocaine and solvents (Moran, 1999). A further addiction disorder in the form of pathological gambling, may also be comorbid (Crockford and el-Guebaly, 1998). Although anxiety disorders were previously not thought to occur together with ASPD, disorders such as simple phobias and obsessive-compulsive disorder show increased prevalence rates in ASPD (Swanson et al., 1994). Rates of affective disorders such as depression and dysthymia are also increased (Sanderson et al., 1992; Swanson et al., 1994). Furthermore, ASPD is associated with increased rates of early and unnatural demise that may be linked to accidental death and suicide (Black et al., 1995; Moran, 1999).

1.7.5. Previous neuroimaging findings

Overall, the current evidence suggests dysfunction in the frontal and medial temporal lobe structures as accounting for aggression and impulsivity associated with both ASPD and psychopathy though other regions such as the corpus callosum have been relatively understudied. The literature currently relates frontal lobe dysfunction in particular to antisocial behaviour (Moffitt, 1993; Raine, 2002) where aggressive
behaviour has been reported to be associated with reduced frontal lobe volume (Raine, 2002). Indeed frontal lobe dysfunction was first reported in the case of Phineas Gage where Gage’s physician, Harlow, described his patient’s personality change following extensive left frontal lobe damage and specifically, his orbitofrontal cortex which has been linked to aggression and impulsivity. Though frontal lobe damage was reported in the case of Gage, a ‘frontal lobe syndrome’ was only delineated some time later based on clinical observations of disturbed behaviour in a group of patients with frontal lobe damage (Lishman, 1998).

Symptoms of the frontal lobe syndrome include apathy, emotional lability, impulsivity, reactive aggression and a lack of social awareness and concern for social rules. There exists much overlap between frontal lobe syndrome, ASPD and psychopathy where the key uniting deficit appears to be frontal lobe dysfunction (Damasio, 2000). Indeed in ASPD, frontal lobe volume reduction has been found in adults (Narayan et al., 2007; Raine et al., 2000) and in conduct disordered children (Huebner et al., 2008). Also, within criminal psychopaths, volume reduction in prefrontal grey matter in particular has been associated with higher total and subfactor psychopathy scores in ‘unsuccessful’ (caught) psychopaths, versus healthy controls (Yang et al., 2005). Furthermore, higher total and subfactor PCL-R scores (arrogant/deceptive, affective, and impulsive/unstable) were associated with reduced prefrontal grey matter volume (ibid). Other studies have identified associations between psychopathic traits and subregions of the PFC. The association found in brain injured patients between ventromedial PFC (vmPFC) damage and reactive aggression (Blair and Cipolotti, 2000; Grafman et al., 1996) is mirrored by vmPFC structural and functional impairments in psychopaths (Tiihonen et al., 2008).
Nevertheless, structural and functional abnormalities in people with psychopathy and ASPD are not restricted to the frontal lobe.

For instance, temporal lobe volume reduction has been found in the amygdala where atrophy has been reported in patients with TLE and aggressive behaviour (van Elst et al., 2000). Reduced volume in temporal lobe structures have also been found in both adults (Barkataki et al., 2006; Dolan et al., 2002; Laakso et al., 2001) and antisocial children (Huebner et al., 2008; Sterzer et al., 2007). Further, volume losses in the temporal lobe and in particular the superior temporal gyrus have been identified as substrates in psychopathy (Muller et al., 2007; Muller et al., 2008). Also, abnormal amygdala structure and function have each been found to correlate with the emotion processing deficits observed in ASPD and psychopathic individuals (Gordon et al., 2004; Kiehl et al., 2001; Yang et al., 2009b), as well as in regions (such as fusiform-extrastriate cortices) known to be modulated by the amygdala (Deeley et al., 2006). Moreover, temporal cortical grey matter thinning in the right hemisphere has been related to elevated PCL-R Factor 1 ‘Affective’ facet scores in psychopathic individuals (Yang et al., 2009a). Therefore, studies of antisocial adults and conduct disordered children (with and without psychopathic traits) illustrate reduced volume of the temporal lobe and its constituent structures, and deficits in amygdala structure and function.

Additionally, it has been postulated that dysfunction in networks connecting the frontal lobe and temporal structures such as amygdala may contribute to moral deficits in psychopathy (Blair, 2007); specifically, impairments in the orbitofrontal cortex and vmPFC which are extensively interconnected with the amygdala and
involved in stimulus-reinforcement learning, instrumental learning and response reversal (Blair et al., 2001; Blair, 2007; Kiehl et al., 2000; Mitchell et al., 2006) suggesting perhaps that the underlying mechanisms of psychopathy may be dependent not only on structural and functional abnormalities but also impairments in a number of neural networks. However, there are only limited studies that have examined the network of abnormalities in ASPD and psychopathy.

A recent study using DT-MRI and tractography focused on FA in the uncinate, inferior longitudinal and inferior fronto-occipital fasciculi and reported a significant reduction of this measure in only the uncinate fasciculus of nine psychopaths compared with age- and IQ-matched controls (Craig et al., 2009). Additionally, a significant negative correlation was found between measures of antisocial behaviour (PCL-R Factor 2 scores) and tract volume within this white matter pathway, suggesting abnormal connectivity in the amygdala-orbitofrontal cortex limbic network. However, as this study was confined to a limited number of white matter tracts, it was not possible to assess white matter networks on a whole brain level. Consequently, the presence of deficits affecting white matter connectivity with other brain regions is yet to be established in either ASPD or psychopathy.

In summary, there is mounting evidence that people with ASPD and psychopathy have significant differences in the structure and function of specific brain regions. Following the account of Gage's neurological trauma and associated disturbed behaviour, the understanding of the links between neurobiology and behaviour has improved with abnormalities in the frontal lobe, especially the orbitofrontal cortex and, the amygdala in the temporal lobe being associated with cognitive and
emotional impairments (Sarkar et al., 2011; Sommer et al., 2006). However, although there appears to be an interaction between frontal and temporal lobe regions, relatively few studies have directly examined the underlying white matter microstructural integrity or connectivity of neural networks subserving these regions. Further, the assessment of white matter networks on a whole-brain level in ASPD and psychopathy has never been conducted previously and therefore abnormalities in white matter pathways involved in social behaviour such as interhemispheric or other brain networks are currently unclear.

1.8. Asperger syndrome

In 1944 Hans Asperger an Austrian paediatrician described ‘autistic psychopathy’ in four boys aged 6-11 years who had presented to him with physical clumsiness, difficulties relating to their peers and social deficits such as poor nonverbal communication skills and limited empathy (Asperger, 1944). However, in the previous year and independent to Asperger’s work, Leo Kanner, a child psychiatrist working at Johns Hopkins in Baltimore, described 11 children with similar impairments of social interaction, communication and behaviour, while he used the term ‘infantile autism’ to describe these children (Kanner, 1943). These descriptions constituted some of the earliest reports in the literature in defining autism, Asperger syndrome and related disorders as distinct diagnoses.

Although Asperger produced his seminal report in German some 70 years ago, it did not gain wider acceptance by the scientific community till it was translated into
English (Frith, 1991); additionally, Lorna Wing described a larger group of individuals with similar characteristics to Asperger's original report which further promoted Asperger's account of the disorder (Wing, 1981). Wing subsequently proposed modifications to the original Asperger description for instance by defining an earlier age of onset i.e. before the age of two years and recognised the significant overlap between Asperger syndrome and autism. Also, while autism in earlier studies had been considered a subset of schizophrenia, Michael Rutter provided robust evidence to counter this argument (Rutter, 1972).

Subsequently in 1989, Christopher Gillberg described a set of criteria to delineate Asperger syndrome from autism based on their social impairment, speech and language abnormalities, and difficulties with nonverbal communication together with narrow interest, repetitive routines, and motor clumsiness (Gillberg, 1989; Gillberg and Gillberg, 1989). In the same year, Szatmari and colleagues also differentiated the diagnosis of autism from Asperger syndrome based on pervasive developmental disorder symptoms, adaptive behaviours, and cognitive measures of language competence (Szatmari et al., 1989).

Currently, Asperger syndrome is classified as a pervasive developmental disorder subtype (World Health Organization, 1993) and falls within the autism spectrum disorders though is distinct to autism. Classical autism is defined as a pervasive developmental disorder usually consisting of a triad of – a) abnormal development of social skills (e.g. social withdrawal, lack of interest in peers, impaired reciprocal social interaction); b) limitations in the use of interactive language (verbal as well as nonverbal communication); and c) restricted, repetitive, and stereotyped patterns of
behaviour, interests, and activities (World Health Organization, 1993). Although Asperger syndrome is characterised by the same qualitative abnormalities that typically occur in autism, it principally differs from autism in that there is no general delay of language or cognitive abilities and indeed, most individuals with Asperger syndrome have general intelligence within the normal range.

While children with autism and Asperger syndrome have tremendous long-term developmental potential, however, they continue to be understudied and underserved throughout their lifetime (Kasari and Rotheram-Fuller, 2005). Consequently, studies are required to assess not only early neurodevelopmental processes but also later brain maturation and any associated neurobiological correlates accounting for persistence of the disorder in later life.

1.8.1. Epidemiology

While pervasive developmental disorders are estimated to occur at a prevalence rate of 63 per 10,000 children (Chakrabarti and Fombonne, 2001), in epidemiological surveys that have investigated Asperger syndrome and other pervasive developmental disorders, Asperger syndrome has been found to be approximately five times less common than autism (Fombonne and Tidmarsh, 2003; Fombonne, 2005a). Data on the prevalence of Asperger syndrome is limited with the prevalence rate often dependent on the stringency of the diagnostic criteria applied (Baird et al., 2006; Ehlers and Gillberg, 1993). Studies that have adopted stringent diagnostic criteria have found conservative estimates for the prevalence of Asperger syndrome
of approximately 2.6 per 10,000 (Fombonne, 2005a; 2007) and similar to autism, Asperger syndrome appears to be more common in males (Khouzam et al., 2004). However, some epidemiological studies have reported far higher rates.

For instance, in an earlier Swedish epidemiological study, a high minimum prevalence rate of 3.6 per 1,000 children with a male to female ratio of 4:1 was found (Ehlers and Gillberg, 1993). Given that approximately half of children with Asperger syndrome reach adulthood without ever being assessed, diagnosed, or treated (Szatmari et al., 1995), when possible cases were included in the Swedish study, the prevalence rate increased to 7.1 per 1,000 children but the male to female ratio dropped to 2.3:1. Further, a recent Finnish epidemiological study reported a rate of approximately 2.7 per 1,000 children aged eight years (Mattila et al., 2007).

In a recent community sample in England examining adults with autism spectrum disorder (including Asperger syndrome), a rate of approximately 1% was reported while rates were higher in males, those without higher educational qualifications as well as those living in social housing (Brugha et al., 2011). Also in this study, although individuals with autism spectrum disorder were identified, none of them were aware that they had the condition. Therefore the evidence suggests that although Asperger syndrome is indeed highly prevalent, it is relatively underdetected. Further, while Asperger syndrome is recognised as a formal psychiatric diagnosis, doubts continue to persist with regard to its distinction from high-functioning autism (HFA) (Kasari and Rotheram-Fuller, 2005; Klin, 2006) and because of these reasons, it is difficult to accurately establish its prevalence.
Overall, prevalence rates in Asperger syndrome vary enormously (Fombonne and Tidmarsh, 2003) and differences in epidemiological estimates is likely to continue not only due to the application of varying clinical criteria but also overlap that is shared with other pervasive developmental disorders as well as varying awareness of the disorder (Foster and King, 2003). Additionally, although rates of Asperger syndrome in recent times have been noted to be increasing, this may not reflect a true increase per se but rather an increased recognition of the disorder (Fombonne, 2005b; 2008). Further, while the classical features of Asperger syndrome persist into adolescence and adult life, a proportion of individuals with the disorder may experience clinical improvement which may also affect estimates of the disorder (Seltzer et al., 2003; Woodbury-Smith and Volkmar, 2009).

1.8.2. Causation

Asperger identified similarities between family members and the patients he assessed and particularly amongst biological fathers, suggesting the importance of genetic factors even some 70 years ago. Although there is much evidence from family and twin studies to account for a genetic basis for autism (Bailey et al., 1995; Bolton et al., 1994; Pericak-Vance, 2003), however less work has focused specifically on the study of Asperger syndrome (Woodbury-Smith and Volkmar, 2009).

In studies examining families for genetic risk of Asperger syndrome, a strong family history of Asperger syndrome among first-degree relatives has been demonstrated; further, evidence of a genetic relationship between autism and Asperger syndrome
has been found (Ghaziuddin, 2005; Volkmar et al., 1998). However, there is currently a lack of genetic association and linkage studies specifically assessing Asperger syndrome. Of the limited studies completed, linkage has been observed at 1q21-22, 3p14-24 and 13q31-33 (Woodbury-Smith and Volkmar, 2009; Ylisaukko-oja et al., 2004). Other chromosomal regions implicated include abnormalities in both the short and long-arms of chromosome 17 at 17p13 and 17q11 (Anneren et al., 1995; Stone et al., 2004). Investigators have also identified an association between certain behavioural traits within children with autism, specifically, repetitive behaviour and insistence on sameness, and with gamma-aminobutyric acid (GABA) A receptor, beta 3 (GABRB3) localised to chromosome 15q11-13 (Shao et al., 2003) and the same genetic loci were implicated in an association analysis of 80 families with autism (Buxbaum et al., 2002). A recent study examining Asperger syndrome and autistic traits (Empathy and Autism Spectrum Quotients) implicated CYP11B1 and NTRK1 as candidate genes (sex hormone synthesis and neurotrophic factor receptor) (Chakrabarti et al., 2009). Further, copy-number variation (CNV) which corresponds to submicroscopic deletions and duplications is the most prevalent type of structural variation in the human genome and has been associated with autism spectrum disorder (Kakinuma and Sato, 2008) but has been less extensively investigated in Asperger syndrome.

Moreover, concerns had been raised with regard to environmental factors such as immunisations and in particular the vaccine to protect against measles, mumps, and rubella (MMR) which had been suggested to precipitate autism (Wakefield et al., 1998); however, such an association has been countered by numerous subsequent reports which have not shown a relationship between vaccines and the future
development of autism or Asperger syndrome (DeStefano and Chen, 2000; Miller et al., 1997; Taylor et al., 1999). Although other environmental factors have been postulated to also contribute to the development of Asperger syndrome after birth, the available evidence has not confirmed an environmental basis but rather that Asperger syndrome may indeed have a significant genetic basis that is more compelling than autism (McPartland and Klin, 2006).

1.8.3. Diagnostic criteria

The clinical characteristics of Asperger syndrome have been discussed earlier in Section 1.8. In summary, the diagnostic criteria for Asperger syndrome are similar to that of classical autism in that deficits encompass problems in social interaction and where individuals with the disorder demonstrate restricted, repetitive and stereotyped patterns of behaviour, interests, and activities but lack the typical language, communication or cognitive delays seen in autism (American Psychiatric Association, 2000; World Health Organization, 1993). Although not required for a diagnosis in either DSM-IV or ICD-10, physical clumsiness and atypical use of language are frequently reported in Asperger syndrome (McPartland and Klin, 2006). Speech may be tangential and verbose or lack normal prosody; additionally, speech may be odd in terms of rate, rhythm or volume (Klin et al., 2005). Some of these supplementary criteria are listed in other classification systems for a diagnosis of Asperger syndrome (Gillberg and Gillberg, 1989). For this thesis however, the DSM-IV and ICD-10 classification systems are used and are explored in more detail next.
The criteria for a diagnosis of Asperger syndrome in both the DSM-IV and ICD-10 overlap significantly and the details of one of the classification systems are listed below so as to elaborate further on these criteria. According to ICD-10 (World Health Organization, 1993), the diagnosis of Asperger syndrome is based on criteria encompassing four broad domains:

1) a lack of any clinically significant general delay in language or cognitive development where the diagnosis requires that single words should have developed by two years of age or earlier and that communicative phrases be used by three years of age or earlier. Self-help skills, adaptive behaviour, and curiosity about the environment during the first three years should be at a level consistent with normal intellectual development. However, motor milestones may be somewhat delayed and motor clumsiness is usual (although motor symptoms are not necessarily diagnostic features). Isolated special skills, often related to abnormal preoccupations, are common, but are not required for diagnosis;

2) qualitative impairments in reciprocal social interaction (criteria for autism);

3) restricted, repetitive, and stereotyped patterns of behaviour, interests, and activities (criteria for autism);

4) the disorder is not attributed to the other varieties of pervasive developmental disorder; schizotypal disorder; simple schizophrenia; reactive and disinhibited attachment disorder of childhood; obsessive personality disorder; obsessive-compulsive disorder.
Currently, the diagnosis of Asperger syndrome is predominantly clinical where the gold standard is a combination of clinical judgment together with diagnostic interviews. The autism diagnostic interview - revised (ADI-R) (Lord et al., 1994) and the autism diagnostic observation schedule (ADOS) (Lord et al., 2000) represent a semi-structured interview with a primary caregiver and a semi-structured interactive/play session respectively; both these schedules require specific training and administration to ensure robust diagnosis.

The ADI-R consists of 93 questions which focus on behaviours in the three central areas on which clinical diagnoses are based:

- communication and language skills (e.g. speech development, appropriate word use or social usage of language);
- social interaction (e.g. social smiling and responding to others, emotion sharing, offering or seeking comfort) and;
- repetitive and obsessive behaviours (e.g. finger/hand mannerisms, unusual sensory interests or unusual preoccupations)

As the ADI-R consists of semi-structured interview, responses are scored by the clinician based on the caregiver's description of the individual's behaviour. Current behaviour forms the focus of the majority of questions with the exception of specific age-related developmental milestones such as language development. Also, reciprocal friendships are scored only for children aged five and above. Apart from exploring current behaviour, each question focuses on the period when abnormal behaviours were likely to be most pronounced which generally corresponds to between the ages of four and five years.
The ADI-R uses a scoring system for each item where scores range from 0 to 3. A score of 0 is given when "behaviour of the type specified in the coding is not present"; a score of 1 is given when “behaviour of the type specified is present in an abnormal form, but not sufficiently severe or frequent to meet the criteria for a 2. A score of 2 indicates "definite abnormal behaviour" meeting the criteria specified while a score of 3 is reserved for "extreme severity" of the specified item. A diagnosis of autism is achieved when scores in all three constituent areas of communication, social interaction, and patterns of behaviour meet or exceed the specified thresholds, and where onset of the disorder is evident by 36 months of age.

The total cut-off score for the communication and language domain is 8 for verbal subjects and 7 for nonverbal subjects. The cut-off for the social interaction domain is 10, and the cut-off for restricted and repetitive behaviours is 3.

When it is not possible to interview a caregiver, the ADOS may be used directly with the individual being assessed and it represents a semi-structured tool which allows for the observation of social and communication behaviours related to the diagnosis of autism spectrum disorder. It takes approximately 40 minutes to administer and the ADOS relies on assessment for instance of emotions, friendships and pictures but also incorporates construction tasks. The cut-off scores for the communication, social interaction and combined (communication and social interaction) domains are 2, 4 and 7 respectively. The ADOS in its current form does not have a cut-off score for restricted interests/repetitive behaviour but these are scored if present; there are proposals to integrate these in a future revision (Oosterling et al., 2010). There are
other limitations to the application of the ADOS in adults as this population is required to have average verbal ability for assessments to be valid.

While these interview schedules are involved in the diagnosis of autism spectrum disorder, there is also growing momentum currently to replace the diagnosis of Asperger syndrome by a diagnosis of autism spectrum disorder on a severity scale in the next iteration of DSM (DSM-V) though some reports argue for a modification of the diagnostic criteria and its continued retention instead in the diagnostic manual (Ghaziuddin, 2010).

1.8.4. Behavioural and psychiatric disorder

The features of Asperger syndrome are described in full detail in the above sections whereby the syndrome constitutes a pervasive developmental disorder. Such disorders on the whole are associated with high rates of behavioural and psychiatric comorbidity whereby classical autism has been studied relatively more extensively than Asperger syndrome though both are associated with significant mental health comorbidity (Klin et al., 2005; Woodbury-Smith and Volkmar, 2009). Psychiatric disorders that occur at a relatively lower frequency in autism spectrum disorder include Tourette’s syndrome and obsessive-compulsive disorder (Baron-Cohen et al., 1999; Bejerot et al., 2001; Bejerot, 2007; Fitzgerald and Corvin, 2001) while psychosis, violent offending, substance misuse and ASPD have also been reported (Hofvander et al., 2009; Langstrom et al., 2009; Raja and Azzoni, 2009; Starling and Dossetor, 2009). Although there are also overlaps in some of these disorders with
Asperger syndrome, psychiatric disorders that have been reported to be highly comorbid with Asperger syndrome include mood and anxiety disorders and ADHD (Ghaziuddin et al., 1998; Tantam, 2003).

For instance, although both Asperger syndrome and obsessive-compulsive disorder share stereotyped patterns of behaviour, the restricted pattern of interests and activities seen in Asperger syndrome differs principally from obsessive-compulsive disorder in that they are more pronounced and are ego-syntonic with Asperger syndrome (Khouzam et al., 2004; Woodbury-Smith and Volkmar, 2009). Similarly, Asperger syndrome differs from adult schizoid personality disorder by the overarching presence of stereotyped behaviour and interests that severely interfere with social interactions (Khouzam et al., 2004; Wing and Gould, 1979).

Additionally, a disorder characterised by deficits in attention, motor control and perception (DAMP) (Gillberg et al., 1982) encompasses a disturbance of attention, gross and fine motor dysfunction and perceptual dysfunction across many situations and is not accounted for by associated mental retardation or cerebral palsy whereby the disorder overlaps significantly with Asperger syndrome (Fitzgerald and Corvin, 2001). Also, although people with Asperger syndrome have deficits suggestive of nonverbal learning disability (Myklebust, 1975), the primary diagnosis of Asperger syndrome is preferred.

1.8.5. Previous neuroimaging findings
One of the earliest indicators that autism spectrum disorders including Asperger syndrome may have a neurobiological basis was the observation by Kanner that five of the 11 children in his report had 'large heads' (Kanner, 1943). Since then, the finding of megalencephaly or increased brain volume has been consistently replicated (Courchesne, 2004; Hazlett et al., 2011; Lainhart et al., 1997; Wallace and Treffert, 2004). Indeed autism spectrum disorder as a group is characterised by initial overgrowth of global grey and white matter tissue in very young children followed by reduced volumes in later childhood (Ben Bashat et al., 2007; Courchesne et al., 2001). Also, differences in white matter are postulated to continue as the brain matures into adulthood due to abnormal neuronal loss and synaptic pruning (Muller, 2007). Additionally, a third with autism will go on to develop comorbid epilepsy suggesting a further neurobiological basis to Asperger syndrome (Danielsson et al., 2005). However, most neuroimaging studies to date have not specifically assessed Asperger syndrome but have instead examined autism or autism spectrum disorders as a single group. Thus the neuroanatomical correlates in Asperger syndrome are presently poorly established.

From the limited literature in Asperger syndrome, people with the disorder have been reported to show significant and widespread white matter abnormalities and differences in grey matter volume of fronto-striatal and cerebellar regions compared to controls (McAlonan et al., 2002). Similarly, studies in neurodevelopmental disorders in general (including autism) have implicated abnormalities in the fronto-striatal system (Bradshaw and Sheppard, 2000). Overall, abnormal regions that have been implicated in studies of autism spectrum disorders include the frontal and
temporal lobes, limbic system, cerebellum and basal ganglia (Sokol and Edwards-Brown, 2004; Sugihara et al., 2007; Toal et al., 2005).

In an early VBM study focusing on adults with HFA who had been diagnosed with autism as children, the authors found decreased grey matter density in anterior regions of the brain such as the right paracingulate sulcus and left inferior frontal gyrus but increased grey matter density in more posterior neuroanatomical areas for instance in the amygdala, middle and inferior temporal gyri, and cerebellum (Abell et al., 1999). In a subsequent VBM study comparing male children and adolescents with HFA and Asperger syndrome relative to healthy controls where only grey matter was assessed, significant differences were reported between groups (Kwon et al., 2004); the HFA and Asperger syndrome groups had a reduction of grey matter density in the ventromedial temporal cortex in comparison to the age-matched healthy comparison group. Additionally, the comparison group had increased grey matter density relative to the HFA and Asperger syndrome groups in the right inferior temporal gyrus, entorhinal cortex, and rostral fusiform gyrus. However, the Asperger syndrome group was reported to have less grey matter density in the body of the cingulate gyrus in comparison to the other groups. The findings of decreased grey matter density in the ventromedial temporal cortex was postulated by the authors to correlate with impaired integration of visual stimuli and affective information in individuals with HFA and Asperger syndrome (Kwon et al., 2004).

A further study attempted to distinguish between low-functioning autism, HFA and Asperger syndrome where cerebral grey matter volume was reported as enlarged in both low and high-functioning autism groups relative to controls (Lotspeich et al.,
2004). However, although non-significant, cerebral grey matter volume in Asperger syndrome was reported as being intermediate between that of HFA and controls. Exploratory analyses conducted in the same study revealed a negative correlation between cerebral grey matter volume and performance IQ within HFA but not with Asperger syndrome while a positive correlation between cerebral white matter volume and performance IQ was reported within Asperger syndrome but not with HFA. Overall, based on their cerebral grey matter findings, the authors suggested that Asperger syndrome may be on the mild end of the autism spectrum. Additionally the authors postulated that differences in brain-IQ correlations may indicate that HFA and Asperger syndrome are neurodevelopmentally distinct (Lotspeich et al., 2004).

In a recent anatomical and functional study of autism, a group of HFA participants was compared to a control group matched with respect to age, gender and IQ where executive function was assessed using a Tower of London task (Just et al., 2007). Although the two groups activated the same cortical areas in general and to similar degrees, however, there were indications of underconnectivity in the group with autism; these were found between the frontal and parietal regions. Additionally, parts of the corpus callosum were found to be reduced in cross-sectional area and the size of the genu of the corpus callosum was correlated with frontal-parietal functional connectivity in the autism group. Although autism spectrum disorder has been proposed to represent a ‘connectivity disorder’ involving abnormalities in fronto-temporal, fronto-limbic, fronto-parietal and interhemispheric connections (Catani and ffytche, 2005; Courchesne and Pierce, 2005; Frith, 2004; Geschwind and Levitt, 2007; Just et al., 2004; Just et al., 2007) that is accompanied by widespread
abnormalities in white matter development (Herbert et al., 2004), these connectivity
deficits have been relatively understudied in Asperger syndrome via DT-MRI.

A prior whole-brain DT-MRI study which assessed autism spectrum disorder instead
of specifically examining Asperger syndrome included seven children with autism
and nine controls (Barnea-Goraly et al., 2004). The authors reported significant and
widespread reduction in FA of white matter adjacent to the vmPFC, anterior
cingulate gyri, temporo-parietal junctions, occipito-temporal tracts, medial and
lateral temporal lobe and in the corpus callosum of the children with autism.
Subsequently, others have also investigated mixed samples of children and adults
with autism (Alexander et al., 2007; Keller et al., 2007; Lee et al., 2007) where
people with autism have been reported to show a significant reduction in FA of areas
within (or near) the corpus callosum (Alexander et al., 2007; Keller et al., 2007),
right retro lent icul ar portion of internal capsule (Keller et al., 2007) and the superior
temporal gyrus and temporal stem (Lee et al., 2007). White matter organisation
abnormalities involving short- and long-range fibres in the frontal lobe have also
been reported in children with autism spectrum disorder (Sundaram et al., 2008).

Although these studies were valuable first steps, they have however included
heterogeneous clinical populations in terms of mixed ages or clinical diagnoses such
as including HFA, low-functioning autism or Asperger syndrome as single groups;
and some did not correct for multiple comparisons. Further, it has been proposed that
autism spectrum disorder may be ‘exponentially distributed’ whereby early brain
abnormalities in the disorder increasingly affect additional regions and functional
systems throughout development (Hammock and Levitt, 2006; Knudsen, 2004;
Muller, 2007). This suggests that differences in brain anatomy become more pronounced as the brain matures into adulthood for those who still have the disorder, underscoring the importance of assessing brain anatomy of people with autism spectrum disorder longitudinally.

1.9. Summary

Psychiatric disorders are recognised to have multifactorial origins that impact on the brain. Therefore, investigation of these abnormal neurobiological processes allows for a better understanding of these pathways. Currently, there is an apparent lack of a biological basis for many psychiatric disorders which hinders the understanding of their aetiology. Consequently, studies into brain structure, function and connectivity are gradually emerging whereby neuroimaging has been applied to the study of psychiatric disorders with the aim of establishing neurobiological correlates (Malhi and Lagopoulos, 2008).

Identification of neural correlates of psychiatric impairment marks the transition of psychiatry towards defining biological substrates driving psychiatric disorders and away from phenomenological-based approaches (Kapur, 2003; Silbersweig and Stern, 1996). Additionally, the advent of new technologies in a variety of fields related to neuroimaging such as physics, computing and mathematics have permitted neuroimaging to develop to a very high level of sophistication for use in the examination of neural tissue (Abou-Saleh, 2006; Bandettini, 2009; Malhi and Lagopoulos, 2008). However, the majority of psychiatric neuroimaging is presently
research oriented and as we gain a better understanding of the underlying neural correlates, the clinical utility of these research findings will gradually become more evident (Malhi and Lagopoulos, 2008).

Using the neuroimaging and analytical techniques based on MRI outlined earlier in this chapter, I will now examine the neurobiology of the following psychiatric disorders using DT-MRI and/or conventional MRI in combination with whole-brain voxel-based methods: 1) psychiatric disorder associated with 22q11DS; 2) psychosis associated with TLE; 3) ASPD; and 4) Asperger syndrome. Further details are provided next in Chapter 2 with regard to the aims and hypotheses tested in each of the experimental chapters in this thesis whereby each chapter is dedicated to the investigation of a specific psychiatric phenotype. Additionally in Chapter 2, details of the standardised MRI acquisition protocols for conventional MRI and DT-MRI data and their subsequent analysis through whole-brain voxel-based methodology are also described.
Chapter 2:

Magnetic resonance imaging acquisition and analytical methodology

The purpose of this chapter is to provide a summary of the aims and hypotheses tested in this thesis in conjunction with a detailed description of the methodology used in conducting neuroimaging analyses.

The chapter begins with a summary of the aims and hypotheses followed by a description of the plan of investigation for each of the four experimental studies performed in the thesis. This is then accompanied by details of brain acquisition sequences based on conventional MRI and DT-MRI and is followed by a description of image processing and voxel-based statistical analyses.
2.1. Aims

Overall, there are several broad aims to this thesis which are summarised as follows:

1) to investigate psychiatric phenotypes especially those where the literature is presently lacking or inconsistent and where endophenotypes for these psychiatric disorders are currently not well-established;

2) to investigate psychiatric disorders via neuroimaging acquisition techniques that encompass conventional MRI and/or DT-MRI methodology so as to enable assessment of structural brain differences and/or white matter tract connectivity;

3) to utilise novel and advanced analytical techniques to study whole-brain abnormalities on a voxel basis through the use of VBM for conventional MRI data (white and/or grey matter tissue content) and voxel-based analyses for DT-MRI data (microstructural tissue integrity/connectivity represented on FA maps) respectively; and

4) to correlate relevant psychometric measures to brain structure or brain connectivity abnormalities.

The hypotheses for each experimental study are provided next.

2.2. Hypotheses

Study 1 - White matter microstructure in 22q11DS: a pilot DT-MRI and VBM study of children and adolescents
This study assesses the structural and connectivity alterations in 22q11DS using conventional MRI and DT-MRI respectively. The main hypothesis tested in this study is that children and adolescents with 22q11DS have significant differences to controls in the microstructural integrity and volume of white matter where these deficits are not co-located. Additional hypotheses tested are that within young people with 22q11DS: 1) severity of schizotypy is correlated with differences in white matter integrity; and 2) polymorphism of the COMT gene is associated with differences in both white matter content and coherence.

Study 2 - Neuroanatomical correlates of psychosis in TLE: a VBM study

This study examines the structural differences in TLE+psychosis based on conventional MRI. In this study the main hypothesis tested is that individuals with TLE and associated psychosis when compared to those with TLE alone (without psychiatric disorder) will demonstrate reduction in total brain and grey and white matter content. Additional hypotheses are that: 1) given TLE is derived from abnormalities in the temporal lobe, grey and white matter reduction will be found only in the temporal lobe; and 2) any structural differences encountered will overlap with those found in schizophrenia.

Study 3 - White matter microstructural abnormalities in adults with ASPD: a preliminary whole-brain DT-MRI study

In this study, white matter connectivity abnormalities in ASPD are investigated with the use of DT-MRI. The main hypothesis tested in this study is that individuals with
ASPDD will have significant differences in white matter integrity compared to healthy controls. These abnormalities are postulated to be found in regions subserving social behaviour and emotional regulation. An additional hypothesis tested is that differences in white matter integrity are correlated with measures of psychopathy.

Study 4 - White matter integrity in Asperger syndrome: a preliminary DT-MRI study in adults

In this final study, the white matter connectivity differences in Asperger syndrome based on DT-MRI are examined. The main hypothesis tested is that adults with Asperger syndrome when compared to healthy adults without the disorder will demonstrate deficits in white matter integrity. These white matter abnormalities are hypothesised to be found in areas important for behaviour and social skills.

2.3. Plan of investigation and study participants

All the studies in the thesis are based on subject images acquired through MRI acquisition protocols. The acquired images are subsequently assessed through modern computerised voxel-based methodology whereby statistical comparisons of differences are performed at the level of each voxel. The thesis includes four experimental chapters which examines four separate phenotypes -

Study 1 - White matter microstructure in 22q11DS: a pilot DT-MRI and VBM study of children and adolescents
A sample of children and adolescents with confirmed genetic deletion at the chromosome 22q11.2 locus was recruited to analyse structural and connectivity differences based on white matter relative to sibling and healthy controls that lack the genetic deletion. Only white matter was assessed as this is more significantly affected in 22q11DS relative to other tissue classes. Given that 22q11DS is significantly associated with schizophrenia-spectrum disorders, an exploratory analysis was conducted where white matter deficits in 22q11DS were correlated with measures of schizotypy. Additionally, the effect of COMT on brain measures of white matter volume and integrity were also assessed. This is the first time that such associations have been examined.

**Study 2 - Neuroanatomical correlates of psychosis in TLE: a VBM study**

A sample containing exclusively adults with TLE was recruited. The variable of interest was psychosis where the structural differences of those with TLE and comorbid psychosis were compared to those with TLE alone. All participants had normal intellectual functioning and a robust diagnosis of TLE based on seizure profile, neuroimaging and electrophysiological criteria. Controls had TLE but were free from comorbid psychiatric disorder. This is the first time that TLE and associated psychosis has been investigated using whole-brain VBM to encompass both grey and white tissue compartments.

**Study 3 - White matter microstructural abnormalities in adults with ASPD: a preliminary whole-brain DT-MRI study**
A population of adults with known convictions and forensic offending who have a
diagnosis of ASPD were recruited to enable the investigation of white matter
connectivity differences relative to healthy age-, gender-, and IQ-matched controls.
Abnormalities in white matter networks in the ASPD group were correlated with
measures of psychopathy. This is the first-ever investigation of ASPD based on DT-
MRI and whole-brain voxel-based analytical methods.

Study 4 - White matter integrity in Asperger syndrome: a preliminary DT-MRI
study in adults

As previous studies have recruited mixed samples of autism spectrum disorder, for
this study a population of adults with only confirmed Asperger syndrome was
recruited. Brain connectivity in the Asperger syndrome group was compared to a
healthy gender- and age-matched sample of adults without the disorder. This study
also represents a novel investigation where a relatively homogeneous cohort of
adults with Asperger syndrome has yet to be examined through DT-MRI and whole-
brain voxel-based methodology.

In summary

Brain structure is examined in 22q11DS and TLE with associated psychosis using
conventional MRI while brain connectivity/microstructural integrity is investigated
in 22q11DS, ASPD and Asperger syndrome using DT-MRI acquisition. As the
samples recruited for each experimental study represent distinct phenotypes,
accordingly detailed descriptions of each sample is provided for in their respective chapters in the thesis.

In the following sections, details of the conventional MRI and DT-MRI acquisition protocols used in this thesis are described. Additionally, preparation of acquired scans for analyses and the non-parametric statistical comparisons at the level of individual voxels are explained.

2.4. Conventional MRI brain acquisition protocol at
Institute of Psychiatry, London

I acquired conventional MRI data for Study 1 (Chapter 3) using a 1.5 Tesla GE Signa LX system MRI scanner (General Electric, Milwaukee, WI, USA). A 3-D inversion recovery prepared spoiled gradient recalled acquisition in the steady state (IR-SPGR) scan [TR of 11.9ms, TE of 5.2ms, inversion time of 450ms] was acquired during the same scanning session as acquisition of DT-MRI data (see Section 2.7.). Images were acquired with a 256 x 192 matrix over a 200 x 160mm field of view, and reconstructed to a 256 x 256 pixel matrix, giving a final in plane pixel size of 0.78125mm. Data were collected, in a coronal orientation, from 124 1.5mm thick sections.

2.5. Conventional MRI brain acquisition protocol at
Beaumont Hospital, Dublin
For each subject in Study 2 (Chapter 4), I acquired a volumetric spoiled gradient recalled acquisition in the steady state (SPGR) MRI brain scan at the Beaumont Hospital 1.5 Tesla MRI scanner (GE Signa Systems, Paris). Coronal thin-cut 1.5mm slices were obtained via a 3-D-volume gradient echo pulse sequence which was radiofrequency spoiled. A sagittal localiser was first acquired and the volume of interest was then arranged to include the whole brain. The 3-D-SPGR sequence was acquired over a period of 14 minutes with the following MRI parameters: TR of 35ms, TE of 15ms, readout bandwidth of 16 kHz and excitation flip angle of 35°. The data were collected with an in plane image matrix of 256 x 256 pixels over a field of view of 240 x 240mm, leading to a voxel size of 0.9375 x 0.9375mm. 128 partitions were collected, of which 4 were discarded during reconstruction to minimise wraparound artefacts, resulting in a final 124 1.5mm slices covering a field of view of 18.6cm.

2.6. VBM analysis of conventional MRI data

Following conventional MRI acquisition and prior to image analysis, voxels representing extracerebral tissue, such as bone and skin were removed. I achieved this by applying an automated computational algorithm to the dataset for each subject (Bullmore et al., 1999). Additionally, global volumes for total brain and grey matter and white matter were extracted via SPM5 and between group differences were compared using Student’s t or Mann-Whitney U tests. If global volumes
significantly differ, these are then entered as further covariates in between-group analyses.

Subsequent to brain tissue extraction, the initial steps of VBM processing i.e. normalisation, segmentation, modulation and smoothing were performed in SPM5 within Matlab 7.0 (The MathWorks, Natick, MA, USA). The Brain Activation and Morphometric Mapping (XBAMM) package based on non-parametric, permutation testing (full details of which can be found at http://www-bmu.psychiatry.cam.ac.uk/software/docs/xbamm/) was then used to test for statistical significance for all conventional MRI studies in this thesis (Studies 1 and 2 in Chapters 3 and 4 respectively). Next, the details of VBM analyses of tissue class maps based on conventional MRI data are provided.

2.6.1. Normalisation, segmentation, modulation and smoothing of conventional MRI data

Chapter 1, Section 1.1.2.2. provides details on the use of VBM with conventional MRI data. With previous versions of SPM, a set of processing steps commonly known as ‘optimised VBM’ (Good et al., 2001) was needed to ensure high quality segmentations. However, the methodology was inherently circular as the registration required an initial tissue classification, and the tissue classification requires an initial registration. Therefore, I used SPM5 where both components are integrated into a single framework and also includes correction of the effects of image intensity non-uniformity termed ‘the bias field’ (Ashburner and Friston, 2005).
Grey and white matter were extracted from the normalised images and ‘modulated’ to compensate for the effects of spatial normalisation. This is achieved by multiplying each voxel value by its relative volume before and after warping, in order to compensate for the fact that spatial normalisation expands/contracts some brain regions (Ashburner and Friston, 2005). After modulation, the total amount of grey (or white) matter is the same as in the original images. Good and colleagues (Good et al. 2001) note that ‘in effect, an analysis of modulated data tests for regional differences in the absolute amount (volume) of grey matter.’ The maps so produced are referred to as images of ‘grey (or white) matter volume’ to distinguish them from the images of ‘concentration’ or ‘density’ which result if the modulation stage is omitted. However, in order to avoid potential confusion with manual volumetry measures such as stereology, the outputs are referred to as maps of grey (or white) matter content, or simply grey (or white) matter maps.

The segmented images were then smoothed with a Gaussian kernel prior to statistical analyses so as to reduce noise, and also allow for the effects of small residual misregistrations. As differing smoothing kernels are applied in this thesis, these are considered separately in Chapter 3, Section 3.4.6.4. and Chapter 4, Section 4.4.4.

2.6.2. Statistical comparisons of maps of tissue content
As structural brain changes are likely to extend over a number of contiguous voxels, I used test statistics incorporating spatial information such as 3-D cluster mass (the sum of suprathreshold voxel statistics) which are generally more powerful than other possible test statistics, which are informed only by data at a single voxel (Bullmore et al., 1999). However, given that no parametric distribution is known for cluster mass, permutation-based testing which is implemented in the XBAMM package, a joint development of the Brain Mapping Unit, Department of Psychiatry, University of Cambridge and the Institute of Psychiatry, London (http://www-bmu.psychiatry.cam.ac.uk/BAMM/index.html), was used to assess statistical significance at both the voxel and cluster levels (Bullmore et al., 1999).

With permutation-based testing, the null hypothesis is tested operationally by permuting observations at each voxel randomly across levels of the factor of interest and the test statistic is re-estimated after each permutation. The procedure is repeated \( x \) number of times and enables an assessment of the distribution of the test statistic under the null hypothesis through the ordering of the set of permuted test statistics. As test statistics may be performed at several thousand voxels in neuroimaging, the computational demands of sampling the permutation distribution may therefore be reduced substantially by permuting the data a small number of times (\( x = 10 \)) at each voxel and subsequently pooling permuted statistics (10 multiplied by the number of voxels) to construct a null distribution of observations as suggested by previous statistical work with MRI and fMRI data (Bullmore et al., 1999; Suckling and Bullmore, 2004). Such an approach is termed global permutation whereby a single threshold for significance is used across the whole brain.
Between-group differences in grey and white matter content were estimated by fitting an analysis of variance (ANOVA) or analysis of covariance (ANCOVA) model (dependent on whether covariates are controlled for) which is regressed onto the data at each voxel in standard space where proportional volume for each tissue class (grey or white matter) was the dependent variable and group classification as the key predictor variable. The statistical model is detailed below:

\[ T = a_0 + a_1V + a_2X_2 + \ldots + a_nX_n + e \]

T is a vector denoting the proportional volume at a given voxel for each individual in the cohort, V is the independent variable vector, X_n's are the covariate vectors and e is random variation. The model is regressed at each intracerebral voxel onto the observed data to yield a test statistic map of the coefficient \( a_1 \) of the independent variables divided by its standard error:

\[ a^* = \frac{a_1}{SE(a_1)} \]

and is known as the voxel-wise test statistic.

The model is additionally regressed 10 times at each voxel after random permutation of the elements of the vector coding the independent variable, V. This generates 10 randomised or permuted effect maps. Both observed and permuted test statistic maps are then thresholded such that if the absolute value of \( a^* \) is less than 1.96, the test statistic at that voxel is set to zero. If the absolute value of \( a^* \) is greater than 1.96, the test statistic of that voxel is set to \( a^* - 1.96 \). This procedure generates clusters of suprathreshold voxels that are spatially contiguous in three spatial dimensions. The sum of suprathreshold voxel-wise test statistics of each 3-D cluster is measured in
each of the 10 permuted cluster-wise test statistic maps; and these measurements are ordered to sample the permutation distribution of cluster mass under the null hypothesis of zero difference between- or correlation within-groups.

The mass of each cluster in the observed effect map is then tested against critical values (or p-values) obtained from the corresponding permutation distribution. The statistical threshold for cluster significance was then adjusted such that the expected number of false positive clusters arising by chance alone would be less than one over the whole imaging volume. The non-parametric or distribution free hypothesis testing procedure utilised in this thesis allows the use of cluster level statistics even if their distribution is non-Gaussian (even after smoothing); further, there is significant evidence in the literature that cluster level statistics incorporating information about the spatial neighbourhood of each voxel may be more sensitive than voxel test statistics (Poline and Mazoyer, 1993; Rabe-Hesketh et al., 1997; Shapleske et al., 2002). As voxel level statistics are associated with multiple comparisons and thus increased risk of Type 1 error, cluster level statistics reduce such error due to the performance of fewer comparisons of several orders of magnitude (Shapleske et al., 2002).

The output from VBM in this thesis is a statistical non-parametric map showing regions where tissue class content differs significantly between groups and regional differences in the content of grey matter or white matter are reported using standard space. As SPM was used initially for segmentation, XBAMM yields coordinates of clusters and cluster localisation labels in Montreal Neurological Institute (MNI).
space; MNI coordinates are subsequently converted to Talairach space via a non-linear transformation (Brett et al., 2002) (further details can be found at http://imaging.mrc-cbu.cam.ac.uk/imaging/MniTalairach) and interpreted with the aid of widely accepted atlases (Talairach and Tournoux, 1988). Fig. 2.1 shows the standard brain template output used by XBAMM for reporting results based on MNI space that is used for all conventional MRI analyses in this thesis.
Fig. 2.1 XBAMM MNI brain template output for conventional MRI data (Z Talairach coordinates) where coloured regions indicate statistically significant tissue class differences.
2.7. DT-MRI brain acquisition protocol at Institute of Psychiatry, London

I acquired DT-MRI data for Studies 1, 3 and 4 (Chapters 3, 5 and 6) using the same 1.5 Tesla GE Signa LX system MRI scanner (General Electric, Milwaukee, WI, USA) as in Section 2.4., with actively shielded magnetic field gradients (maximum amplitude 40mT m\(^{-1}\)). A standard quadrature birdcage head coil was used for both radiofrequency transmission and signal reception. Each DT-MRI volume was acquired using a multi-slice peripherally-gated EPI sequence, optimised for precise measurement of the diffusion tensor in brain parenchyma, from 60 contiguous 2.5mm thick slices with field of view of 240 x 240mm and matrix size 96 x 96, zero-filled during reconstruction to 128 x 128, giving a final in-plane voxel size of 1.875 x 1.875mm\(^2\) (Jones et al., 2002a; Kyriakopoulos et al., 2008).

Image acquisition was synchronised to the cardiac cycle using a peripheral gating device placed on the subject’s forefinger. TE was 107ms while the effective repetition time was 15 R-R intervals. Duration of the diffusion encoding gradients was 17.3ms giving a maximum diffusion-weighting of 1,300 s mm\(^{-2}\). At each slice location, seven images were acquired with no diffusion gradients applied \((b = 0)\), together with 64 diffusion-weighted images in which gradient directions were uniformly distributed in space (Jones et al., 2002b). Total scan time was approximately 15 minutes and the relative orientations of the diffusion gradient vectors were based on the electrostatic repulsion algorithm (Jones et al., 1999; Jones et al., 2002a). Table 2.1 provides a summary of acquisition parameters. Following DT-MRI acquisition, datasets are prepared for whole-brain voxel-based analyses.
Table 2.1 Summary of DT-MRI pulse sequence acquisition parameters (adapted from [Jones et al., 2002a])

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Echo time</td>
<td>107 ms</td>
</tr>
<tr>
<td>Repetition time</td>
<td>15 R-R intervals</td>
</tr>
<tr>
<td>Duration of diffusion gradients</td>
<td>17.3 ms</td>
</tr>
<tr>
<td>Separation of diffusion gradients</td>
<td>49 ms</td>
</tr>
<tr>
<td>Amplitude of diffusion gradients</td>
<td>40 mT m(^{-1})</td>
</tr>
<tr>
<td>Maximum diffusion weighting</td>
<td>1300 s mm(^{-2})</td>
</tr>
<tr>
<td># Measurements without diffusion gradients</td>
<td>7</td>
</tr>
<tr>
<td># Measurements with diffusion gradients</td>
<td>64</td>
</tr>
<tr>
<td>Field of view</td>
<td>24 cm (\times) 24 cm</td>
</tr>
<tr>
<td>Acquisition matrix</td>
<td>96 (\times) 96</td>
</tr>
<tr>
<td>Slice thickness/gap</td>
<td>2.5 mm/0 mm</td>
</tr>
<tr>
<td>Number of slice locations</td>
<td>60</td>
</tr>
</tbody>
</table>
2.8. Whole-brain voxel-based analysis of DT-MRI data

Following acquisition, the diffusion-weighted images were corrected for the effects of eddy-current-induced image distortions introduced by the diffusion-weighting gradients by in-house software. Initially, a reference image was constructed by calculating the mean intensity within each voxel from all the images with no diffusion-weighting applied; subsequently, for each diffusion-weighted image, the downhill simplex method (Press et al., 1992) was used to select the optimal magnification, shear, and displacement of the diffusion-weighted images so as to give optimum registration with the reference image. The 'mutual information' (Studholme et al., 1997) was used to assess the registration between the 'reference' image and the corrected image. Following correction of image distortion, the data were masked from the background using a semi-automated thresholding procedure and subsequently, the diffusion tensor was calculated for each voxel using multivariate linear regression after logarithmic transformation of the signal intensities (Basser et al., 1994a; Jones et al., 2002a).

The tensor in each voxel was diagonalised so as to determine the eigenvectors and eigenvalues; the principal eigenvector (associated with the largest eigenvalue), trace (sum of the three eigenvalues or total diffusivity), and the FA of the tensor in each voxel were computed as discussed in Chapter 1, Section 1.1.4. Following calculation of the diffusion tensor and FA in each voxel, a multi-slice FA image was created (Basser and Pierpaoli, 1996). As a result of the DT-MRI acquisition scheme, a series of discrete estimates of the diffusion tensor field on a regular grid (i.e. at the centre of each image voxel) was obtained. Although the diffusion tensor was estimated at
the centre of the image voxel, a continuous approximation of the diffusion tensor
field from the voxel-wise measurements was possible using the B-spline-based
continuous tensor field approach (Aldroubi and Basser, 1999; Pajevic et al., 2001)
that permits the assessment of FA at any arbitrary point within the imaged region.

Brain extraction was next performed using the software package Brain Extraction
Tool (BET) which is part of the Functional Software Library (FSL) package (Oxford
Centre for Functional Magnetic Resonance Imaging of the Brain, Oxford University,
Oxford, UK; http://www.fmrib.ox.ac.uk/analysis/research/bet). A connectivity
algorithm based on intensity thresholding was then applied which was then followed
by the use of the extracted T2-weighted volume as a binary mask on the tensor
volume dataset (Catani et al., 2002; Jones et al., 2002b). Subsequently, I conducted
voxel-based analyses of the FA maps.

In terms of an overview of FA analyses performed, study images are initially
spatially normalised with SPM2 using the mapping parameters from the averaged \( b \)
= 0 and FA images. Images are then segmented into grey and white matter tissue
components and white matter masks are applied to the FA data. This serves to limit
statistical analyses to a skeletal core of white matter and therefore minimises errors
at the grey-white matter interface. The next step involves smoothing with a filter so
as to improve the signal-to-noise ratio and inter-subject matching and also to reduce
confounds that arise due to individual variations in neuroanatomy. Finally, statistical
comparisons are made on a voxel-by-voxel level on maps of FA involving non-
parametric statistics and FA maps are examined at both the voxel and cluster levels.
Given that a parametric distribution has not been demonstrated for cluster mass data,
permutation-based, non-parametric statistical methods are used (Bullmore et al., 1999) as with conventional MRI data. Locally developed software (XBAM) was used to measure between group differences at each intracerebral voxel in standard space by either fitting an ANOVA or ANCOVA model. Significant findings are reported using standard space i.e. Talairach coordinates (Talairach and Tournoux, 1988) and corrected for multiple comparisons. In the next section, details of normalisation, segmentation, masking, smoothing, and statistical comparisons of FA maps are provided.

2.8.1. Normalisation

Scans were examined for image corruption or motion artefacts prior to inclusion in the imaging pipeline and excluded if they demonstrated these abnormalities. To facilitate a voxel-based comparison of FA between subjects, images were pre-processed using SPM2 (Wellcome Department of Imaging Neuroscience, University College London) within MATLAB 6.5.2 (The MathWorks, Natick, MA, USA) and used a two-stage approach. DT-MRI group mapping techniques (derived from VBM analysis methods developed for structural T1 and/or T2-weighted images) aligned DT-MRI data into standard space. The default normalisation parameters for SPM2 were used (medium regularisation; 16 non-linear iterations; trilinear interpolation).

As a two-stage normalisation was performed, the first step was to align all the scans into standard MNI space using the T2-weighted (nondiffusion-weighted, \( b = 0 \)) images of the DT-MRI data. The mean T2-weighted images from each subject were
initially registered to the standard EPI template provided by SPM2. The derived mapping parameters for each subject were then applied to the (inherently co-registered) FA images. The normalised FA images of all subjects were then averaged and smoothed (8mm full-width at half maximum Gaussian filter) to create a new, study-specific, intermediate template to which each subject’s FA images were then re-registered. Use of such a study specific, intermediate template reduces the bias that would otherwise exist due to the larger degree of warping needed to match the subject brains than the control brains to a standard (control subject based) template.

2.8.2. Segmentation and Masking

The registered FA images were segmented (using SPM’s default *a priori* tissue probability information) to give maps of the probability of a tissue being either white or grey matter, and these segmented images were thresholded at a low level (10%) to provide a (deliberately slightly overinclusive) binary mask of white matter. Visual inspection of example datasets confirmed that masking at this threshold produced maps which included all major white matter areas, and did not suffer from any unexpected ‘holes’ (e.g. in low FA regions caused by crossing fibres).

2.8.3. Smoothing

Scans need to be smoothed in order to reduce confounds due to individual variation in white matter anatomy. Smoothing the data in order to coerce it into the
appropriate statistical distribution is also a prerequisite for some analysis approaches, but is not necessary for the non-parametric approach used in all the experimental studies of this thesis (Hayasaka and Nichols, 2003). The degree of smoothing to apply is still a subject of much discussion as different smoothing levels result in varying results (Jones et al., 2005); in the absence of a specific hypothesis about the spatial extent of any abnormalities, a smoothing filter (Gaussian, 5mm full-width at half maximum) was applied to aid between-subject anatomical matching and improve the signal-to-noise ratio for all DT-MRI studies in this thesis.

2.8.4. Statistical comparisons of FA maps

The statistical significance of between group differences in FA was examined using a non-parametric permutation-based method via locally developed software, XBAM (version 3.4, Institute of Psychiatry, London; http://www.brainmap.co.uk/) which measured between group differences at each intracerebral voxel in standard space. XBAM makes no distributional assumptions, uses median statistics to control outlier effects and uses permutation rather than normal theory based inference. Furthermore, its test statistics are computed by standardising for individual differences in residual noise before embarking on second level, multi-subject testing using robust permutation-based methods which allow a mixed effects approach to analysis. The statistical model in XBAM is described below:

\[ Y = a + bX + e \]
Chapter 2: Magnetic resonance imaging acquisition and analytical methodology

$Y$ is the vector of effect sizes for each individual, $X$ is the contrast matrix for FA between groups, $a$ is the mean effect across all individuals in the various groups, $b$ is the computed group difference and $e$ is a vector of residual errors. In order to reduce outlier effects, the model is fit by minimising the sum of absolute deviations rather than the sums of squares. The null distribution of $b$ is computed by permuting data between groups and refitting the above model. Group difference maps are generated at voxel and cluster levels by appropriate thresholding of the null distribution of $b$.

With this statistical model, XBAM typically uses an ANOVA testing paradigm where FA is the dependent variable while group classification is the key predictor variable. In some of the experimental studies conducted in the thesis, covariates were added into the statistical model e.g. IQ or age using an ANCOVA framework.

At this stage, only those voxels at which all subjects contribute data were considered and which, along with the masking procedure, restricted the analysis to core white matter regions. This reduced the search volume (and thus the number of comparisons made) and also avoided testing at the grey/white interfaces, where the high grey/white contrast of FA images exacerbates any edge effects. As with assessment of maps of tissue content, higher-level statistics in the form of cluster-based inference were used in the assessment of FA maps. These involved the application of double-thresholding to statistical maps whereby an initial threshold was performed at the voxel level; subsequently, suprathreshold clusters were kept whenever their size was statistically significant whereby such non-parametric tests improve the sensitivity and reproducibility of analyses as well as allow additional sensitivity to low-power experiments (Suckling and Bullmore, 2004; Thirion et al., 2007).
At both the voxel and cluster levels of analyses, 1000 permutations were performed. Local permutation testing was initially conducted at the voxel level so as to determine a voxel-specific threshold whereby testing involves randomly permuting participant labels between groups so as to achieve the null hypothesis of no main effect of group membership on FA. Permutation was carried out 1000 times at each voxel to allow the construction of a voxel-level null distribution of FA differences and the significance of each voxel was assessed against its own null distribution (Bullmore et al., 1999). Local permutation is less susceptible to contamination by statistics from across the brain and is more sensitive in the situation where the magnitude of group differences is spatially variable as is the case with FA. This permutation approach is also necessary with DT-MRI data given that any normalisation error will otherwise produce a strongly bimodal distribution of FA in areas close to tissue boundaries (Kanaan et al., 2009). As an identical permutation strategy was applied at all voxels, it is valid to subsequently form clusters of spatially contiguous significant voxels.

The sum of voxel statistics within each cluster was computed for each randomisation to form a distribution of cluster mass under the null hypothesis. As no parametric distribution is known for cluster mass (sum of voxel statistics within each cluster) (Poline et al., 1997), the mass of each cluster in the observed data was compared to this randomised distribution, and significant clusters were defined as those that had a greater cluster mass than the randomised distribution at a particular significance level. The voxel-wise statistic images were initially thresholded at a relatively lenient level of \( p \leq 0.05 \), and voxels that were spatially contiguous in three dimensions in
the thresholded maps were assigned to the same cluster. At the cluster level, rather than setting a single *a priori* p-value below which the findings are regarded as significant, for a range of p-values, the number of clusters which would be expected by chance alone was calculated. The statistical threshold was then set for cluster significance for each analysis such that the expected number of false-positive clusters by chance alone would be less than one. Additionally, significant findings were considered at a threshold for cluster size that was set at larger than 10 voxels.

As SPM was used for image registration, XBAM yielded coordinates of significant clusters in MNI space and the identification of significant clusters and white matter tracts intersecting them was based on an atlas approach where several white matter atlases were used in conjunction to determine the projection and localisation of white matter bundles; these atlases were based on both single and multiple subject DT-MRI datasets in MNI space (Catani and Thiebaut de Schotten, 2008; Mori et al., 2005; Thiebaut de Schotten et al., 2011; Wakana et al., 2004). Given that the Talairach coordinate system is most commonly used for reporting findings in the neuroimaging literature, MNI coordinates were subsequently converted to Talairach space via a non-linear transformation (Brett et al., 2002) (details at http://imaging.mrc-cbu.cam.ac.uk/imaging/MniTalairach) and the localisation of white matter tracts and their respective anatomical course within cerebral lobes was additionally confirmed using the Talairach atlas (Talairach and Tournoux, 1988). Where significant differences were found on FA maps, the p-value of each significant cluster is reported alongside the anatomical locations of their respective white matter tracts in Talairach space.
Given that brain changes are likely to extend over a number of contiguous voxels, test statistics incorporating spatial information such as 3-D cluster mass (the sum of suprathreshold voxel statistics), are generally more powerful than other possible test statistics, which are informed only by data at a single voxel (Bullmore et al., 1999; Hayasaka and Nichols, 2003). Also, with cluster-based testing, the number of statistical tests performed are substantially fewer than the number of voxels tested which therefore allows for more relaxed probability thresholds and reduced Type 2 error rates for a given number of false positive tests. Further, unlike voxels, which may be correlated under the null hypothesis, clusters are regarded as independent events under the null hypothesis. Additionally, the non-parametric methods used also overcome the assumption that parametric methods adopt in that the residuals of the model tested will follow a Gaussian distribution (which has been shown to not always be true for DT-MRI data, even after extensive smoothing) (Jones et al., 2005). Finally, although variance of the data may differ with brain area (Carew et al., 2007), the clusters were evaluated over the whole brain and voxel level maps were concurrently inspected in order to check for any gross discrepancies between voxel and cluster levels. Fig. 2.2 is the standard brain output template used in reporting FA differences for DT-MRI data based on MNI space at the Institute of Psychiatry.
Fig. 2.2 Institute of Psychiatry MNI template for DT-MRI data - 74 x 2.0 mm axial slices (AC-PC=anterior commissure-posterior commissure plane)
2.8.5. Post hoc correlations

Where significant 3-D clusters are found on DT-MRI voxel-based analyses, post hoc tests are carried out to determine if significant differences in white matter FA are associated with behavioural variation within the subject group. As the clusters detected by XBAM may encompass multiple anatomical regions (i.e. were not constrained to lie only within particular white matter tracts), mean FA was first extracted for each subject. To do this, mask images were created from each of the clusters found by the group mapping analysis and applied to each subject’s normalised FA images thereby enabling mean FA values to be calculated over each region for each subject. These were then correlated with relevant behavioural scores using Pearson product-moment correlation coefficients with Statistical Package for the Social Sciences (SPSS 14.0 for Windows, SPSS Inc, Chicago, IL, USA). Significant correlations are reported where a Bonferroni adjusted alpha was applied.

2.9. Summary of analytical methods used in this thesis

For every experimental study in the thesis, I acquired subject images based on MRI methods. Where conventional MRI is acquired on study subjects, I applied whole-brain VBM analyses to white and/or grey matter tissue segments. For experimental studies where I acquired DT-MRI on study participants, I applied whole-brain voxel-based analyses to FA maps of white matter. The statistical analyses I used for both VBM of conventional MRI data and voxel-based analyses of DT-MRI data
respectively are based on a non-parametric permutation-based statistical testing model that assesses data at both voxel and cluster levels.

For VBM of conventional MRI data, I aligned and segmented images using SPM5 where both components are integrated into a single framework. Following this, I modulated and smoothed grey and white matter compartments. Finally, I used non-parametric statistical comparisons of tissue differences between brains of study participants using XBAMM.

For voxel-based analyses of DT-MRI data, I aligned images using SPM2 to a customised brain template derived from all study participants based on a two-stage normalisation procedure which initially aligns acquired T2-weighted images to tissue probability maps derived from SPM and these mapping parameters are subsequently applied to registration of FA maps and a customised group-averaged FA template is then created. This is followed by segmentation and masking of white matter maps of FA and smoothing with a Gaussian kernel. Finally, I conducted non-parametric statistical comparisons of FA differences across brains of study participants using XBAM.

For both VBM and voxel-based analyses, correction for multiple comparisons is conducted such that analyses are reported at a stringent adaptive cluster level threshold where the expected number of false-positive clusters is less than one per analysis. Further, localisation of brain differences is based on known neuroanatomical pathways and ultimately reported in Talairach space. As I examined four distinct phenotypes in this thesis, detailed descriptions of the: recruitment
strategy, ethical approval, subject groups examined, psychometric assessments, psychometric correlations and findings are discussed in individual chapters according to the respective phenotype under investigation. Each of the experimental studies represents a novel investigation that has never been performed previously and therefore results from these investigations may significantly contribute to the literature where understanding of these disorders is presently lacking.

The next chapter constitutes the first experimental chapter (Chapter 3, Study 1) which compares a cohort of 22q11DS children and adolescents relative to healthy matched controls using both conventional MRI and DT-MRI acquired datasets where VBM and voxel-based analyses are respectively conducted. Of note, these same VBM analytical techniques of conventional MRI data are applied in Chapter 4 while the voxel-based analyses of DT-MRI data utilised in Chapter 3 are also used in Chapters 5 and 6.
Chapter 3:

Study 1
White matter microstructure in 22q11 deletion syndrome: a pilot diffusion tensor imaging and voxel-based morphometry study of children and adolescents

The main aim of this experimental chapter is to examine a cohort of children and adolescents with 22q11DS relative to healthy sibling and unrelated controls. Neuroimaging datasets based on conventional MRI and DT-MRI are concurrently investigated for structural and connectivity differences respectively. Given that a significant number of individuals with 22q11DS develop psychiatric disorder (in particular, schizophrenia spectrum disorders) and that white matter is affected to a greater extent in 22q11DS, it is therefore important to identify neuroanatomical correlates within white matter potentially contributing to the development of such disorder. Additionally, the influence of COMT genotype on white matter measures of volume and FA is also assessed.
3.1. Summary of chapter

Young people with 22q11DS are at substantial risk for developing psychosis and have significant differences in brain anatomy particularly involving white matter volume. However, there are few in-vivo studies of both white matter microstructural integrity (as measured using DT-MRI) and white matter volume in the same individual. In the present study, DT-MRI and conventional MRI datasets were analysed respectively with voxel-based analysis and VBM to compare white matter FA and volume of 11 children and adolescents with 22q11DS and 12 healthy controls. Also, within 22q11DS differences in white matter were related to severity of schizotypy, and polymorphism of the COMT gene.

People with 22q11DS were found to have significantly lower FA in interhemispheric and brainstem and frontal, parietal and temporal lobe regions after covarying for IQ. Significant white matter volumetric increases were concurrently found in the internal capsule, anterior brainstem and frontal and occipital lobes after covarying for IQ. There was a significant negative correlation between increased schizotypy scores and reduced white matter FA in the right posterior limb of internal capsule and the right body and left splenium of corpus callosum. Finally, the Val allele of COMT was associated with a significant reduction in both white matter FA and volume in the frontal lobes, cingulum and corpus callosum. The findings suggest that young people with 22q11DS have significant differences in both white matter microstructure and volume. Also, there is preliminary evidence that within 22q11DS, some regional differences in FA are associated with allelic variation in COMT and may perhaps also be associated with schizotypy.
3.2. Introduction

People with 22q11DS are reported to have an increased incidence of midline and regional volumetric neuroanatomical abnormalities which have been summarised in Chapter 1, Section 1.5.5. While the cerebellum and posterior brain structures have been previously implicated in volumetric MRI studies of 22q11DS, and that age and choice of brain template may affect measures of brain anatomy, however, two prior DT-MRI studies have either used a healthy paediatric brain template or have excluded the posterior brain structures from analysis. Additionally, examination of white matter anatomy in a relatively homogeneous group of children and adolescents has yet to be conducted in 22q11DS using DT-MRI where prior studies have used a combination of adults and children as a single group instead.

In the present study, I assessed white matter connectivity and white matter volume differences simultaneously and specifically in a cohort of children and adolescents. Also, I determined if differences in white matter FA and volume demonstrated any overlap as assessed by voxel-based analysis of FA maps and VBM respectively. Analyses included the posterior brain structures and a customised brain template derived from all subjects under study was used for normalisation.

Additionally, as high rates of schizophrenia spectrum disorders have been reported in 22q11DS during adulthood but with psychotic symptoms beginning even earlier, white matter differences were related to measures of schizotypy. Schizotypy is a
particular risk factor in children for the future development of psychosis and white matter integrity abnormalities have been related to schizotypy in the non-deleted population (Nakamura et al., 2005). Further, given that white matter is significantly affected in 22q11DS, I correlated schizotypy with white matter disruption for the first time in a young 22q11DS population at risk of developing psychosis. Moreover, as COMT polymorphism may modulate brain maturation through dopamine metabolism, its effect on white matter integrity was also studied in children and adolescents with 22q11DS.

3.3. Aims, objectives and hypotheses

This study aims to assess white matter integrity, connectivity and volume in a cohort of children and adolescents with confirmed chromosome 22q11.2 deletion who are at high risk of developing schizophrenia spectrum disorders.

The objectives of this experimental chapter include the:

- recruitment and assessment of a cohort of children and adolescents with and without chromosome 22q11.2 deletion
- screening of children and adolescents with confirmed 22q11.2 deletion to rule out the presence of comorbid psychiatric disorder with the exception of schizotypal traits
- analysis of the whole brain and to encompass posterior brain structures
- analysis of white matter using a customised brain template derived from all subjects under study instead of a healthy paediatric template
analysis of white matter integrity, connectivity and volume

The main hypothesis tested in this experimental chapter is that children and adolescents with 22q11DS have significant differences from healthy non-deleted controls in white matter measures of microstructural integrity and volume that are not co-located. Additionally, preliminary post hoc investigations are performed to test further hypotheses that, within young people with 22q11DS: 1) severity of schizotypy is correlated with differences in white matter integrity; and 2) polymorphism of the COMT gene is associated with differences in white matter volume and coherence.

3.4. Methods

The following subsections provide details of the study participants, screening of psychiatric and medical disorders, diagnosis of schizotypal traits, genetic testing, assessment of COMT genotype influence on brain anatomy and finally, correlation of schizotypy with any neuroanatomical abnormalities found.

3.4.1. Subjects

Subjects were recruited from the UK 22qDS support group and the Behavioural Genetics Clinic at the South London and Maudsley NHS Foundation Trust. The study was approved by the Ethics Committee at the Institute of Psychiatry, King's
College London (067/00) and the Multicentre Research Ethics Committee (MREC) MT/AB/MREC/00/7/57. Funding was provided by the Healthcare Medical Trust.

Written informed consent was obtained from parents/carers, and assent from children, after full description of the study. All participants (including controls) were medication free, had English as their first language, and were right handed as assessed by the Annett Handedness Questionnaire.

Parents and carers of individuals with 22qDS and controls were contacted via postal correspondence and invited to participate in the study. This was followed up by telephone contact and an information sheet was discussed that provided details of the study. Additionally, consent forms were also provided to those interested in participating. Families that provided consent were later contacted so as to coordinate a visit to the family home to provide supplementary information and where consent was obtained, individuals were invited to participate in MRI acquisition at the Institute of Psychiatry, King's College London. While 12 children and adolescents with 22q11DS successfully underwent structural MRI and DT-MRI at the same scanning session, a DT-MRI scan for one 22q11DS subject demonstrated excessive motion artefact and his scans were excluded from further analysis.

Therefore, 11 young people (5 male and 6 female) with clinical features of 22q11DS and an established genetic 22q11.2 deletion (mean age: 12 years, SD ± 2.2, range 9-17 years; mean FSIQ: 66, SD ± 8.0, range 56-84) were included and form the focus of this experimental chapter. Exclusion criteria were a 22q11DS clinical phenotype but without the large 3Mb 22q11.2 deletion, a clinically detectable medical disorder known to affect brain structure (e.g. epilepsy), a history of head injury or
contraindications to MRI scanning. See Table 3.1 for a summary of subject characteristics.

22q11DS cases were compared to 12 healthy controls (8 male and 4 female) with a non-deleted 22q11.2 region (mean age: 13 years, SD ± 3, range 9-17 years; mean FSIQ: 116, SD ± 16, range 90–141). Controls in this study were a combination of sibling (n = 7) and non-sibling (n = 5) controls. All healthy controls were free from genetic and physical disorders affecting brain anatomy or function.

3.4.2. Medical and psychiatric disorders

Children and parents were examined through semi-structured interview to evaluate past medical history and all subjects had a detailed physical examination. Copies of past assessments from their local health services were also obtained. Controls were free from medical illness while 22q11DS subjects (as expected) had a range of palatal (n = 9) and cardiac/vascular anomalies (n = 6).

The Wechsler Intelligence Scale for Children-III (WISC-III) (Wechsler, 1991) was used to assess general intellectual functioning while the strengths and difficulties questionnaire (SDQ) (Goodman et al., 2000) measured emotional symptoms, hyperactivity/inattention, conduct problems, peer problems and pro-social behaviour in all subjects. The short-version of the autism screening questionnaire (ASQ) (Berument et al., 1999) was used to measure autistic symptoms with a cut-off score of 7 for individuals who may have autism.
A previously published schizotypy scale designed for young people with learning disability to include 22q11DS was also administered (Campbell et al., 2006); this scale was derived from DSM-IV (American Psychiatric Association, 2000). All behavioural assessments were completed by a primary caregiver or parents. Also, due to the high incidence of comorbid neuropsychiatric disorder in 22q11DS and the potential for non-homogeneity, only subjects that were free from Axis One psychiatric disorder (as far as could be best determined) were recruited.

3.4.3. Genetic testing

DNA was extracted from blood samples collected on all subjects. FISH (Oncor Inc, Gaithersburg, MD, USA) confirmed 3Mb 22q11.2 deletion in 22q11DS cases while chromosome 22q11.2 deletion was excluded in all controls. The COMT Val\textsuperscript{158}Met polymorphism was genotyped using the SNaPshot technique of single base extension (Applied Biosystems, Foster City, CA, USA). The initial PCR reaction was performed using a Touchdown-PCR-protocol, with the following primers: forward: 5'-ACTGTGGCTACTCAGCTGTG-3' and reverse: 5'-CCTTTTTCCAGGTCTGACAA-3'. The allele at the single nucleotide polymorphism (SNP) position was determined by use of a 30bp extension primer (5'-ATCACCCAGCGGATGGGTGATTTGCCTGGC-3'). All alleles were resolved on an ABI 3100 sequencer (Applied Biosystems, Foster City, CA, USA).
### Table 3.1 Demographic, cognitive and genetic data and global volume per 22q11DS subject

<table>
<thead>
<tr>
<th>22q11DS subject</th>
<th>Gender</th>
<th>Age</th>
<th>Grey matter volume (ml)</th>
<th>White matter volume (ml)</th>
<th>Total brain volume (ml)</th>
<th>Full scale IQ</th>
<th>Performance IQ</th>
<th>Verbal IQ</th>
<th>COMT genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>15</td>
<td>759.745</td>
<td>423.744</td>
<td>1183.489</td>
<td>71</td>
<td>70</td>
<td>79</td>
<td>Met</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>17</td>
<td>786.875</td>
<td>422.782</td>
<td>1209.657</td>
<td>56</td>
<td>56</td>
<td>63</td>
<td>Met</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>12</td>
<td>814.155</td>
<td>403.656</td>
<td>1217.811</td>
<td>66</td>
<td>76</td>
<td>61</td>
<td>Val</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>12</td>
<td>650.714</td>
<td>372.946</td>
<td>1023.66</td>
<td>67</td>
<td>70</td>
<td>68</td>
<td>Val</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>13</td>
<td>660.107</td>
<td>361.804</td>
<td>1021.911</td>
<td>65</td>
<td>70</td>
<td>65</td>
<td>Val</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>11</td>
<td>760.499</td>
<td>374.218</td>
<td>1134.717</td>
<td>84</td>
<td>75</td>
<td>96</td>
<td>Val</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>13</td>
<td>798.654</td>
<td>435.796</td>
<td>1234.45</td>
<td>69</td>
<td>76</td>
<td>67</td>
<td>Met</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>11</td>
<td>654.007</td>
<td>321.227</td>
<td>975.234</td>
<td>56</td>
<td>69</td>
<td>46</td>
<td>Val</td>
</tr>
<tr>
<td>9</td>
<td>F</td>
<td>13</td>
<td>648.87</td>
<td>349.425</td>
<td>998.295</td>
<td>58</td>
<td>53</td>
<td>68</td>
<td>Val</td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>9</td>
<td>776.956</td>
<td>407.82</td>
<td>1184.776</td>
<td>68</td>
<td>60</td>
<td>83</td>
<td>Met</td>
</tr>
<tr>
<td>11</td>
<td>M</td>
<td>11</td>
<td>806.555</td>
<td>356.033</td>
<td>1162.588</td>
<td>63</td>
<td>59</td>
<td>73</td>
<td>Val</td>
</tr>
</tbody>
</table>
3.4.4. Conventional MRI and DT-MRI acquisition protocol

Conventional MRI was acquired as per scan protocol in Chapter 2, Section 2.4. Furthermore, DT-MRI was concurrently acquired during the same scanning session on the same MRI scanner as per protocol in Chapter 2, Section 2.7.

3.4.5. Conventional MRI and DT-MRI data pre-processing

As mentioned earlier, a DT-MRI scan for one 22q11DS subject demonstrated excessive motion artefact and as a result, both structural and DT-MRI scans for this individual were excluded from the imaging pipeline. Therefore, whole-brain voxel-based analyses and VBM were performed as per Chapter 2, Sections 2.6. and 2.8. on the DT-MRI and conventional MRI data of the 11 subjects with 22q11DS and 12 healthy controls.

3.4.6. Statistics

In the following subsections, details are provided on the statistical tests used for examining behavioural, genetic and demographic data. Descriptions of the DT-MRI and VBM statistical comparisons performed at the individual voxel level are given in Chapter 2 in Sections 2.6.2., 2.8.4. and 2.8.5.
3.4.6.1. Demographic, genetic, behavioural and global brain volume data

Statistical analysis of these data was performed in SPSS 14.0 for Windows (SPSS Inc, Chicago, IL, USA). Student’s $t$-tests ($p = 0.05$, two-tailed) for independent samples were used to examine between-group differences on age, IQ, ASQ, SDQ and schizotypy scores while the binomial test assessed COMT and gender distribution ($p = 0.05$, two-tailed).

Further, global total brain, grey matter and white matter volumes were extracted via SPM5 and between group differences were compared using Student’s $t$-tests (see Table 3.2). Although there was a trend for significance involving global brain volumes, these were not entered as covariates in the statistical testing model used with either VBM or XBAM as the primary measure of interest was white matter which did not demonstrate a statistical difference between groups.

3.4.6.2. Summary of neuroimaging analyses conducted

Below is a summary of the neuroimaging analyses conducted between study groups, within 22q11DS and on white matter measures of FA and/or volume:

- 22q11DS vs. controls FA and white matter volume (not controlled for IQ)
- 22q11DS vs. controls FA and white matter volume (controlled for IQ)
- Within 22q11DS, a preliminary correlation of FA and schizotypy scores
3.4.6.3. Whole-brain voxel-based analysis of DT-MRI data

The statistical significance of between group differences in FA was initially examined using a non-parametric permutation-based method based on ANOVA. Further details are found in Chapter 2, in Section 2.8. and in particular, Section 2.8.4. The analysis was corrected for false positives where a cluster significance threshold of $p = 0.0025$ was used. When IQ was used as a covariate, an ANCOVA model was used and the false positive corrected analysis used a cluster significance threshold of $p = 0.0005$ So as to minimise IQ as a source of potential variance and also to reduce heterogeneity between study groups, it was entered as a covariate and results are presented both with IQ controlled and uncontrolled.

3.4.6.4. VBM of white matter content

In this study, although global and total white and grey matter content were extracted, only white matter was further assessed by VBM. Following image registration and segmentation, white matter was extracted from the normalised images and modulated to compensate for the effects of spatial normalisation via SPM5 (Chapter 2, Section 2.6.1.). Images were then smoothed by convolution with a 5mm Gaussian kernel. The maps so produced are referred to as images of white matter content and a
cluster significance threshold of $p = 0.006$ was used to correct for false positives.

Chapter 2, Section 2.6.2. provides full details of the testing procedure on white matter content. While IQ was added as a covariate in further between-group analyses, however, as global volumes did not significantly differ, these were not entered as further covariates in between-group analyses.

3.4.6.5. Post hoc analysis of schizotypy scores

A preliminary analysis was carried out to determine if significant differences in white matter FA were associated with behavioural variation within people with 22q11DS. To do this, mean FA values were extracted from each of the clusters that were significantly different from healthy controls (see Chapter 2, Section 2.8.5.); of note, only FA values from the clusters in the analysis whereby IQ was entered as a covariate were examined (results found in Section 3.5.2.2. and Table 3.4). These values were then related to severity of schizotypy (Campbell et al., 2006) and a Bonferroni adjusted alpha of 0.025 was subsequently applied.

3.4.6.6. Post hoc analysis of COMT status

A second preliminary investigation within people with 22q11DS was performed by comparing the white matter FA and volume of those with Val-COMT to their counterparts with Met-COMT. The FA analysis used a false positive corrected
cluster significance threshold of $p = 0.00175$ while the VBM analysis used a level of $p = 0.005$.

3.5. Results

The following subsections expand on the results of behavioural, genetic and demographic data as well as global brain volumes. Additionally, results from DT-MRI voxel-based analyses and VBM are provided. Preliminary post hoc analyses based on COMT genotype and schizotypy and their correlation with FA and/or white matter volume differences in only the 22q11DS group is also given.

3.5.1. Demographic, genetic, behavioural data and global brain volume data (Table 3.2)

The groups did not differ significantly in age, or gender distribution. However, as expected, the mean FSIQ of those with 22q11DS was significantly lower; also, mean SDQ, ASQ and schizotypy scores were higher in 22q11DS individuals than controls. Six 22q11DS subjects and one healthy subject scored above the cut-off of 7 in the ASQ. With regard to COMT status, seven subjects with 22q11DS had Val-COMT while four had Met-COMT.
### Table 3.2 Summary of demographic, genetic, behavioural and global brain volume data

<table>
<thead>
<tr>
<th></th>
<th>22q11DS (n = 11)</th>
<th>Controls (n = 12)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender male/female</strong></td>
<td>5/6</td>
<td>8/4</td>
<td>1.0, 0.39</td>
</tr>
<tr>
<td><strong>Mean Age</strong></td>
<td>12 (SD ± 2.2; Range: 9 - 17)</td>
<td>13 (SD ± 2.5; Range: 9 - 17)</td>
<td>0.83</td>
</tr>
<tr>
<td><strong>Mean FSIQ</strong></td>
<td>66 (SD ± 8.0; Range: 56 - 84)</td>
<td>116 (SD ± 15.9; Range: 90 - 141)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td><strong>Handedness</strong></td>
<td>11 Right</td>
<td>12 Right</td>
<td>-</td>
</tr>
<tr>
<td><strong>Strengths and Difficulties</strong></td>
<td>15.2 (SD ± 7.1; Range: 6 - 25)</td>
<td>4.9 (SD ± 3.4; Range: 0 - 11)</td>
<td>0.002</td>
</tr>
<tr>
<td><strong>Schizotypy mean score</strong></td>
<td>2.0 (SD ± 2.3; Range: 0 - 7)</td>
<td>0 (SD ± 0; Range: 0)</td>
<td>0.034</td>
</tr>
<tr>
<td><strong>Autism Screening</strong></td>
<td>6.8 (SD ± 4.3; Range: 0 - 13)</td>
<td>2 (SD ± 4.0; Range: 0 - 10)</td>
<td>0.044</td>
</tr>
<tr>
<td><strong>COMT status</strong></td>
<td>7 Val-COMT vs. 4 Met-COMT</td>
<td>-</td>
<td>0.549</td>
</tr>
<tr>
<td><strong>Mean global grey matter volume (ml)</strong></td>
<td>738 (SD ± 69.1; Range: 649 - 814)</td>
<td>790 (SD ± 59.0; Range: 692 - 896)</td>
<td>0.067</td>
</tr>
<tr>
<td><strong>Mean global white matter volume (ml)</strong></td>
<td>385 (SD ± 36.5; Range: 321 - 436)</td>
<td>418 (SD ± 62.2; Range: 327 - 512)</td>
<td>0.138</td>
</tr>
<tr>
<td><strong>Mean total brain volume (ml)</strong></td>
<td>1,122 (SD ± 97.8; Range: 975 - 1234)</td>
<td>1,207 (SD ± 108.3; Range: 1018 - 1408)</td>
<td>0.062</td>
</tr>
</tbody>
</table>
3.5.2. Group contrasts of FA using DT-MRI voxel-based analyses

In the following subsections, results of DT-MRI voxel-based analyses comparing 22q11DS and controls are provided. Results of analyses controlled and not controlled for IQ are detailed. These are followed by results of preliminary post hoc analyses involving COMT and schizotypy data. In summary, the results of following analyses are found below:

- 22q11DS vs. controls FA (not controlled for IQ)
- 22q11DS vs. controls FA (controlled for IQ)
- Within 22q11DS, a preliminary correlation of FA and schizotypy scores
- Within 22q11DS, a preliminary analysis of COMT influence on FA

3.5.2.1. 22q11DS vs. controls FA (not controlled for IQ) (Fig. 3.1, Table 3.3)

Relative to controls, white matter FA in 22q11DS subjects was significantly reduced in the frontal, parietal and temporal lobes of the left hemisphere. The centre of the most significant cluster was localised to the white matter of the superior thalamic radiation and the cluster encompassed the: 1) projections from the thalamus to the parietal lobe via the posterior limb of the internal capsule; 2) projections from the motor cortex of the frontal lobe to the posterior limb of the internal capsule via the superior region of the corona radiata; 3) tapetum (Crosby et al., 1962; Dejerine,
1895) lateral to the posterior horn of the lateral ventricle; 4) posterior thalamic radiation; and 5) the fronto-parietal course of the arcuate fasciculus.

In contrast, people with 22q11DS had a significantly increased FA relative to controls (again, exclusively in the left hemisphere) in regions that were anatomically more anterior and inferior to the FA decreases described above. The most significant clusters of FA increases included the white matter of the genu/anterior limb of the internal capsule together with the anterior and superior portions of the corona radiata.

When gender was added as a covariate, a small cluster was localised to the left frontal lobe but subsequently did not reach statistical significance when correction for Type 1 error was applied.
Fig. 3.1 FA in 22q11DS subject group vs. control group (not controlled for IQ). Lower FA (orange) and higher FA (blue) in 22q11DS subjects. Ascending 2mm transverse sections; (reversed where L=R, R=L)
Chapter 3: Study 1 - White matter microstructure in 22q11 deletion syndrome: a pilot diffusion tensor imaging and voxel-based morphometry study of children and adolescents

Table 3.3 DT-MRI voxel-based analyses of white matter FA of 22q11DS subjects vs. controls (cluster significance threshold p = 0.0025)
– Not controlled for IQ

<table>
<thead>
<tr>
<th>Cluster label</th>
<th>Cluster size (number of voxels)</th>
<th>Talairach and Tournoux coordinates</th>
<th>Tract(s) within cluster</th>
<th>Region</th>
<th>Cluster mean FA 22q11DS (SD)</th>
<th>Cluster mean FA Control (SD)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cluster 1</td>
<td>378</td>
<td>-23 -22 23</td>
<td>Internal capsule</td>
<td>Temporo-parietal &amp; sublobar</td>
<td>0.355 (0.027)</td>
<td>0.399 (0.031)</td>
<td>0.001547</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(posterior limb)/superior region of the corona radiata/caputem/posterior thalamic radiation/arcuate fasciculus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**White matter FA deficits in 22q11DS subjects**

<table>
<thead>
<tr>
<th>Cluster 2</th>
<th>463</th>
<th>-13 5 0</th>
<th>Genu and anterior limb of internal capsule/anterior and superior portions of the corona radiata</th>
<th>Fronto-parietal &amp; sublobar</th>
<th>0.389 (0.025)</th>
<th>0.352 (0.026)</th>
<th>0.001208</th>
</tr>
</thead>
</table>

**White matter FA excesses in 22q11DS subjects**
3.5.2.2. 22q11DS vs. controls FA (controlled for IQ) (Fig. 3.2, Table 3.4)

Significant widespread FA deficits were found in the 22q11DS group relative to healthy controls that extended from the brainstem to more superior brain sections. These deficits were mainly bilateral and found in structures close to the midline such as the corpus callosum, internal capsule and cingulum but also more laterally in temporal and parietal lobe regions. There were no regions of increased FA in the 22q11DS group relative to controls.

FA deficits in the brainstem encompassed the right corticopontine, corticospinal and middle cerebellar peduncle while the corpus callosum was found to have reduced FA especially in the splenium, tapetum and body and to a lesser extent in the genu. FA deficits were found in the cingulum adjacent to the body of the corpus callosum and in the uncinate fasciculus in the temporal lobe. The hippocampi and inferior longitudinal and inferior fronto-occipital fasciculus in the temporal lobes were found to have reduced FA bilaterally while the bilateral corona radiata in the occipital and parietal lobes also showed FA deficits.
Fig. 3.2 FA in 22q11DS subject group vs. control group (controlled for IQ). Significant FA deficits in 22q11DS (red). Ascending 2mm transverse sections; (reversed where L=R, R=L)
Table 3.4 DT-MRI voxel-based analyses of white matter FA of 22q11DS subjects vs. controls (cluster significance threshold $p = 0.0005$) – Controlled for IQ

<table>
<thead>
<tr>
<th>Cluster label</th>
<th>Cluster size (number of voxels)</th>
<th>Talairach and Tournoux coordinates $x/y/z$</th>
<th>Tract(s) within cluster</th>
<th>Region</th>
<th>Cluster mean FA 22q11DS (SD)</th>
<th>Cluster mean FA Control (SD)</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cluster 3</td>
<td>201</td>
<td>-13/-15/-11</td>
<td>Corticopontine and corticospinal tracts and middle cerebellar peduncle</td>
<td>Brainstem</td>
<td>0.415 (0.026)</td>
<td>0.430 (0.021)</td>
<td><strong>0.000254</strong></td>
</tr>
<tr>
<td>Cluster 4</td>
<td>280</td>
<td>30/-25/-4</td>
<td>Internal capsule (retrolenticular and posterior limbs)/inferior fronto-occipital fasciculus/inferior longitudinal fasciculus/hippocampus</td>
<td>Temporal lobe &amp; sublobar</td>
<td>0.391 (0.034)</td>
<td>0.406 (0.026)</td>
<td><strong>0.000157</strong></td>
</tr>
<tr>
<td>Cluster 5</td>
<td>180</td>
<td>-21/-20/5</td>
<td>Posterior limb of internal capsule/hippocampus</td>
<td>Temporal lobe &amp; sublobar</td>
<td>0.392 (0.036)</td>
<td>0.409 (0.032)</td>
<td><strong>0.000350</strong></td>
</tr>
<tr>
<td>Cluster 6</td>
<td>699</td>
<td>-9/-37/-11</td>
<td>Splenium of corpus callosum/superior corona radiata</td>
<td>Parietal lobe &amp; sublobar</td>
<td>0.414 (0.030)</td>
<td>0.426 (0.028)</td>
<td><strong>0.000056</strong></td>
</tr>
</tbody>
</table>
### Cluster 7
- **Cluster ID**: 752
- **Coordinates**: X: 14, Y: -39, Z: 13
- **Region**: Splenium of corpus callosum/superior corona radiata
- **Parietal lobe**
  - **Difference**: 0.402 (0.037)
  - **p-value**: 0.000051

### Cluster 8
- **Cluster ID**: 150
- **Coordinates**: X: 9, Y: -2, Z: 22
- **Region**: Body of corpus callosum/cingulum
- **Fronto-parietal lobe**
  - **Difference**: 0.432 (0.057)
  - **p-value**: 0.000448
3.5.2.3. Within 22q11DS, a preliminary analysis of schizotypy and differences in FA

There was a significant negative correlation between high schizotypy scores and decreased white matter FA (clusters found in Table 3.4) in the right internal capsule (retrolenticular and posterior limbs), inferior fronto-occipital fasciculus, inferior longitudinal fasciculus and hippocampus (Cluster 4, $r = -0.822$, $p = 0.023$); left splenium of corpus callosum and superior corona radiata (Cluster 6, $r = -0.851$, $p = 0.015$); and right body of corpus callosum and cingulum (Cluster 8, $r = -0.827$, $p = 0.022$).

3.5.2.4. Within 22q11DS, a preliminary analysis of COMT variation (Fig. 3.3, Table 3.5)

22q11DS individuals with Val-COMT had significantly lower FA than their counterparts with Met-COMT (Cluster 9). The most significant differences were found bilaterally in the: 1) anterior cingulum; 2) frontal lobe; and 3) corpus callosum. However, 22q11DS people with Val-COMT also had a unilateral FA reduction in right hemisphere regions incorporating the: 1) forceps minor; 2) inferior fronto-occipital and uncinate fasciculi; and 3) anterior/superior corona radiata.
Fig. 3.3 FA deficits within 22q11DS group in subjects with Val-COMT relative to Met-COMT. Ascending 2mm transverse sections; (reversed where L=R, R=L)
Table 3.5 White matter FA reduction of 22q11DS subjects with Val-COMT relative to Met-COMT (cluster significance threshold \( p = 0.00175 \))

<table>
<thead>
<tr>
<th>Cluster label</th>
<th>Cluster size (number of voxels)</th>
<th>Talairach and Tournoux coordinates</th>
<th>Tract(s) within cluster</th>
<th>Region</th>
<th>Cluster mean FA 22q11DS Val-COMT (SD)</th>
<th>Cluster mean FA 22q11DS Met-COMT (SD)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cluster 9</td>
<td>2721</td>
<td>-7 -6 26</td>
<td>Anterior cingulum/corpus callosum Forceps minor/inferior fronto-occipital and uncinate fasciculi/anterior and superior corona radiata</td>
<td>Frontal lobe</td>
<td>0.374 (0.030)</td>
<td>0.460 (0.027)</td>
<td>0.001211</td>
</tr>
</tbody>
</table>
3.5.3. Group contrasts of white matter volume using VBM

In the following subsections, results of conventional MRI VBM analyses assessing white matter volume are provided whereby analyses that are both controlled and uncontrolled for IQ are detailed. This will be followed by results of a preliminary post hoc analysis involving COMT data. In summary, results of the following analyses are presented:

- 22q11DS vs. controls white matter volume (not controlled for IQ)
- 22q11DS vs. controls white matter volume (controlled for IQ)
- Within 22q11DS, a preliminary analysis of the effect of COMT genotype

While mean global white matter volume did not differ significantly between young people with 22q11DS and controls (mean = 385ml, SD ± 36.5ml vs. mean = 418ml, SD ± 62.2ml respectively; p = 0.138) (Table 3.2), however, there were significant differences in the regional distribution of white matter which are described below.

3.5.3.1. 22q11DS vs. controls white matter volume (not controlled for IQ) (Fig. 3.4, Table 3.6)

Young people with 22q11DS, compared to controls, had a significant reduction bilaterally in the 1) middle cerebellar peduncle of the cerebellum and brainstem; 2) optic radiation and lingual, middle and inferior occipital gyri; 3) cuneus and precuneus; 4) posterior thalamic radiation; 5) body, genu and tapetum of the corpus
6) hippocampus; and 7) paracentral lobule. In contrast, 22q11DS people had a significantly greater white matter volume bilaterally in the 1) anterior limb and genu of the internal capsule; 2) white matter tracts from the basal ganglia; 3) medial frontal gyrus and cingulum; and 4) body and splenium of the corpus callosum.

3.5.3.2. 22q11DS vs. controls white matter volume (controlled for IQ) (Fig. 3.5, Table 3.7)

White matter volume was found to be significantly increased relative to healthy controls. These regional increases were mainly bilateral and found in the brainstem, the internal capsule (posterior limb and genu) and superior corona radiata while unilateral increases were found in the posterior corona radiata in the right occipital lobe and in the left pre-central gyrus of the frontal lobe. There were no regions of white matter volume reduction in 22q11DS.

3.5.3.3. Within 22q11DS, a preliminary analysis on the effect of COMT genotype (Fig. 3.6, Table 3.8)

22q11DS individuals with Val-COMT had a significantly decreased white matter volume relative to those with Met-COMT bilaterally in the 1) frontal lobes; 2) cingulum; 3) corpus callosum; 4) internal capsule; 5) hippocampus; and 6) superior and middle temporal gyri; and unilaterally in the right inferior temporal and supramarginal gyri.
Fig. 3.4 White matter volume in 22q11DS subjects vs. controls (not controlled for IQ). Volumetric excesses in 22q11DS (red/yellow). Volumetric deficits in 22q11DS (blue/purple). Ascending transverse sections; (reversed where L=R, R=L)
Table 3.6 VBM analysis of white matter volume of 22q11DS subjects vs. controls (cluster significance threshold $p = 0.006$) – Not controlled for IQ

<table>
<thead>
<tr>
<th>Cluster Size (Number of Voxels)</th>
<th>Talairach and Tournoux Coordinates</th>
<th>Region</th>
<th>Hemisphere</th>
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<tbody>
<tr>
<td></td>
<td>$x$  $y$  $z$</td>
<td></td>
<td></td>
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<tr>
<td><strong>White matter volume deficits in 22q11DS subjects</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4218</td>
<td>1  -36  -11</td>
<td>Brainstem/cerebellum</td>
<td>Right</td>
</tr>
<tr>
<td>149</td>
<td>34  -19  -9</td>
<td>Hippocampus</td>
<td>Right</td>
</tr>
<tr>
<td>77</td>
<td>-33  -35  -2</td>
<td>Hippocampus</td>
<td>Left</td>
</tr>
<tr>
<td>533</td>
<td>28  -71  6</td>
<td>Middle occipital gyrus/optic radiation and lingual, middle and inferior occipital gyrus/cuneus and precuneus</td>
<td>Right</td>
</tr>
<tr>
<td>688</td>
<td>8   -11  23</td>
<td>Body, genu and splenium of corpus callosum</td>
<td>Interhemispheric</td>
</tr>
<tr>
<td>489</td>
<td>-12  -17  52</td>
<td>Medial frontal gyrus/paracentral lobule</td>
<td>Left</td>
</tr>
<tr>
<td><strong>White matter volume excesses in 22q11DS subjects</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>133</td>
<td>-8   28  -15</td>
<td>Corpus callosum/cingulum/medial frontal gyrus</td>
<td>Left</td>
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<tr>
<td>459</td>
<td>-13</td>
<td>5</td>
<td>1</td>
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<tr>
<td>396</td>
<td>20</td>
<td>1</td>
<td>3</td>
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<tr>
<td>341</td>
<td>2</td>
<td>-13</td>
<td>25</td>
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<td><strong>Right</strong></td>
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<td></td>
<td></td>
<td></td>
<td><strong>Interhemispheric</strong></td>
</tr>
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</table>
Fig. 3.5 White matter volume in 22q11DS subjects vs. controls (controlled for IQ). Significant volumetric excesses in 22q11DS (red/yellow). Ascending transverse sections: (reversed where L=R, R=L)
Table 3.7 VBM analysis of white matter volume of 22q11DS subjects vs. controls (cluster significance threshold p = 0.006) – Controlled for IQ

<table>
<thead>
<tr>
<th>Cluster Size (Number of Voxels)</th>
<th>Talairach and Tournoux Coordinates</th>
<th>Region</th>
<th>Hemisphere</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>x</td>
<td>y</td>
<td>z</td>
</tr>
<tr>
<td><strong>White matter volume excesses in 22q11DS subjects; no deficits found</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>108</td>
<td>3</td>
<td>-19</td>
<td>-23</td>
</tr>
<tr>
<td>602</td>
<td>-17</td>
<td>-12</td>
<td>7</td>
</tr>
<tr>
<td>771</td>
<td>23</td>
<td>-13</td>
<td>8</td>
</tr>
<tr>
<td>210</td>
<td>22</td>
<td>-75</td>
<td>19</td>
</tr>
<tr>
<td>85</td>
<td>-36</td>
<td>-2</td>
<td>30</td>
</tr>
</tbody>
</table>
Fig. 3.6 White matter volume deficits within 22q11DS group in subjects with Val-COMT relative to Met-COMT. Ascending transverse sections; (reversed where L=R, R=L)
Table 3.8  White matter volume reduction of 22q11DS subjects with Val-COMT relative to Met-COMT (cluster significance threshold p = 0.005)

<table>
<thead>
<tr>
<th>Cluster Size (Number of Voxels)</th>
<th>Talairach and Tournoux Coordinates</th>
<th>Region</th>
<th>Hemisphere</th>
</tr>
</thead>
<tbody>
<tr>
<td>212</td>
<td>-36  -35  -2</td>
<td>Hippocampus and superior/middle/inferior temporal gyri</td>
<td>Left</td>
</tr>
<tr>
<td>374</td>
<td>7  20  6</td>
<td>Anterior limb of internal capsule</td>
<td>Right</td>
</tr>
<tr>
<td>329</td>
<td>25  -2  11</td>
<td>Retrolenticular limb of internal capsule</td>
<td>Right</td>
</tr>
<tr>
<td>4924</td>
<td>8  -14  28</td>
<td>Cingulum and corpus callosum</td>
<td>Bilateral</td>
</tr>
<tr>
<td>213</td>
<td>30  -36  31</td>
<td>Supramarginal gyrus</td>
<td>Right</td>
</tr>
</tbody>
</table>
3.6. Discussion

In this cross sectional study, measures of white matter microstructural integrity and volume using DT-MRI voxel-based analyses and VBM respectively were compared in young people with 22q11DS and healthy controls. Also, within 22q11DS, a preliminary analysis of the relationship between white matter abnormalities and schizotypy, and with variation in COMT genotype was performed.

In young people with 22q11DS, reduced FA was found in numerous brain regions as compared to controls. However, and consistent with previous DT-MRI studies (Barnea-Goraly et al., 2003; Simon et al., 2005), these predominantly affected the parietal lobe. When IQ was included as a covariate in the analysis, FA deficits in the corpus callosum, internal capsule, arcuate fasciculus and thalamic radiations remained significant. It has been suggested that a reduction in FA is caused by damage to highly-aligned axonal structures or replacement of axonal fibres with less tightly organised cells (Horsfield and Jones, 2002). Therefore these differences in FA may be associated with impaired ‘connectivity’ in a number of neural systems, and confirm earlier reports of widespread deficits in microstructural integrity in 22q11DS.

Higher FA was also found in 22q11DS relative to controls (prior to controlling for IQ) and localised to regions distinct from the FA reductions described above. These occurred exclusively in the left hemisphere and particularly in the left internal capsule. Higher FA has previously been reported (Barnea-Goraly et al., 2003; Simon et al., 2005) but it has been suggested that higher FA reported by others in 22q11DS
may be artefactual - because 22q11DS brains are abnormally shaped and so may not register perfectly to the brain template that is typically used in imaging studies (e.g. healthy child or adult templates) during the normalisation step in SPM. As noted in the methods section (see Chapter 2, Section 2.8.), this potential confound was addressed by employing a customised FA brain template derived from all the subjects under study, aiming to minimise both the degree of warping needed for any individual subject, and bias in such warping between the groups. However, increased FA did not remain significant in 22q11DS when IQ was included as a covariate, although this may simply reflect a loss of sensitivity (i.e. a Type 2 error) due to the inclusion of two strongly correlated variables (IQ and group) in the analysis (Miller and Chapman, 2001). Compared to previous DT-MRI reports in 22q11DS, the current study is the first to covary for IQ, and this combined with template considerations (i.e. use of a study-specific template), may have resulted in findings differing from other work.

In the VBM analyses of white matter proportion, regional increases and decreases of volume were found within young people with 22q11DS prior to controlling for IQ. A bilateral reduction in white matter was found in regions such as the cerebellum, corpus callosum, and occipital and medial temporal lobes, consistent with prior reports (Campbell et al., 2006; Eliez et al., 2000; Kates et al., 2001; van Amelsvoort et al., 2001; van Amelsvoort et al., 2004). Although, these findings did not remain significant when covaried for IQ, increased white matter proportion bilaterally in regions close to the midline cerebral structures did; and particularly in the posterior limb of the internal capsule and superior corona radiata. Unilateral increases were also found in the right posterior corona radiata and left pre-central gyrus. The finding
of increased white matter volume in the frontal lobe contrasts with a previous study which concurrently examined white matter FA and volume in 22q11DS (Simon et al., 2005) that reported right middle frontal gyrus volume reduction but supports the finding of relatively increased frontal white matter volume in other studies of 22q11DS (Antshel et al., 2008; Eliez et al., 2000).

Overall, a number of findings for FA and volume were not co-located after IQ was covaried for e.g. reduced FA in the corpus callosum where reduction in white matter volume was absent. Therefore in such regions, the differences in FA cannot be accounted for simply by differences in white matter volume (and vice versa). However, there were also regions where reduced FA but increased volume both occurred for instance, in the frontal lobes. It is possible that given 22q11DS is associated with cortical dysgenesis, an increased number of ectopic neurones in white matter may account for a reduced FA in association with a relatively increased volume that may be attributed to a degradation of white matter with loss of directional organisation while cell density is relatively preserved or increased (Trivedi et al., 2006; Wieshmann et al., 1999). Therefore, (wherever possible) both conventional MRI and DT-MRI should be used together to investigate white matter pathology given that the latter may be able to detect abnormalities in normal appearing white matter on conventional MRI (Makki et al., 2007; Rugg-Gunn et al., 2001).

As some of the differences found in white matter microstructural integrity may affect behaviour and/or risk for developing psychosis, the relationship between schizotypy and white matter FA in brain regions which differed significantly between young
people with 22q11DS and controls was investigated for the first time. A significant negative correlation was found between increased schizotypy scores and reduced FA in the clusters that encompassed white matter of the right posterior limb of internal capsule and the right body and left splenium of corpus callosum as well as cingulum, inferior fronto-occipital and inferior longitudinal fasciculi within young people with 22q11DS. These white matter tracts may be relevant to the non-deleted general population as for example the internal capsule contains reciprocal white matter fibres from the thalamus to the cerebral cortex and FA deficits in the corpus callosum, cingulum, internal capsule and white matter fibres between the frontal and temporal lobes have been previously reported in schizophrenia (Buchsbaum et al., 2006; Mitelman et al., 2007). The pilot evidence from this study provides tentative support for the suggestion that microstructural abnormalities in these white matter tracts may partially explain some schizotypic behaviours in 22q11DS but these results however require replication in future studies.

A further preliminary investigation was conducted on the effect of COMT polymorphism within 22q11DS youngsters where Val-COMT was found to be associated with significant reduction in local white matter volume; and perhaps especially in the frontal lobes, cingulum and corpus callosum. Similarly, preliminary evidence was also found for decreased FA in these same regions. As COMT activity is largely responsible for dopamine modulation in the PFC (Tunbridge et al., 2004), haploinsufficiency of the COMT gene means that individuals with 22q11DS are exposed to high levels of prefrontal dopamine (Gothelf et al., 2008). COMT polymorphism is known to affect global brain development in both healthy adults (Zinkstok et al., 2006) and adults with 22q11DS (van Amelsvoort et al., 2008) where
the latter whole-brain investigation of 22q11DS adults reported that variation in
COMT activity not only affects the anatomy of the frontal lobes but also a number of
non-frontal regions. Most studies so far in children and adolescents have only
assessed the PFC (Gothelf et al., 2005; Kates et al., 2006) and the results from the
current study involving children and adolescents with 22q11DS demonstrate the
widespread effects of COMT polymorphism that affects both the volume and
microstructural integrity of white matter which extends beyond frontal lobe anatomy.

3.7. Limitations and Conclusions

This study has a number of methodological considerations including, the sample
size, cross-sectional design, lack of an FSIQ-matched control group, and the multiple
comparisons carried out (and therefore the increased risk for Type 1 error). However,
a learning disabled control group was deliberately not included as this study was
designed to address the question how people with 22q11DS differ from those with
healthy brain development. It would be virtually impossible to recruit a ‘perfect’
control group of non-22q11DS controls with the same degree of learning disability
as the 22q11DS probands. With regard to genetics, only COMT was investigated
because it modulates both brain maturation and psychosis although a number of
other important genes that impact on brain development are also deleted in the
22q11.2 region e.g. TBX-1 and PRODH. While this experimental study has a
number of weaknesses, it does however have some strengths. For instance a
customised FA brain template was used in order to reduce registration errors, and the
level of significance was adapted in order to yield less than one false-positive cluster
over the entire imaging volume investigated. Therefore, Type I errors are unlikely to fully explain the results. Further, attempts were made to recruit a relatively homogeneous cohort of children and adolescents that was relatively free from psychiatric comorbidity which would otherwise confound results and misattribute any potential differences found.

In conclusion, this study demonstrates that people with 22q11DS have widespread differences in both the microstructural integrity and volume of white matter that were found in largely independent regions. Although white matter volume may appear relatively normal as measured using standard MRI techniques, this does not necessarily indicate that the underlying white matter microstructural integrity is similarly unaffected. Further studies on the relationship of white matter differences to the behavioural phenotype of 22q11DS and to genetic variation are required.

While in the present chapter white matter is exclusively assessed in a genetic disorder associated with high rates of psychosis using both VBM and voxel-based analyses of conventional MRI and DT-MRI respectively, in the next chapter, an organic disorder (TLE) that is also associated with high rates of psychotic disorders is investigated through the acquisition of structural MRI datasets whereby neuroimaging analyses are expanded to include both grey and white matter compartments as assessed by VBM.
Chapter 4:

Study 2
Neuroanatomical correlates of psychosis in temporal lobe epilepsy: a voxel-based morphometry study

This chapter examines a cohort of individuals all of whom have a neurological diagnosis of TLE. The aim is to specifically assess the neuroanatomical differences in those with TLE with comorbid psychosis relative to those with TLE only through VBM techniques applied to conventional MRI datasets. As TLE contributes significantly to the development of psychiatric disorder and in particular psychosis, potential regions within the temporal lobe and beyond are explored that may be driving such disorder.
4.1 Summary of chapter

Although TLE is associated with a significant risk of psychosis, there are only limited studies that have investigated the underlying neurobiology contributing to the development of psychosis. Therefore, the aim of this chapter is to characterise the neuroanatomical changes in TLE and comorbid psychosis. The study population comprised all individuals with TLE on the epilepsy database at the National Centre for Epilepsy and Epilepsy Neurosurgery in Ireland (Beaumont Hospital) between 2002 and 2006. 10 people with TLE+psychosis were matched for age, gender, handedness, epilepsy duration, seizure laterality, severity of epilepsy and antiepileptic medication with 10 comparison participants with TLE only. Participants received a standardised conventional MRI brain scan based on an epilepsy acquisition protocol and VBM analyses were applied to grey and white matter anatomy.

Significant grey matter reduction was found bilaterally in those with TLE+psychosis in the temporal lobes in the inferior, middle and superior temporal gyri and fusiform gyri, and unilaterally in the left parahippocampal gyrus and hippocampus. Significant extratemporal grey matter reduction was found bilaterally in the insula, cerebellum, caudate nuclei and in the right cingulum and left inferior parietal lobule. Additionally, significant white matter reduction in those with TLE+psychosis was found bilaterally in the hippocampus, parahippocampal/fusiform gyri, middle/inferior temporal gyri, cingulum, corpus callosum, posterior thalamic radiation, anterior limb of internal capsule and white matter fibres from the caudate
nuclei, and unilaterally in the left lingual gyrus and right midbrain and superior temporal gyrus.

Overall, significant grey and white matter deficits occur in TLE+psychosis. These encompass the medial temporal lobe structures but also extend to lateral temporal and extratemporal regions. Some of these deficits overlap with those also found in schizophrenia.

4.2 Introduction

TLE carries a substantial risk of psychosis which is several times greater than that seen in the general population. However, although TLE represents one of the highest known risk factors for the development of psychosis, very little is known about the neurobiology of TLE+psychosis. Given that TLE+psychosis presents with a phenotype that resembles schizophrenia and that these conditions also share neurodevelopmental pathways, studying TLE+psychosis provides an opportunity to understand the evolution of psychosis in both the epileptic and non-epileptic populations. Currently, MRI methods have the potential to improve the understanding of pathogenesis of such disorder.

While there have been several neuroimaging studies in schizophrenia, there have only been a limited number of MRI studies that have assessed regional volumetric differences in subjects with TLE+psychosis and so far, most studies have been based
on manual volumetry. Indeed, three previous studies used manual ROI volumetry to examine subjects with TLE+psychosis. Overall, there have been a variety of findings which have included reduction of volume in the temporal, frontal and parietal lobes and grey matter volumes in the superior temporal gyrus and left hippocampus as well as bilateral amygdala enlargement (Marchetti et al., 2003; Marsh et al., 2001; Tebartz Van Elst et al., 2002). The findings from each of these studies are summarised next while an expanded description is provided in Section 4.6.2.

In the earliest manual volumetry study of TLE+psychosis, Marsh et al. (2001) provided evidence of ventricular enlargement and smaller temporal, frontal and parietal lobes and superior temporal gyrus grey matter volumes in TLE, TLE+psychosis and schizophrenia where TLE+psychosis was associated with the most prominent changes. Subsequently, Tebartz Van Elst and colleagues (2002) found that patients with TLE+psychosis had smaller total brain volumes than TLE alone and healthy volunteers, but reported no group differences in hippocampal volumes; additionally, bilateral amygdala enlargement was observed in TLE+psychosis. Marchetti and associates (2003) in their manual volumetry study demonstrated left lateralised hippocampal volume deficits in patients with TLE+psychosis.

As manual volumetry is associated with difficulties in reproducibility (see Chapter 1, Section 1.1.2.1.), a more recent study attempted to overcome such issues by using automated whole-brain VBM. However, this study only limited assessment to grey matter where no cortical differences were observed between groups (Rusch et al.,
2004). Overall, the neuroanatomical literature suggests that medial temporal lobe structures are implicated in TLE+psychosis although results are not entirely consistent and involvement of regional brain volumes and laterality remain uncertain. Also, although reports thus far have inconsistently reported neuropathology in several brain regions and the application of ROI volumetry to investigate structural abnormalities in patients with TLE is difficult to reproduce and time consuming (Keller et al., 2002a), the application of unbiased, automated, quantitative voxel-based techniques in the study of TLE+psychosis to encompass both grey and white matter measures on a whole-brain level has yet to be performed.

4.3 Aims, objectives and hypotheses

This study aims to assess a cohort of adults with TLE and to characterise global and regional grey and white matter volume differences in those who have comorbid psychosis.

The objectives of this chapter include the following:

- Assessment of a cohort of adults with epilepsy
- Identification of patients who have a neurological diagnosis consistent with TLE based on seizure semiology, EEG and neuroimaging data
- Of those with TLE, to establish patients who develop psychosis
• Characterisation of the neuroanatomical differences in those with TLE with comorbid psychosis versus those with TLE only

• These differences are characterised through analysis of the whole brain using VBM to encompass both grey and white matter volume measures

As this is the first study of its kind, VBM was considered the most appropriate because it permits a hypothesis-free survey of the entire brain. This may subsequently allow for hypotheses generation and areas to be investigated in future studies or the further application of advanced neuroimaging techniques. Given previous findings with manual volumetry, I hypothesised in the current study that individuals with TLE+psychosis when compared to those with TLE but without psychiatric disorder demonstrate: 1) reduction in total brain and grey and white matter content; 2) grey and white matter reduction in the temporal lobe; and 3) structural differences that overlap with those found in schizophrenia.

4.4 Methods

The following subsections detail the study setting as well as participants. Further, the diagnostic and exclusion criteria based on neurological and psychiatric parameters are provided. Details of 1:1 subject matching in order to control for potential confounders are also given.
4.4.1 Study population

The study was carried out at the National Centre for Epilepsy and Epilepsy Neurosurgery, Beaumont Hospital, Dublin, Ireland. A retrospective approach was used to identify patients with TLE and ethics approval was provided by the Beaumont Hospital medical research ethics committee (Protocol Number: 04/55). The study population included all patients on the hospital’s research and clinical epilepsy database. The database represents ongoing efforts to develop a register of patients with epilepsy within the Irish Republic (more details available at http://www.epilepsyprogramme.ie). In total, there were 860 patients on the database attending the inpatient and outpatient neurology service at Beaumont Hospital between 2002 and 2006. As the centre has an epilepsy surgery programme where appropriate candidates receive presurgical evaluation, the epilepsy population under study represents a combination of medically managed and those with medically intractable or surgically remediable epilepsies.

4.4.2 Subjects

A combination of seizure semiology, EEG, video-EEG telemetry and neuroimaging data was examined for the 860 patients on the epilepsy database to identify potential subjects. A diagnosis of epilepsy was based on the International League Against
Chapter 4: Study 2 - Neuroanatomical correlates of psychosis in temporal lobe epilepsy: a voxel-based morphometry study

Epilepsy classification system (1989) and the study participants have already participated in prior studies (Kinirons et al., 2006; Ronan et al., 2006; Ronan et al., 2007) and had consented to further assessment. The neurology service routinely refers people with epilepsy and suspected psychiatric disorder for neuropsychiatric assessment and these assessments are comprehensive, documented in detail and include diagnoses/neuropsychiatric formulation based on the ICD-10 classification system (World Health Organization, 1993).

Exclusion criteria for participation were a clinically detectable medical disorder known to affect gross brain structure (e.g. tumour, haemorrhage), pervasive developmental disorders (e.g. autism spectrum disorder), patients with an extratemporal epileptic focus, unclassified seizures, an IQ < 70 on Wechsler Adult Intelligence Scale - Revised (WAIS-R) (Wechsler, 1981), age < 18 years, previous neurosurgery, non-right-handedness and individuals with contraindications to MRI scanning or no suitable MRI scan. As software on the MRI scanner was upgraded in 2002, people scanned prior to this date were excluded to ensure homogeneity of scan parameters.

4.4.2.1 TLE+psychosis cases

The clinical syndrome of interest was defined as complex partial seizures with clinical findings and investigations (EEG and MRI) compatible with a diagnosis of
TLE. Further, the presence of delusions and/or hallucinations resulting in an ICD-10 diagnosis for psychosis was essential. Acute confusional states or depressive symptomatology alone were not deemed sufficient. Patients with a drug-induced psychosis or episodes of psychosis provoked by excessive alcohol consumption or those representing complex partial status were also excluded.

Of the 860 individuals with epilepsy identified on the database, 280 had a diagnosis of TLE of whom 26 had been diagnosed with psychosis by the neuropsychiatric service. However, of these 26 with TLE+psychosis, 7 had previous neurosurgery for medically intractable seizures, 2 had an existing tumour, 1 was left-handed, 4 had an MRI scan at another institution either in Ireland or abroad and 2 had incomplete MRI scans. Consequently, 10 individuals with TLE+psychosis were considered suitable for participation in the study. Fig. 4.1 below summarises patient selection.

4.4.2.2 TLE only control group

Using the Beaumont Hospital research and clinical epilepsy database, each TLE+psychosis case included in the study was then matched for age (SD ± 5 years), gender, handedness, epilepsy duration, seizure laterality, severity of epilepsy and antiepileptic medication with a patient with TLE, but no psychosis who was attending the same neurology service. None of the comparison group had a lifetime history of prior psychosis and all were free of comorbid psychiatric disorder in the preceding year.
Fig. 4.1 Summary of patient selection
4.4.2.3 Chart review

Chart reviews were conducted for the 10 TLE+psychosis cases and 10 TLE only subjects. The clinical assessments of the 10 TLE+psychosis subjects completed by the specialised neuropsychiatry service was objectively assessed using the Operational Criteria Checklist for Psychotic Illness (OPCRIT) (McGuffin et al., 1991). OPCRIT was used as it offers a polydiagnostic classification system that yields operationally defined psychiatric diagnoses with good reliability (Williams et al., 1996).

All cases had to fulfil ICD-10 criteria for psychotic disorder which encompassed schizophrenia, persistent delusional disorder or other non-organic psychotic disorder; affective psychoses were excluded. With regard to timing of psychoses relative to seizure events, postictal psychoses were included where MRI and neuropsychiatric assessments were performed within a week of psychotic episodes while interictal psychoses were included where the same assessments were completed within a month of commencement of psychosis in the absence of antecedent seizure activity. Neither cases nor controls had features of an intellectual disability, non-epileptic seizures or a history of poor compliance with their antiepileptic medication. Refer to Tables 4.1 and 4.2 for a summary of patient characteristics.
### Table 4.1 Characteristics of cohort: TLE+psychosis vs. TLE only

<table>
<thead>
<tr>
<th></th>
<th>TLE+psychosis (n = 10)</th>
<th>TLE only (n = 10)</th>
<th>p²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age, years (SD)</td>
<td>35 (± 5.2)</td>
<td>33 (± 6.1)</td>
<td>0.36</td>
</tr>
<tr>
<td>Male/Female</td>
<td>7/3</td>
<td>7/3</td>
<td></td>
</tr>
<tr>
<td>Handedness</td>
<td>All right handed</td>
<td>All right handed</td>
<td></td>
</tr>
<tr>
<td>Mean age at diagnosis of epilepsy, years (SD)</td>
<td>12 (± 11.0)</td>
<td>16 (± 8.2)</td>
<td>0.41</td>
</tr>
<tr>
<td>Mean epilepsy duration, years (SD)</td>
<td>23 (± 12.4)</td>
<td>17 (± 8.6)</td>
<td>0.20</td>
</tr>
<tr>
<td>Site of epileptic focus</td>
<td>6 right sided</td>
<td>6 right sided</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4 left sided</td>
<td>4 left sided</td>
<td></td>
</tr>
<tr>
<td>Lesional versus non-lesional</td>
<td>6 mesial temporal sclerosis (4 right and 2 left) vs. 4 non-lesional</td>
<td>5 mesial temporal sclerosis (4 right and 1 left) vs. 5 non-lesional</td>
<td></td>
</tr>
<tr>
<td>Mean age at first psychotic event, years (SD)</td>
<td>30 (± 5.6)</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Mean duration of epilepsy at time of first psychotic event, years (SD)</td>
<td>18 (± 9.9)</td>
<td>N/A</td>
<td></td>
</tr>
</tbody>
</table>

a. All values represent t-tests
Table 4.2 Clinical characteristics and prescribed antipsychotic and antiepileptic medication in TLE+psychosis and antiepileptic medication in TLE only groups

<table>
<thead>
<tr>
<th>Patient</th>
<th>Timing of psychosis relative to seizure event</th>
<th>Antipsychotic medication</th>
<th>Daily dosage</th>
<th>Epilepsy clinical data</th>
<th>TLE+psychosis patient antiepileptic medication</th>
<th>Matched TLE only control antiepileptic medication</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Interictal</td>
<td>Haloperidol</td>
<td>20mg</td>
<td>1/week</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>2</td>
<td>Postictal</td>
<td>Amisulpride</td>
<td>800mg</td>
<td>1/month</td>
<td>N</td>
<td>Y</td>
</tr>
<tr>
<td>3</td>
<td>Interictal</td>
<td>Risperidone</td>
<td>6mg</td>
<td>1/week</td>
<td>N</td>
<td>Y</td>
</tr>
<tr>
<td>4</td>
<td>Interictal</td>
<td>Haloperidol</td>
<td>2mg</td>
<td>2/day</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>5</td>
<td>Postictal</td>
<td>Olanzapine</td>
<td>5mg</td>
<td>1/day</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>6</td>
<td>Postictal</td>
<td>Thioridazine/Olanzapine</td>
<td>400mg/30mg</td>
<td>1/day</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>7</td>
<td>Postictal</td>
<td>Haloperidol/Olanzapine</td>
<td>5mg/20mg</td>
<td>1/2weeks</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>8</td>
<td>Postictal</td>
<td>Olanzapine</td>
<td>20mg</td>
<td>3-4/week</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td></td>
<td>Interictal</td>
<td>Olanzapine</td>
<td>20mg</td>
<td>1/month</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>---</td>
<td>------------</td>
<td>------------</td>
<td>------</td>
<td>---------</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>9</td>
<td>Interictal</td>
<td>Olanzapine</td>
<td>12.5mg</td>
<td>2-3/week</td>
<td>Y</td>
<td>Y</td>
</tr>
</tbody>
</table>

CPS - complex partial seizures; SG - secondary generalisation; Refract - refractory to medical treatment
4.4.3 MRI brain acquisition

Each subject had a volumetric SPGR MRI brain scan at the Beaumont Hospital 1.5 Tesla scanner (GE Signa Systems, Paris) as per details in Chapter 2, Section 2.5.

4.4.4 MRI brain analysis with VBM

Each scan was checked for movement artefact and corruption prior to inclusion in the image processing pipeline. In brief, VBM was used which offers an unbiased and fully automated whole brain measurement technique that normalises all the images to the same stereotactic space and subsequently segments, modulates and smoothes images and finally, a non-parametric statistical analysis is performed on the smoothed images to localise, and make inferences about group differences (as described in more detail in Chapter 2, Section 2.6.).

Smoothing the data in order to coerce it into the appropriate statistical distribution is a prerequisite for some analytical approaches, but is not necessary for the non-parametric approach used in this study (see Chapter 2, Section 2.6.). However, smoothing was primarily applied to reduce confounds due to individual variation in neuroanatomy and to enhance between-subject anatomical matching and the signal-to-noise ratio (Ashburner and Friston, 2000; Mechelli et al., 2005). While the degree of smoothing to apply is still a subject of much discussion as different smoothing
levels may alter findings, a smoothing filter was applied (Gaussian, 8mm full-width at half maximum) where the literature suggests it would aid the detection of potentially widespread changes in the neocortex and also changes in smaller subcortical structures (Keller and Roberts, 2008).

Additionally, total global, grey matter and white matter volumes were extracted via SPM5 and between group differences for these global measures were compared using non-parametric Mann-Whitney U tests. However, between-group differences in regional grey and white matter content were estimated by fitting an ANCOVA model at each intracerebral voxel in standard space using XBAMM where proportional volume for each tissue class (grey or white matter) was the dependent variable and group classification as the key predictor variable. Although total brain volume approached but did not achieve statistical significance (Tables 4.3 and 4.4), it was entered as a covariate because both grey and white matter compartments were investigated in this study. However, while epilepsy duration is related to severity of epilepsy, it was not entered as a covariate as it did not demonstrate a trend nor achieve statistical significance. The statistical threshold for cluster significance was then adjusted such that the expected number of false positive clusters arising by chance alone would be less than one over the whole imaging volume; consequently, \( p = 0.002 \) and \( p = 0.006 \) were respectively applied for grey and white matter VBM analyses (as per Chapter 2, Section 2.6.2.).
4.5 Results

The sections below detail the results of the analyses performed including tissue class volumes (total brain as well as total grey and white matter) and VBM of grey and white matter tissue content.

4.5.1 Total tissue volumes

There were no significant differences in median total global, grey or white matter volumes between the groups at the \( p = 0.05 \) level although there was an approximate reduction of 6, 5 and 7\% respectively in these tissue classes in the TLE+psychosis group (see Table 4.4).
Table 4.3 Total volume of tissue classes in TLE+psychosis subjects and TLE only controls

<table>
<thead>
<tr>
<th>Patient / Control P</th>
<th></th>
<th>Total grey matter volume</th>
<th></th>
<th>Total white matter volume</th>
<th></th>
<th>Total brain volume</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P</td>
<td>C</td>
<td>P</td>
<td>C</td>
<td>P</td>
<td>C</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>707.97</td>
<td>664.94</td>
<td>476.40</td>
<td>468.99</td>
<td>1184.37</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>683.49</td>
<td>570.78</td>
<td>437.52</td>
<td>376.15</td>
<td>1121.01</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>534.56</td>
<td>756.31</td>
<td>328.06</td>
<td>513.28</td>
<td>862.62</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>613.37</td>
<td>741.99</td>
<td>397.63</td>
<td>629.56</td>
<td>1011.00</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>714.13</td>
<td>734.66</td>
<td>447.12</td>
<td>462.43</td>
<td>1161.25</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>661.14</td>
<td>730.56</td>
<td>463.02</td>
<td>457.93</td>
<td>1124.17</td>
</tr>
<tr>
<td>7</td>
<td>7</td>
<td>744.95</td>
<td>714.65</td>
<td>508.20</td>
<td>512.68</td>
<td>1253.15</td>
</tr>
<tr>
<td>8</td>
<td>8</td>
<td>525.94</td>
<td>675.68</td>
<td>368.74</td>
<td>431.70</td>
<td>894.68</td>
</tr>
<tr>
<td>9</td>
<td>9</td>
<td>713.70</td>
<td>750.55</td>
<td>510.49</td>
<td>526.36</td>
<td>1224.19</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>691.55</td>
<td>713.43</td>
<td>470.23</td>
<td>516.67</td>
<td>1161.78</td>
</tr>
</tbody>
</table>
### Table 4.4 Median total volume of tissue classes in TLE+psychosis vs. TLE only

<table>
<thead>
<tr>
<th></th>
<th>TLE+psychosis, median (SD)</th>
<th>TLE only, median (SD)</th>
<th>p^a</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total brain volume (ml)</strong></td>
<td>1142.7 (± 133.8)</td>
<td>1212.1 (± 114.9)</td>
<td>0.07</td>
</tr>
<tr>
<td><strong>Total grey matter volume (ml)</strong></td>
<td>687.5 (± 76.6)</td>
<td>722.6 (± 56.0)</td>
<td>0.08</td>
</tr>
<tr>
<td><strong>Total white matter volume (ml)</strong></td>
<td>455.1 (± 59.5)</td>
<td>490.8 (± 67.6)</td>
<td>0.11</td>
</tr>
</tbody>
</table>

a. All values represent asymptotic significance (2-tailed) Mann-Whitney U tests
4.5.2 VBM of grey and white matter content

Significant regional deficits were found affecting both grey and white matter content but were only confined to subjects with TLE+psychosis. Significant regional grey matter reduction was found unilaterally in the medial temporal lobe structures such as the left parahippocampal gyrus and hippocampus. Deficits also extended to the lateral temporal lobes encompassing the bilateral inferior, middle and superior temporal gyri and inferiorly in the fusiform gyri. However, grey matter reduction was not limited to the temporal lobe structures but also extended to extratemporal regions. The most significant extratemporal deficits were distributed bilaterally and included the insula, cerebellum and caudate nuclei and unilaterally, in the right cingulum and left inferior parietal lobule (see Fig. 4.2).

Significant regional white matter reduction was also found in the TLE+psychosis group. Within the medial temporal lobe, these deficits were distributed bilaterally in the hippocampus and parahippocampal gyrus. White matter deficits were also found in the lateral temporal lobes bilaterally in the middle and inferior temporal gyri and inferiorly in the fusiform gyri while unilateral deficits were found in the right superior temporal gyrus. Reduction of white matter extended beyond the boundaries of the temporal lobes and bilaterally involved the cingulum, corpus callosum (genu, splenium and tapetum), anterior limb of internal capsule, posterior thalamic radiation and white matter fibres from the caudate nuclei while unilateral deficits were found in the left lingual gyrus and right midbrain (Fig. 4.3). Refer to Table 4.5 for a summary of the anatomical locations of regional grey and white matter deficits.
Fig. 4.2 Ascending transverse sections demonstrating regional grey matter reduction (blue) in participants with TLE+psychosis (image is flipped so left is right and right is left)
Fig. 4.3 Ascending transverse sections demonstrating regional white matter reduction (blue) in participants with TLE+psychosis (image is flipped so left is right and right is left)
Table 4.5 Regional grey and white matter reduction in TLE+psychosis versus TLE only

<table>
<thead>
<tr>
<th>Cluster Size (Number of Voxels)</th>
<th>Talairach and Tournoux Coordinates</th>
<th>Region</th>
<th>Hemisphere</th>
</tr>
</thead>
<tbody>
<tr>
<td>1770</td>
<td>-25 -6 -33</td>
<td>Inferior temporal gyrus</td>
<td>Left</td>
</tr>
<tr>
<td></td>
<td>-40 -65 -27</td>
<td>Posterior lobe of cerebellum</td>
<td>Left</td>
</tr>
<tr>
<td></td>
<td>-21 -10 -20</td>
<td>Parahippocampal gyrus</td>
<td>Left</td>
</tr>
<tr>
<td></td>
<td>-37 -34 -15</td>
<td>Fusiform gyrus</td>
<td>Left</td>
</tr>
<tr>
<td></td>
<td>-39 -3 -13</td>
<td>Hippocampus</td>
<td>Left</td>
</tr>
<tr>
<td></td>
<td>-56 -17 -9</td>
<td>Middle temporal gyrus</td>
<td>Left</td>
</tr>
<tr>
<td></td>
<td>475 49 -13</td>
<td>Inferior temporal gyrus</td>
<td>Right</td>
</tr>
<tr>
<td></td>
<td>44 -30 -21</td>
<td>Fusiform gyrus</td>
<td>Right</td>
</tr>
<tr>
<td></td>
<td>41 1 -14</td>
<td>Superior temporal gyrus</td>
<td>Right</td>
</tr>
<tr>
<td></td>
<td>52 12 8</td>
<td>Superior temporal gyrus</td>
<td>Right</td>
</tr>
</tbody>
</table>

Significant grey matter deficits in TLE+psychosis subjects relative to TLE only

(Cluster significance threshold \( p = 0.002 \))
### Significant white matter deficits in TLE+psychosis subjects relative to TLE only

(Cluster significance threshold \( p = 0.006 \))

<table>
<thead>
<tr>
<th>Cluster Size (Number of Voxels)</th>
<th>Talairach and Tournoux Coordinates</th>
<th>Region</th>
<th>Hemisphere</th>
</tr>
</thead>
<tbody>
<tr>
<td>1733</td>
<td>-41 -8 -27</td>
<td>Fusiform gyrus</td>
<td>Left</td>
</tr>
<tr>
<td></td>
<td>-36 -6 -23</td>
<td>Inferior temporal gyrus</td>
<td>Left</td>
</tr>
<tr>
<td></td>
<td>-36 -8 -20</td>
<td>Parahippocampal gyrus</td>
<td>Left</td>
</tr>
<tr>
<td></td>
<td>-36 -18 -13</td>
<td>Hippocampus</td>
<td>Left</td>
</tr>
<tr>
<td>X</td>
<td>Y</td>
<td>Z</td>
<td>Neuron</td>
</tr>
<tr>
<td>------</td>
<td>------</td>
<td>------</td>
<td>-------------------------------</td>
</tr>
<tr>
<td>-44</td>
<td>-7</td>
<td>-10</td>
<td>Middle temporal gyrus</td>
</tr>
<tr>
<td>-29</td>
<td>-50</td>
<td>3</td>
<td>Lingual gyrus</td>
</tr>
<tr>
<td>-13</td>
<td>16</td>
<td>3</td>
<td>Anterior limb of internal capsule</td>
</tr>
<tr>
<td>-33</td>
<td>-43</td>
<td>6</td>
<td>Posterior thalamic radiation</td>
</tr>
<tr>
<td>-14</td>
<td>22</td>
<td>8</td>
<td>Genu of corpus callosum</td>
</tr>
<tr>
<td>-14</td>
<td>16</td>
<td>10</td>
<td>Caudate</td>
</tr>
<tr>
<td>-21</td>
<td>-41</td>
<td>17</td>
<td>Splenium of corpus callosum</td>
</tr>
<tr>
<td>-14</td>
<td>-15</td>
<td>27</td>
<td>Cingulum</td>
</tr>
<tr>
<td>1106</td>
<td>-4</td>
<td>-23</td>
<td>Inferior temporal gyrus</td>
</tr>
<tr>
<td>49</td>
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</tr>
<tr>
<td>32</td>
<td>-16</td>
<td>-9</td>
<td>Hippocampus</td>
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<td>31</td>
<td>-18</td>
<td>-6</td>
<td>Parahippocampal gyrus</td>
</tr>
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<td>41</td>
<td>-27</td>
<td>2</td>
<td>Posterior thalamic radiation</td>
</tr>
<tr>
<td>45</td>
<td>-27</td>
<td>5</td>
<td>Superior temporal gyrus</td>
</tr>
<tr>
<td>22</td>
<td>18</td>
<td>6</td>
<td>Anterior limb of internal capsule</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Anatomical Structure</td>
</tr>
<tr>
<td>---</td>
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<td>---</td>
<td>--------------------------------------</td>
</tr>
<tr>
<td>21</td>
<td>20</td>
<td>14</td>
<td>Genu of corpus callosum</td>
</tr>
<tr>
<td>28</td>
<td>-36</td>
<td>20</td>
<td>Splenium of corpus callosum</td>
</tr>
<tr>
<td>18</td>
<td>-1</td>
<td>26</td>
<td>Cingulum</td>
</tr>
</tbody>
</table>
4.6 Discussion

The discussion below encompasses the main findings from the current experimental study in light of studies investigating schizophrenia. This is then followed by a review of neuroimaging findings from the limited number of studies that have examined TLE+psychosis. Finally, the limitations of the present study are considered.

4.6.1 Main findings

Although Slater and Beard observed the occurrence of schizophrenia-like psychoses in association with epilepsy some 50 years ago, very few MRI studies have been undertaken since then and even fewer have attempted to accurately quantify in-vivo the brain changes seen in the psychoses related to TLE. In this study I conducted the first ever comparison (on a whole-brain level) of grey and white matter tissue classes of a well-matched group of adults with TLE+psychosis versus those with TLE only using unbiased automated voxel-based statistical methods. Each subject had a robust diagnosis of TLE based on clinical, neuroimaging and electrophysiological criteria. Further, cases that had been clinically diagnosed with psychosis were then confirmed objectively via a validated polydiagnostic classification system and were subsequently matched with TLE only controls that were free from psychiatric disorder. MRI scans were based on a standardised epilepsy brain imaging protocol to ensure homogeneity of scan parameters where the timing of MRI scan acquisition was close to the development of psychosis and therefore the relationship between
acute (rather than chronic) psychosis and potential brain changes was explored. Significant temporal and extratemporal lobe deficits were found among those with comorbid psychosis.

Both grey and white matter regional deficits were found in TLE+psychosis compared to TLE only where deficits were mainly localised to the temporal lobe. For example, significant grey matter deficits were found in the medial temporal lobes in the left hippocampus and parahippocampal gyrus. These findings are compatible with the neuroanatomical and neuropsychological deficits described in schizophrenia (Pantelis et al., 2003; Seidman et al., 2003; Wright et al., 2000). However, within the current study, evidence of deficits bilaterally in the lateral temporal lobes and in extratemporal regions were also found perhaps suggesting that the psychosis seen in TLE is the result of more widespread abnormalities.

Strengthening the argument that psychosis in TLE may be a more widely distributed disorder is the finding of grey matter deficits in the cingulum, insula and cerebellum in this study; grey matter reduction in the insula and cingulum have previously been associated with psychosis (Pantelis et al., 2003; Shapleske et al., 2002) while the cerebellum is emerging as an organ not only involved in motor coordination but also in higher cortical functions. Abnormalities in the cerebellum have previously been postulated to result in a ‘cognitive dysmetria’ (Andreasen et al., 1998) characterised by impairments in coordination of the perception, encoding, retrieval and prioritisation of experience and information and may arise from a defect in circuitry connecting the thalamus, frontal cortex and cerebellum. Although cerebellar grey
matter deficits have been previously reported in patients with TLE without comorbid psychiatric disorder (Sandok et al., 2000), given that there are reciprocal neural pathways between the hippocampus and cerebellum, the cerebellum in those with psychosis may perhaps be particularly vulnerable to excitotoxic damage as a result of its connectivity with a pathological hippocampus. Currently, cerebellar abnormalities are also recognised to contribute to the development of schizophrenia (Andreasen and Pierson, 2008; Pantelis et al., 2003) and while evidence of grey matter reduction in the cerebellum was found in the present study, these were not specifically quantified and should be thus considered preliminary.

Pronounced white matter deficits were also found in TLE+psychosis in the current study in regions that included the corpus callosum, hippocampi, cingulum and parahippocampal gyri. Impaired intra and interhemispheric connectivity has been suggested to play a major role in the development of schizophrenia (Hoffman and McGlashan, 1998; Shapleske et al., 2002) and currently there is strong evidence in the literature of widespread altered cortico-cortical and transcallosal connections between homologous brain regions in schizophrenia (Brambilla and Tansella, 2007). Abnormalities in such white matter tracts may also contribute to the development of psychosis seen in TLE. Given the finding in the present study of greater white matter content deficits relative to grey matter in TLE+psychosis, assessment of white matter microstructure and connectivity should therefore be considered in future studies using a combination of VBM and advanced neuroimaging techniques (e.g. DT-MRI) as has been applied in other disorders such as 22q11DS where white matter is preferentially affected (Sundram et al., 2010b).
4.6.2 Findings from other studies

Though the left temporal lobe has been associated with TLE+psychosis in older reports (Currie et al., 1971), the literature is not entirely consistent (Sachdev, 1998) as suboptimal matching may have contributed to this inconsistency. Furthermore, the majority of studies have employed manual ROI volumetric techniques e.g. hand-tracing or stereology that may not be easily reproducible. One such manual volumetry study which assessed TLE with chronic interictal psychosis compared TLE, TLE+psychosis and schizophrenia to healthy controls via hand-tracing methods reported ventricular enlargement and smaller temporal, frontal and parietal lobes and superior temporal gyrus grey matter volumes in all groups with the most pronounced differences being found in the TLE+psychosis group (Marsh et al., 2001). Based on their findings, the authors concluded that cortical grey matter deficits in TLE+psychosis and schizophrenia predispose to chronic psychosis.

In another manual volumetry study using a retrospective patient identification approach, patients with TLE and comorbid psychoses (both postictal and interictal) were reported to have smaller total brain volumes than either TLE alone or healthy volunteers. No group differences were observed in hippocampal volumes although bilateral amygdala enlargement of the order of 16-18% was reported in
TLE+psychosis (Tebartz Van Elst et al., 2002). However, as some of the TLE only subjects were dysthymic, this may have confounded findings.

In a subsequent manual volumetry study based on hand-tracing, hippocampal volume deficit was found in those who had epilepsy and comorbid psychosis relative to healthy controls where the left hippocampus was reported as significantly smaller than the right (Marchetti et al., 2003). However, as the timing of psychosis in relation to epileptic events was unclear and additionally, the epilepsy group contained both individuals with TLE and other partial epilepsies, there was heterogeneity in the patient population examined. Overall manual volumetry based studies of TLE+psychosis have not reported consistent findings due to heterogeneity of populations investigated or methodological issues with manual assessment of brain volumes.

Keller and colleagues (Keller et al., 2002a) argue that there are inherent difficulties with manual volumetry (for instance stereology) that may account for such inconsistent evidence. For example, there is subjectivity associated with point counting on MR images and in judgement of boundaries of structures under investigation (e.g. delineation between hippocampus, white matter and cerebrospinal fluid). These differences in judgement may lead to differing volume estimates between raters. Additionally, the calibration of MR images with settings such as image brightness may influence the perception of brain tissue contrasts. Despite the inconsistencies reported with manual volumetry, automated techniques have only been applied on a limited basis in TLE+psychosis.
In one such automated computerised statistical study (Rusch et al., 2004), the authors retrospectively explored cortical grey matter differences between 26 patients with TLE+psychosis, 24 with TLE only and 20 healthy comparison subjects. This was the same cohort as previously examined by Tebartz Van Elst and colleagues (2002) (Tebartz Van Elst et al., 2002) whereby both postictal and interictal psychoses were investigated; VBM based on SPM99 was used to assess for morphometric differences and no significant cortical grey matter differences between the TLE+psychosis and the TLE only groups were found. However, the TLE only group showed a significant increase in grey matter concentration in the right temporal lobe relative to healthy controls. The authors concluded that since they observed no grey matter volume deficits between TLE+psychosis and the TLE only groups, and since cortical pathology is prominent in schizophrenia (Wright et al., 2000), TLE+psychosis may represent a clinically distinct entity from schizophrenia.

In another VBM analysis, 20 patients with TLE and interictal psychosis were compared with 20 non-psychotic TLE patients where they were matched with respect to conventional MRI findings (Flugel et al., 2006). Global and hippocampal volumes were specifically assessed. No significant differences were found between the psychotic and non-psychotic subjects but significant reductions of magnetization transfer ratio (an index of signal loss derived from magnetization transfer imaging) in the absence of atrophy was found in the left superior and middle temporal gyri in patients with psychosis.
While the finding of cortical grey matter abnormalities in the present study is in keeping with those of Flugel and colleagues (2006), they however contrast with those of Rusch and associates (2004). The sample size in the current study was smaller than that of Rusch and colleagues (26 vs. 10 with TLE+psychosis) but the subjects in the current study were more tightly matched for psychiatric disorder and handedness which may have accounted for differences. Furthermore, Rusch and colleagues utilised a neuroimaging protocol involving a brain template derived from healthy subjects. Given that individual variability of brains in a study population is substantially increased by injury or disease, achieving satisfactory alignment across individual brains may have resulted in registration difficulties in that study. Additionally, in the present study, widespread grey and white matter changes were found that were not confined to the temporal lobes whereby these findings may contrast with previous automated statistical methods as the current approach utilised instead unified segmentation which combined tissue classification, bias correction, and non-linear warping within the same framework (Ashburner and Friston, 2005) and the non-parametric or distribution free hypothesis testing procedure permits the use of cluster level statistics even if their distribution is non-Gaussian (even after smoothing).

4.7 Limitations

There are other methodological considerations and limitations to the present study. Only MRI datasets from 2002 onwards were used so as to ensure homogeneity of
scans while applying strict exclusion criteria for recruitment into the study; while this may have limited the number of subjects and affected the power of this study, there are several other studies that have applied VBM for instance in schizophrenia where significant differences in grey and white matter have been reported using similar sample sizes (Kubicki et al., 2002b; Sowell et al., 2000). Therefore it is unlikely that Type 1 error is fully accounting for findings in the current study.

The clinical psychiatric diagnoses that were obtained through neuropsychiatric assessment were not achieved through formal structured clinical interviews but they however contained comprehensive clinical information that could be retrospectively evaluated through OPCRIT. Future studies investigating TLE+psychosis may wish to consider a methodological design that prospectively examines individuals with TLE through structured clinical interviews e.g. Structured Clinical Interview for DSM-IV (American Psychiatric Association, 2000) and further, to utilise objective rating scales for psychosis e.g. Positive and Negative Syndrome Scale (Kay et al., 1987) or Brief Psychiatric Rating Scale (Overall and Gorham, 1962). However, to recruit reasonable numbers prospectively would take many years; despite the retrospective recruitment in this study, finding appropriate subjects once exclusion criteria have been applied (surgery, tumours, etc.) has left only 10 cases in a four year period and thus poses an important issue in the study of TLE+psychosis.

Moreover, in a recent study, although widespread neocortical abnormalities were found in both TLE with and without mesial temporal sclerosis (MTS), the pattern of thinning in the former contrasted to the latter which led the authors to suggest that
these might constitute two distinct TLE syndromes (Mueller et al., 2009). The authors found that in TLE with MTS, the inferior medial and posterior temporal regions were most prominently affected whereas the lateral temporal and opercular regions were more significantly affected in TLE without MTS. Therefore, future studies may wish to separately characterise the neurobiology of these disorders.

Similarly, while postictal and interictal forms of psychoses occur in TLE, the assessment of these psychoses related to TLE should perhaps be investigated independently in future studies. However, when the above factors are taken together, they may restrict further the overall number of study participants. Therefore, given that it is not uncommon for the postictal form to progress to the interictal variant (Tarulli et al., 2001), assessing both forms of psychoses in the same study may represent a valid approach (Rusch et al., 2004).

Normal controls were not included in the present study as I was primarily interested in the brain changes of TLE specifically associated with psychosis rather than the effects of epilepsy. Further, the absence of a control group is due to the fact that this was a study based on an epilepsy patient database and therefore no non-epilepsy subjects were recruited. However, the lack of a healthy control group limits the extent to which the findings can be interpreted in the context of the general population. The current literature would suggest that TLE patients will have some regional volume loss compared to healthy controls, usually ipsilaterally and predominantly in temporal lobe regions. As tissue loss commonly affects disease groups, and tissue gain would be less likely (apart from for instance a developmental disorder which might be accounted for by 'differential pruning'), in this study, however, clearly both groups are 'disease groups' where tissue loss is most likely
occurring in both groups. Even if the differences seen are being driven by the non-psychosis group (i.e. grey matter increase), this still implies that these regions are somehow affected. It may also reflect that the psychosis group could be lacking a potential 'neuroprotective response' that is preventing the TLE only controls from developing psychosis. Although this is one possibility, another is that SPM99 has been used in previous VBM reports in TLE while I have used SPM5 with better algorithms for tissue classification and bias correction. Indeed, Keller and colleagues (Keller et al., 2002a) suggest that the grey matter concentration excesses they found using SPM99 in their study reflects diminished grey-white matter demarcation, underlying white matter atrophy, or structural displacement due to cerebrospinal fluid expansion. This may also have accounted for an apparent increase in grey matter concentration in TLE relative to healthy controls in the report by Rusch and colleagues (2004).

4.8 Conclusions

Overall, I recruited a population of patients with TLE with strong diagnostic validity (both neurological and psychiatric) and through VBM, I conducted an unbiased computerised statistical analysis of grey and white matter measures. The findings from this study show that subjects with TLE+psychosis have marked cortical, subcortical and extratemporal grey and white matter deficits compared to those with TLE alone and thus provide support for the psychosis literature which also shows this pattern of change. This study has provided evidence allowing specific
hypotheses to be tested in future studies though recruitment of suitable cases to investigate may prove to be an issue. Due to such difficulties, it is important to conduct 'pilot' work such as this to determine very specific hypotheses before attempts are made to recruit even larger cohorts.

The current chapter has focused on assessment of volume in both grey and white matter tissue compartments in a physical disorder significantly associated with psychosis. In the next chapter, a group of adult males with a diagnosis of ASPD is assessed for abnormalities in white matter microstructural integrity and connectivity relative to healthy matched controls using whole-brain voxel-based analyses of FA maps derived from DT-MRI acquired datasets. While this represents a novel investigation, the DT-MRI examination techniques utilised in Chapter 3 are similarly applied but to another cohort.
Chapter 5:

Study 3
White matter microstructural abnormalities in adults with antisocial personality disorder: a preliminary whole-brain diffusion tensor magnetic resonance imaging study

The main aim of this chapter is to compare a cohort of adult males with a diagnosis of ASPD to a matched group of healthy adults. Whole-brain neuroimaging datasets based on DT-MRI are investigated for connectivity differences based on changes in white matter FA. Additionally, objective measures of psychopathy are correlated with any differences in FA found which may be accounting for the disorder. Assessing such changes in white matter on a whole brain level represents a novel investigation and potentially inform on neuroanatomical abnormalities that contribute to the development of such disorder.
5.1. Summary of chapter

ASPD and psychopathy involve significant interpersonal and behavioural impairments. However, little is known about their underlying neurobiology and in particular, abnormalities of white matter microstructure. A preliminary DT-MRI study of adult psychopaths employing tractography of frontal lobe white matter tracts revealed abnormalities in the right uncinate fasciculus (Craig et al., 2009), indicating fronto-limbic disconnectivity. However, it is not clear whether white matter abnormalities are restricted to this tract or are or more widespread and extend beyond the frontal lobe. In this study, whole-brain voxel-based analyses were performed for the first time on white matter FA maps acquired with DT-MRI to compare 15 adults with ASPD to healthy age-, handedness-, and IQ-matched controls. Also, within ASPD subjects, differences in FA were related to objective measures of psychopathy.

Significant white matter FA reduction was found in ASPD subjects relative to controls. In the anterior brain, FA was bilaterally reduced in the genu of corpus callosum while in the right frontal lobe FA reduction was found in the uncinate fasciculus, inferior fronto-occipital fasciculus, anterior corona radiata and anterior limb and genu of the internal capsule. FA reduction was also found in posterior regions of the left hemisphere in the temporo-occipital course of the inferior longitudinal fasciculus and inferior fronto-occipital fasciculus, in addition to the retrolenticular section and posterior thalamic radiation of the internal capsule.
However, only FA differences in anterior brain regions negatively correlated with objective measures of psychopathy.

The present study confirms a previous report of reduced FA in the uncinate fasciculus. Additionally, for the first time, FA deficits are reported in tracts involved in interhemispheric and posterior brain networks as well as frontal lobe connectivity. While FA deficits specifically in the frontal lobe correlated negatively with psychopathy measures, the current study provides evidence of significant white matter microstructural abnormalities not only in frontal brain areas but also interhemispheric and posterior brain regions in ASPD and psychopathy.

5.2. Introduction

While abnormalities in fronto-temporal structure and function have been implicated as contributing to the development of ASPD, other regions potentially giving rise to ASPD or psychopathy have been less extensively investigated. For instance, there have been only limited MRI studies assessing the corpus callosum, a major white matter bundle supporting interhemispheric functional integration, where abnormalities have included increases in volume and length but reduction in thickness (Raine et al., 2003). Further, while reductions in functional connectivity between prefrontal and limbic regions may contribute to antisocial traits (de Oliveira-Souza et al., 2008; Volkow et al., 1995; Yang et al., 2005), their underlying
microstructural basis remains unknown. Overall, neural disconnectivity and the wider network of abnormalities in ASPD/psychopathy have been relatively understudied although such investigation has been made possible using DT-MRI (Basser et al., 1994a). Only one previous study has examined psychopathy using DT-MRI but based on tractography (Craig et al., 2009) (see Chapter 1, Section 1.1.4.1. for more details on in-vivo fibre tractography). The study was confined to a limited number of frontal white matter tracts and therefore it was not possible to assess white matter networks on a whole-brain level. Consequently, the presence of deficits affecting white matter connectivity with other brain regions and impairments in white matter networks extending beyond the frontal lobe is yet to be established in either ASPD or psychopathy.

5.3. Aims, objectives and hypotheses

This study aims to assess a cohort of adult males with ASPD relative to healthy matched controls and to characterise white matter microstructural and connectivity differences. I undertook the first DT-MRI investigation on a whole-brain level of ASPD and psychopathy whereby the objectives of this chapter encompass the:

- recruitment of an adult male cohort with ASPD and a comparison group of healthy matched controls
- screening of all subjects so as to rule out comorbid psychiatric disorder
• analysis of the whole brain rather than limiting assessment to fronto-temporal structures only (as has been previously conducted)

• analysis of white matter using a customised brain template from all subjects under study

• characterisation of differences in white matter integrity and connectivity based on FA maps derived from DT-MRI acquisition

In this experimental chapter I tested the main hypothesis that people with ASPD and psychopathy have significant differences, based on DT-MRI derived measures of FA, in microstructural integrity and connectivity as compared to healthy matched controls. Also, I tested an additional hypothesis that within ASPD, severity of psychopathy (as measured by PCL-R) is related to differences in FA.

5.4. Methods

In the following subsections, details are provided on participant recruitment, screening for psychiatric and neurological disorders, diagnosis of ASPD and correlation of psychopathic traits with differences in FA.

5.4.1. Subjects
Study participants were recruited from three specialist forensic inpatient units in south-east London (South London and Maudsley National Health Service Foundation Trust) and south-west London (St George's Healthcare NHS Trust) as part of longitudinal work in assessing psychopathy, over a period of eight years.

Healthy controls were recruited from the general population through the Institute of Psychiatry, King's College London by advertisement. Ethical approval was obtained from the Ethics Committee of the South London and Maudsley Trust and Institute of Psychiatry, and St George's Healthcare Trust.

Subjects were invited to participate through their local treating teams. If subjects agreed to participate, an information sheet was discussed in person that provided details of the study. Additionally, consent forms were also provided to those interested in participating and written informed consent was obtained from participants after full description of the study. Subjects were subsequently assessed through semi-structured interview at their respective forensic units while these interviews were completed at the Institute of Psychiatry for controls.

Participants in both groups were medication free, spoke English as their first language, and were right-handed as assessed by the Annett Handedness Questionnaire. The WAIS-R (Wechsler, 1981) was used to measure IQ. All participants (in both groups) were examined by formal psychiatric semi-structured interview using ICD-10 research criteria (World Health Organization, 1993) in addition to assessment of case notes for a diagnosis of ASPD. Assessment for the
presence of comorbid psychiatric illness (e.g. anxiety disorders, substance misuse, schizophrenia, major depression), neurological and extracerebral disorders that may affect brain function, and contraindications to MRI scanning was also performed. Though many individuals with ASPD have a past history of alcohol and/or substance misuse, attempts were made to recruit (as far as possible) subjects without comorbidities rather than to control for these post hoc. None of the participants fulfilled criteria for substance misuse or dependence syndrome six months prior to recruitment, with the exception of one subject who had harmful use of cocaine.

45 participants were initially recruited into the study (20 ASPD vs. 25 controls). However, five with ASPD were unsuitable for further assessment following exclusion for comorbid psychiatric disorder and contraindications to MRI procedures while the remaining 15 subjects with ASPD were matched 1:1 with healthy controls for age, IQ and handedness. PCL-R scores were obtained from case notes derived from assessments based at their specialist forensic unit by forensic psychologists fully trained in the administration of the PCL-R, or by the research group where the PCL-R had not been administered but where subjects otherwise met inclusion criteria. Student’s t-tests (two-tailed) were used to compare the distribution of continuous data between the two groups.

Therefore 30 normal intelligence right-handed adult male subjects were included: 15 with ASPD and a mean PCL-R score of 26 (SD ± 7; range 13 - 34) aged 39 ± 10 years and with FSIQ of 92 ± 13, and 15 healthy controls aged 37 ± 11 years, with FSIQ of 99 ± 12. There were no significant differences in age or IQ between
participant groups. Those with ASPD had a history of violent offending that encompassed manslaughter, attempted murder and multiple rape with strangulation. In the UK it is accepted practice to define psychopathy as a score of 25 or above on the PCL–R (Cooke, 1996; Cooke and Michie, 1999) and 10 of the 15 in the ASPD group scored above this threshold. However, while it was possible to obtain total PCL-R scores for the entire patient cohort, it was only possible to acquire Factor 1 and Factor 2 subscores for 12 of the 15 subjects from case notes. Refer to Tables 5.1 and 5.2 for a summary of participant details.
### Table 5.1 Characteristics of cohort: ASPD vs. healthy controls

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>ASPD (n = 15)</th>
<th>Controls (n = 15)</th>
<th>p&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Age (SD)</td>
<td>39 (± 10)</td>
<td>37 (± 11)</td>
<td>0.43</td>
</tr>
<tr>
<td>Age range (years)</td>
<td>21 – 57</td>
<td>20 – 52</td>
<td>-</td>
</tr>
<tr>
<td>Mean FSIQ (SD)</td>
<td>92 (± 13)</td>
<td>99 (± 12)</td>
<td>0.14</td>
</tr>
<tr>
<td>FSIQ range</td>
<td>70 – 118</td>
<td>77 – 117</td>
<td>-</td>
</tr>
<tr>
<td>Handedness</td>
<td>15 Right</td>
<td>15 Right</td>
<td>-</td>
</tr>
<tr>
<td>Mean total PCL-R (SD)</td>
<td>26 (± 7)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

<sup>a</sup> All values represent t-tests
Table 5.2 Clinical and demographic characteristics per ASPD subject

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age</th>
<th>IQ</th>
<th>Total PCL-R score</th>
<th>Factor 1 score</th>
<th>Factor 2 score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>48</td>
<td>83</td>
<td>13</td>
<td>1</td>
<td>9</td>
</tr>
<tr>
<td>2</td>
<td>34</td>
<td>96</td>
<td>34</td>
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<td>3</td>
<td>24</td>
<td>88</td>
<td>26</td>
<td>9</td>
<td>16</td>
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<tr>
<td>4</td>
<td>41</td>
<td>89</td>
<td>25</td>
<td>-</td>
<td>-</td>
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<tr>
<td>5</td>
<td>42</td>
<td>118</td>
<td>16</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>6</td>
<td>39</td>
<td>89</td>
<td>30</td>
<td>16</td>
<td>12</td>
</tr>
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<td>7</td>
<td>39</td>
<td>95</td>
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<td>21</td>
<td>91</td>
<td>28</td>
<td>8</td>
<td>17</td>
</tr>
<tr>
<td>10</td>
<td>48</td>
<td>87</td>
<td>34</td>
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<td>15</td>
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<td>87</td>
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<td>16</td>
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<td>12</td>
<td>43</td>
<td>105</td>
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<td>-</td>
<td>-</td>
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<td>13</td>
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<td>70</td>
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<td>14</td>
<td>57</td>
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<td>13</td>
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<tr>
<td>15</td>
<td>46</td>
<td>74</td>
<td>28</td>
<td>9</td>
<td>15</td>
</tr>
</tbody>
</table>
5.4.2. MRI acquisition Protocol

All participants agreed to undergo DT-MRI scanning and for further examination of their scans. DT-MRI datasets were acquired for all participants as per Chapter 2, Section 2.7.

5.4.3. Pre-processing DT-MRI data

Scans were examined for image corruption or motion artefacts prior to inclusion in the imaging pipeline and none of the acquired scans demonstrated these abnormalities. Details of preparation for DT-MRI voxel-based analyses are provided in Chapter 2, Sections 2.7. and 2.8.

5.4.4. Statistics

In the following subsections, details are provided on the statistical tests used for examining behavioural and demographic data. Descriptions of the DT-MRI statistical comparisons performed on FA maps and post hoc correlations are given in Chapter 2, Sections 2.8.4. and 2.8.5.
5.4.4.1. Demographic and behavioural data

Statistical analysis of these data was performed in SPSS (SPSS 14.0 for Windows, SPSS Inc, Chicago, IL, USA). Student's t-tests ($p = 0.05$, two-tailed) for independent samples were used to examine between-group differences on age and IQ-scores (see Table 5.1).

5.4.4.2. Whole-brain voxel-based analysis of DT-MRI data

The statistical significance of between group differences in FA was examined using non-parametric permutation-based tests based on ANOVA. As there were no differences in the mean values in either age or IQ between groups, and the ranges similar, these were not entered as covariates and so the ANOVA model utilised FA as the dependent variable while group classification was the key predictor variable. Details are found in Chapter 2, in Section 2.8. and in particular, Section 2.8.4. The analysis was corrected for false positives where a cluster significance threshold of $p = 0.0025$ was used.

5.4.4.3. Post hoc analysis of PCL-R scores
As per Chapter 2 (Section 2.8.5.), mask images were created from each of the clusters found by the DT-MRI voxel-based analyses and applied to each subject’s normalised FA images thereby enabling mean FA values to be calculated over each region for each subject. These were then correlated with PCL-R scores (Factor 1: 'emotion dysfunction'; Factor 2: 'antisocial behaviour' and; total PCL-R) using Pearson product-moment correlation coefficients with SPSS 14.0. Significant correlations are reported where a Bonferroni adjusted alpha of 0.025 was applied.

5.5. Results

The following subsections expand on the results from DT-MRI voxel-based analyses of FA maps between groups. Additionally, preliminary post hoc analyses based on PCL-R scores and their correlation with FA differences in only the ASPD group is also provided.

5.5.1. ASPD vs. Controls white matter FA

People with ASPD, relative to controls, had a significant reduction in white matter FA; 1) bilaterally in the frontal lobe in the anterior portion of the corpus callosum (genu); 2) in the right hemisphere, only in anterior regions of the brain and in white
matter tracts that included the genu of corpus callosum, anterior corona radiata and anterior limb and genu of the internal capsule, and frontal course of the uncinate and inferior fronto-occipital fasciculus; 3) in the left hemisphere in both anterior and posterior regions of the brain including respectively the genu of corpus callosum and temporo-occipital course of the inferior longitudinal and inferior fronto-occipital fasciculus, and the retrolenticular part of the internal capsule and posterior thalamic radiation. Refer to Fig. 5.1 and Table 5.3 for cluster localisation of FA deficits.

5.5.2. Within ASPD, correlation of Factor 1, Factor 2 and total PCL-R with differences in white matter FA

In the ASPD group, there were significant correlations between PCL-R scores and white matter FA. Mean FA of the cluster in the frontal lobe (Cluster 2 in Table 5.3) was negatively correlated with Factor 2 ($r = -0.771, p = 0.003, n = 12$) and total PCL-R ($r = -0.685, p = 0.005, n = 15$) scores. There were no significant correlations between the cluster in the temporo-occipital cortex (Cluster 1, Table 5.3) and total or subfactor PCL-R scores. See Figs. 5.2 and 5.3 for correlation outputs.
Fig. 5.1 Reduced FA in ASPD relative to healthy controls [ascending 2mm transverse sections] where the image is displayed such that the subject's right is to the left and left is right.
### Table 5.3 White matter FA deficits in ASPD relative to healthy controls (cluster significance threshold for FA maps $p = 0.0025$)

<table>
<thead>
<tr>
<th>Cluster label</th>
<th>Cluster size (number of voxels)</th>
<th>Talairach and Tournoux coordinates</th>
<th>Tract(s) within cluster</th>
<th>Region</th>
<th>Cluster mean FA ASPD (SD)</th>
<th>Cluster mean FA Control (SD)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cluster 1</td>
<td>504</td>
<td>-36 -42 -6</td>
<td>Temporo-occipital course of left inferior longitudinal fasciculus and inferior fronto-occipital fasciculus</td>
<td>Temporal lobe</td>
<td>0.433 (0.022)</td>
<td>0.466 (0.029)</td>
<td>0.001378</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-32 -59 4</td>
<td>Left posterior thalamic radiation and retrolenticular part of internal capsule</td>
<td>Occipital lobe</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cluster 2</td>
<td>1027</td>
<td>16 47 -8</td>
<td>Right inferior fronto-occipital fasciculus and genu of corpus callosum</td>
<td>Frontal lobe</td>
<td>0.383 (0.020)</td>
<td>0.409 (0.033)</td>
<td>0.000469</td>
</tr>
<tr>
<td></td>
<td></td>
<td>19 30 -8</td>
<td>Right uncinate fasciculus</td>
<td>Frontal lobe</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>-14 36 2</td>
<td>Left genu of corpus callosum</td>
<td>Frontal lobe</td>
<td>0.383 (0.020)</td>
<td>0.409 (0.033)</td>
<td>0.000469</td>
</tr>
<tr>
<td></td>
<td></td>
<td>18 2 6</td>
<td>Right genu of internal capsule</td>
<td>Sublobar</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>18 32 7</td>
<td>Right genu of corpus callosum</td>
<td>Frontal lobe</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>23 11 9</td>
<td>Right anterior limb of internal capsule and anterior corona radiata</td>
<td>Sublobar</td>
<td></td>
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</tbody>
</table>
Chapter 5: Study 3 - White matter microstructural abnormalities in adults with antisocial personality disorder: a preliminary whole-brain diffusion tensor magnetic resonance imaging study

Fig. 5.2 Significant negative correlations between Cluster 2 mean FA and total PCL-R scores

$R^2$ Linear = 0.469
Fig. 5.3 Significant negative correlations between Cluster 2 mean FA and PCL-R Factor 2 scores
DT-MRI was used in conjunction with whole-brain voxel-based analyses of FA maps to compare white matter microstructural integrity of 15 adult males with a diagnosis of ASPD and 15 healthy controls matched for age, handedness and IQ. Reductions of FA were present in the frontal lobe of the ASPD group, and these were significantly and inversely correlated with severity of psychopathy (Factor 2, and total PCL-R scores).

The frontal cluster (Cluster 2, Table 5.3) that showed significant FA reduction and negative correlations with severity of psychopathy in the ASPD group included the genu of corpus callosum. Furthermore, it included right hemisphere structures of the anterior corona radiata and anterior limb and genu of the internal capsule in addition to the frontal course of the uncinate fasciculus and inferior fronto-occipital fasciculus. Although FA reduction was also found in posterior regions of the left hemisphere in the temporo-occipital course of the inferior longitudinal fasciculus and inferior fronto-occipital fasciculus, in addition to the retrolenticular section and posterior thalamic radiation of the internal capsule (Cluster 1, Table 5.3), these did not correlate significantly with measures of psychopathy. Overall, the findings suggest that people with ASPD have white matter microstructural abnormalities involving frontal white matter networks, and particularly of the right hemisphere.
In a previous report employing DT-MRI tractography examining FA and streamlines (a proxy measure of tract volume) of the uncinate, inferior longitudinal and inferior fronto-occipital fasciculi of nine individuals with psychopathy, FA was found to be reduced only in the right uncinate fasciculus; additionally the number of streamlines in the uncinate fasciculus bilaterally was correlated negatively with Factor 2 and total PCL-R scores though FA did not show any statistically significant correlations (Craig et al., 2009). The present finding of reduced FA in the right frontal lobe encompassing the uncinate fasciculus reproduces the original finding by Craig et al. (2009), noting that these same nine subjects were also included in the larger sample of 15 individuals in the current study where the incorporation of more subjects may have provided additional power to detect further significant correlations with FA.

Apart from FA deficits in the uncinate fasciculus, the frontal lobe of ASPD subjects in the current study also showed FA deficits in the corpus callosum, internal capsule, anterior corona radiata and inferior fronto-occipital fasciculus; this is likely to indicate information relay impairments between prefrontal and other brain regions - e.g. interhemispherically and with the cingulum, pons, thalamus and temporo-occipital cortices (Catani et al., 2002; Schmahmann and Pandya, 2008). Impaired functioning of the corpus callosum in psychopaths has previously been postulated on the basis of evidence of prolonged interhemispheric transfer time relative to controls (Hiatt and Newman, 2007). Lesion studies of the corpus callosum have also revealed its role in supporting sensory-motor functional integration, attention, language, interhemispheric transfer of associative learning, and emotional regulation (Bellani et al., 2009; Glickstein and Berlucchi, 2008; Zaidel and Iacoboni, 2003).
Consequently, the present findings of reduced FA in the corpus callosum of adults with ASPD, which also negatively correlate with measures of psychopathy, may suggest that functions mediated predominantly by the left hemisphere (e.g. approach behaviour, language processing) may be relatively unmodulated by functions mainly mediated by the right hemisphere (including behavioural inhibition and emotion processing) as postulated by Hiatt and Newman (2007). This proposed mechanism may also help explain the association of reduced FA in the corpus callosum of dependent cocaine users with increased impulsivity (Moeller et al., 2005) and white matter abnormalities in the corpus callosum of adolescents engaged in dangerous behaviour (Berns et al., 2009).

With regard to frontal lobe tracts, abnormalities in the uncinate fasciculus are associated with impairments of conditional associative learning (Gaffan and Wilson, 2008; Gutnikov et al., 1997; Parker and Gaffan, 1998). Individuals with ASPD and/or psychopathy demonstrate deficits in reversal learning, a form of conditional associative learning characterised by a failure to 'reverse' a previously rewarded response when it is punished relative to controls. Reversal learning deficits may contribute to the perseveration of antisocial behaviour and high levels of recidivism characteristic of the disorder. Further, fibres of the uncinate fasciculus connect the orbitofrontal cortex and amygdala, while impaired regulation of amygdala activity by the orbitofrontal cortex may contribute to the behavioural disinhibition encountered both in 'acquired sociopathy' (Brower and Price, 2001) and ASPD and psychopathy (Sarkar et al., 2011). Although abnormalities in the inferior fronto-occipital fasciculus and anterior corona radiata were also found in the frontal lobe in
the current study, functional impairments attributable to these tracts are examined later in the discussion.

Additionally, FA deficits were found in the present study in the anterior limb of the internal capsule which contains thalamo-frontal (anterior thalamic radiation), frontothalamic and corticopontine fibres, and interconnects the dorsomedial and anterior thalamic nuclei with both the prefrontal and cingulate cortices (Chapter 1, Section 1.3.2.). Lesions of the anterior limb of the internal capsule have been associated with ‘acquired sociopathy’ (Moll et al., 2003). Further, deficits in the anterior limb of the internal capsule have been suggested to lead to impairments in attention, perception and working memory (Buchsbaum et al., 2006; Sepulcre et al., 2008). The anterior limb of the internal capsule together with the anterior corona radiata (which connects the striatum with the anterior cingulate cortex) have been postulated respectively to contribute to attention impairments of alerting and conflict processing (Niogi et al., 2010). Disruption in tracts supporting the functional integration of cortical and subcortical regions involved in memory, attention, volition, learning and visual integration may contribute to the problems of people with ASPD in adaptively responding to altered contingencies in the social and physical environment (including social cues such as facial expressions), expressed in traits such as impulsivity or difficulty inhibiting motivated responses; a low threshold for the discharge of aggression; and failure to learn from aversive experiences.

As frontal abnormalities in the current study are lateralised and involve the right hemisphere, there may be a relationship between lateralisation of abnormalities and
emergence of ASPD. Indeed, FA deficits found in the right frontal lobe indicates a less coherent underlying white matter microstructure that may have arisen due to abnormal development of glial cells, axons or cell membranes in the right hemisphere (Dong et al., 2004; Herve et al., 2005). Supporting this theory are findings from a previous study that demonstrated impairments in social conduct, social cognition and emotion processing in those with right-sided vmPFC lesions rather than left-sided damage (Tranel et al., 2002). Similarly, right-sided inferior frontal cortex lesions are associated with loss of inhibitory control (Aron et al., 2004) where disinhibition may result from a direct consequence of frontal lobe damage (Brower and Price, 2001) or indirectly through loss of frontal inhibition on temporal lobe structures, particularly, the amygdala (Hoffer et al., 2007). Given the lateralisation of brain functions noted earlier in the discussion of the corpus callosum (see also (Doron and Gazzaniga, 2008)), white matter microstructural abnormalities localised to the right frontal lobe may further exacerbate the impaired modulation of left hemispheric processing that is potentially associated with abnormalities of the corpus callosum (Hiatt and Newman, 2007); this in turn may additionally contribute to emotion dysfunction (such as emotional shallowness and lack of empathy) and poor impulse control.

Although white matter tracts in the posterior brain also showed FA deficits, these did not correlate significantly with psychopathy scores. The inferior longitudinal and inferior fronto-occipital fasciculi share projections at the posterior temporal and occipital lobes and are involved in connecting the visual association areas of the occipital lobe, and the auditory and visual association areas and the PFC respectively.
(Catani et al., 2002; Catani et al., 2003; Kier et al., 2004). Disconnection of the inferior longitudinal fasciculus has been associated with impaired communication between the occipital and temporal lobes (including the amygdala) - for instance involving the occipital and fusiform face areas (Catani and Thiebaut de Schotten, 2008) - which may lead to prosopagnosia and deficits in face processing (Fox et al., 2008) as well as visual memory disturbances (Shinoura et al., 2007). Further, the inferior fronto-occipital fasciculus in humans represents the only direct long range association tract connecting the frontal and occipital lobes (Catani, 2006) and damage to the occipital portion of the inferior fronto-occipital fasciculus has been associated with visual neglect as a result of impaired modulation by the frontal cortex (Urbanski et al., 2008). FA reduction in the present study was also found in the retrolenticular section and posterior thalamic radiation of the internal capsule which carries fibres of the optic radiation and is thus involved in the visual system. Overall, reduced microstructural integrity of these posterior tracts may contribute to deficits in face processing in antisocial populations, who show significant deficits in recognising fearful, sad, and surprised expressions, with a significantly greater deficit in fear recognition relative to other expressions (Marsh and Blair, 2008) and greater abnormalities of fusiform-extrastriate cortical responses to fearful than happy expressions relative to controls (Deeley et al., 2006). Deficits in fear processing in antisocial populations have been hypothesised to contribute to impaired moral socialisation, in which the ‘at risk’ child fails to learn to avoid behaviour that engenders distress in others (Marsh et al., 2008).
5.7. Limitations and Conclusions

There were several limitations to the present work. As this was a cross-sectional study, it is unclear whether the frontal deficits encountered are present early in life as a consequence of abnormal brain maturation and thus predispose to the emotional dysfunction and antisocial behaviour of psychopathy and ASPD, or whether they represent cumulative effects of later biopsychosocial factors such as the experience of recurrent involvement in antisocial behaviour or substance misuse. Future studies should therefore aim to assess child cohorts so as to longitudinally characterise white matter integrity; and to identify whether white matter anomalies predate detrimental lifestyle factors such as substance use, that frequently coexist in antisocial populations. Further, in order to match groups more closely, future studies would benefit from using non-psychopathic/non-ASPD offenders or patients as controls, rather than the healthy community sample used here. This would minimise potential confounds, including the higher incidence of substance misuse disorders, and differing lifestyle and socio-demographic factors. Overall, the current study represents the largest cohort of adults with ASPD analysed by DT-MRI to date, whilst recognising that recruiting suitable participants poses a challenge.

With regard to the PCL-R, while all subjects had total PCL-R scores, a limited number of participants did not have factor subscores (see Section 5.4.1.). Additionally, while controls were not assessed on the PCL-R, they were assessed through semi-structured interview using ICD-10 research criteria to assess for the presence of comorbid ASPD. Future studies may wish to implement the PCL-R or
the shorter PCL-SV (Hart et al., 1995) to screen for psychopathy in control groups. Another weakness in the current study is that volumetry and neuropsychology measures were not assessed. The inclusion of neuropsychological tests within the present study may have helped to elucidate the type of information processing deficits that mediate the link between neural structural deficits and the behavioural profile of ASPD and psychopathy. Also, as white matter FA and structural volume may not be directly related (Lim et al., 1999), where possible, both conventional MRI and DT-MRI should be used in conjunction to investigate potential white matter deficits as DT-MRI may be better able to detect pathology in normal appearing white matter tissue while conventional MRI is able to ascertain independently occurring white matter volumetric changes (Makki et al., 2007; Neil et al., 2002; Rugg-Gunn et al., 2001; Sundram et al., 2010b). Moreover, when considering the results of the correlation analyses, it must be remembered that these may not be representative of other brain areas. In particular, as the regions over which these correlations were measured were defined by differences between ASPD subjects and controls, there is by definition, some relationship within these areas between FA and overall behavioural scores, and measurements cannot be considered truly independent. Future studies should therefore consider the use of ROIs based on the present study employed in independently acquired samples.

In summary, the current study showed reduced white matter FA in areas consistent with a number of white matter tracts within the frontal and temporo-occipital lobes in a group of adult males with ASPD compared to controls. Furthermore, only frontal FA abnormalities in the right hemisphere showed significant correlations
with severity of psychopathy. Taken together, these findings suggest that frontal lobe white matter microstructural abnormalities in ASPD particularly involve the right hemisphere while white matter deficits also encompass posterior brain and interhemispheric networks. Given this pilot investigation, future work is required to longitudinally evaluate abnormalities in frontal and other brain regions through methods assessing brain structure, function and connectivity.

While this chapter assessed a group of adult males with ASPD relative to healthy controls using DT-MRI, similarly, in the next chapter, a group of adult males with a diagnosis of Asperger syndrome instead is assessed for abnormalities in white matter microstructural integrity and connectivity relative to healthy matched adult controls using voxel-based analyses of FA maps derived from DT-MRI acquired datasets. The next chapter constitutes the final experimental chapter in this thesis and utilises the same voxel-based analytical methodology as used in Chapter 3 and the present chapter.
Chapter 6:

Study 4
White matter integrity in Asperger syndrome: a preliminary diffusion tensor magnetic resonance imaging study in adults

The microstructural integrity of white matter has yet to be investigated specifically in a group of adult individuals with Asperger syndrome. The main aim of this experimental chapter is to examine a cohort of adults with the syndrome relative to healthy adult controls where there are no significant differences in overall intelligence or age. DT-MRI datasets were acquired for all subjects and their FA maps were analysed via voxel-based analyses. This study contributes significantly to the literature as prior studies have assessed autism spectrum disorder as a single group rather than Asperger syndrome while this study also investigates adults who may have reached the peak of brain maturation.
6.1. Summary of chapter

Although autism spectrum disorder including Asperger syndrome is a highly genetic neurodevelopmental disorder, the microstructural integrity of white matter in people specifically with Asperger syndrome has never been previously investigated on a whole brain level. Moreover, so far, children and adolescents with autism spectrum disorder have been predominantly investigated as a single group while brain maturation in adults specifically with Asperger syndrome has not been assessed. As a result, abnormalities in the connectivity of white matter networks in adults with Asperger syndrome are presently unclear. In this chapter, FA maps derived from DT-MRI datasets of 13 adults with Asperger syndrome are compared to 13 healthy age-, gender-, and IQ-matched controls using whole-brain voxel-based analyses.

Adults with Asperger syndrome were found to have significantly lower FA than controls in a number of white matter tracts where these abnormalities were largely bilateral and FA deficits were found in all cerebral lobes (frontal, temporal, parietal and occipital lobes). Further, FA deficits were also found in sublobar regions such as the internal capsule and cingulum while significant FA reduction was also found in the corpus callosum suggesting extensive white matter disconnectivity. Overall, the findings from the present study suggest that people with Asperger syndrome not only demonstrate impairments in white matter microstructural integrity and connectivity in regions relevant to social skills and behaviour but also have more widespread differences where these deficits appear to persist into adulthood.
6.2. Introduction

The description of autism spectrum disorder includes autism, Asperger syndrome and pervasive developmental disorder—not otherwise specified (Lord and Bishop, 2010). Autism spectrum disorder is increasingly recognised as a highly genetic disorder with neurodevelopmental origins (Bailey et al., 1995; Frith, 2001) and is usually characterised by a triad of deficits encompassing significant difficulties in social interaction, communication, and unusual or stereotyped routines and behaviour (World Health Organization, 1993). Although autism spectrum disorder is also frequently associated with intellectual disability, however, Asperger syndrome is characterised by the same qualitative impairments as classical autism but where cognitive and language abilities are largely preserved (see Chapter 1, Section 1.8.).

Although there is increasing evidence in the literature that Asperger syndrome is associated with neurobiological deficits, however, most reports so far have examined autism or autism spectrum disorder as a single group instead of specifically assessing Asperger syndrome (see Chapter 1, Section 1.8.5.). While these reports have contributed to the understanding of white matter connectivity abnormalities within autism spectrum disorder, they have nonetheless included heterogeneous clinical populations such as mixed adult and child cohorts. Given that Asperger syndrome is proposed to represent an ‘underconnectivity’ disorder where there is impaired communication between a number of brain networks and that the trajectory of abnormal brain development continues on into adulthood, it is important that white
Chapter 6: Study 4 - White matter integrity in Asperger syndrome: a preliminary diffusion tensor magnetic resonance imaging study in adults

matter integrity across the whole brain is assessed in a relatively homogeneous population of adults specifically with Asperger syndrome.

6.3. Aims, objectives and hypotheses

This study aims to characterise white matter microstructural integrity and connectivity differences in a cohort of adult males with Asperger syndrome relative to healthy matched adult controls.

In this chapter, the first ever investigation of adults specifically with Asperger syndrome based on DT-MRI was undertaken whereby the objectives of the study are to:

- recruit a group of adults exclusively with Asperger syndrome (as opposed to autism spectrum disorder) and to compare them to healthy matched controls
- screen subjects and to exclude comorbid psychiatric disorder
- acquire whole-brain DT-MRI scans in both groups based on a standardised acquisition protocol and analyse white matter using a customised brain template derived from all subjects under study
- compare white matter network differences between groups based on whole-brain voxel-based analyses of maps of FA

The main hypothesis tested in this experimental study is that adults with Asperger syndrome when compared to healthy adults without the disorder will demonstrate
deficits in white matter integrity. These abnormalities are hypothesised to be present in white matter networks subserving behaviour and social skills.

6.4. Methods

The subsections below provide details on subject recruitment and assessment. In particular, the psychological and behavioural testing procedures are expanded on. Finally, statistical comparisons of demographic and neuroimaging data are outlined.

6.4.1. Subjects

People with Asperger syndrome were recruited through local support groups, and an ongoing clinical research programme in autism (Autism Imaging Multicentre Study or AIMS) which is supported by the Medical Research Council, UK. Ethics approval was provided by the Joint Institute of Psychiatry and South London and Maudsley NHS Trust Research Ethics Committee. Potential participants were invited to partake whereby details of the study were discussed in person. Those that were willing to proceed for further investigation gave informed consent and the study was conducted in accordance with the Declaration of Helsinki. Subjects were diagnosed based on a combination of clinical interview, collateral information from family members, and review of other information available for example school reports. Assessments were
conducted blind to MRI scan data and classified according to ICD-10 clinical research criteria (World Health Organization, 1993).

Individuals with Asperger syndrome were excluded if they had a prior history of: head injury, addiction, exposure to toxic substances, major psychiatric disorder (e.g. psychosis) and, medical or genetic disorder associated with autism (e.g. tuberous sclerosis or Fragile X syndrome). Additionally, people with gross abnormalities of brain parenchyma on conventional MRI were excluded due to potential interference with neuroimaging analyses. All subjects had a structured clinical exam and routine clinical blood tests to exclude biochemical, haematological or chromosomal abnormalities. Overall, 13 adult males fulfilling ICD-10 clinical research criteria for autism spectrum disorder were recruited after three scans were excluded due to data quality issues. As subjects did not have a history of language or cognitive delay, they were therefore defined as having Asperger syndrome where each subject was ≥18 years (mean age: 39 years, SD ± 9.8, range: 23 - 54 years; mean FSIQ: 110, SD ± 15.7, range: 88 - 133). Additionally, 13 adult male controls were recruited locally at the Institute of Psychiatry by advertisement (mean age: 37 years, SD ± 9.6, range: 25 - 52 years; mean FSIQ: 115, SD ± 14.4, range: 89 - 133). Table 6.1 provides a summary of participants and study groups did not differ in age or FSIQ.
### Table 6.1 Characteristics of cohort: Asperger syndrome vs. healthy controls

<table>
<thead>
<tr>
<th></th>
<th>Asperger syndrome (n = 13)</th>
<th>Healthy controls (n = 13)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (SD)</td>
<td>39 years (± 9.8)</td>
<td>37 years (± 9.6)</td>
<td>0.66</td>
</tr>
<tr>
<td>Age range</td>
<td>23 - 54 years</td>
<td>25 - 52 years</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>13 Male</td>
<td>13 Male</td>
<td></td>
</tr>
<tr>
<td>Full scale IQ (SD)</td>
<td>110 (± 15.7)</td>
<td>115 (± 14.4)</td>
<td>0.35</td>
</tr>
<tr>
<td>Full scale IQ range</td>
<td>88 - 133</td>
<td>89 - 133</td>
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</tr>
</tbody>
</table>

a. All values represent t-tests
6.4.2. Psychological testing

Currently, the diagnosis of Asperger syndrome is largely dependent on clinical assessment while the gold standard consists of a combination of clinical judgment in conjunction with diagnostic interviews (see Chapter 1, Section 1.8.3.). Therefore, where possible, the ADI-R (Lord et al., 1994) and/or the ADOS-G (Lord et al., 2000) were used to confirm diagnoses where these psychometric tools represent a semi-structured interview with a primary caregiver and a semi-structured interactive/play session respectively. When it was not possible to complete the ADI-R (for example if a parent was not available), completion of the ADOS-G was attempted.

Diagnosis was confirmed through clinical consensus with the ADI-R (Lord et al., 1994) in six individuals with Asperger syndrome (when their parental informants were willing or available) while another five subjects were assessed on the ADOS-G (Lord et al., 1989; Lord et al., 2000). However, two subjects did not receive additional confirmation of their diagnosis using these methods as there were no parent informants or they were unwilling to participate in an assessed interactive session. While the above interview schedules tested/confirmed the presence of an autism spectrum disorder, overall intelligence was measured using the vocabulary, comprehension, similarities, block design and object assembly subtests of the WAIS-R (Wechsler, 1981). As mentioned in Chapter 1, Section 1.8., people with Asperger syndrome lack the cognitive and language deficits typically found in autism. Table 6.2 summarises the ADI-R and ADOS-G scores for the subjects diagnosed with Asperger syndrome.
Table 6.2 ADI-R and ADOS-G scores for Asperger syndrome subjects

<table>
<thead>
<tr>
<th>Asperger syndrome subject</th>
<th>ADI-R Communication</th>
<th>ADI-R Social</th>
<th>ADI-R Repetitive Behaviours</th>
<th>ADI-R All</th>
<th>ADOS-G Communication</th>
<th>ADOS-G Social</th>
<th>ADOS-G Repetitive Behaviours</th>
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<tbody>
<tr>
<td>1</td>
<td>16</td>
<td>8</td>
<td>3</td>
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</table>
6.4.3. MRI acquisition protocol

DT-MRI scans were acquired for all participants in this study at the Institute of Psychiatry MRI scanner. Details of the DT-MRI acquisition protocol are provided in Chapter 2, Section 2.7.

6.4.4. DT-MRI data pre-processing

All data were visually inspected to ensure suitable quality for further processing. As mentioned in Section 6.4.1., three scans were excluded due to data quality issues. DT-MRI pre-processing was conducted, the details of which are found in Chapter 2, Section 2.8.

6.4.5. Statistics

In the following subsections, details are provided on the statistical tests used for examining demographic data while detailed descriptions of the statistical comparisons performed on FA maps derived from DT-MRI acquisitions are given in Chapter 2, Section 2.8.4.

6.4.5.1. Demographic data
SPSS (SPSS 14.0 for Windows, SPSS Inc, Chicago, IL, USA) was used for statistical analysis of non-imaging data. Student’s \( t \)-tests (\( p = 0.05 \), two-tailed) for independent samples were used to examine between-group differences on age and IQ-scores (Table 6.1).

### 6.4.5.2. Whole-brain voxel-based analysis of DT-MRI data

Differences in white matter FA between the Asperger group and controls were evaluated by fitting an ANCOVA model at each intracerebral voxel in standard space. Although age and IQ did not show any statistical difference between groups, these were added as covariates given that IQ levels encompassed normal, superior and very superior intelligence ranges. The statistical threshold for cluster significance was set for each analysis at \( p = 0.0025 \) such that the expected number of false-positive clusters by chance alone would be less than one. Full details of the testing procedure are provided in Chapter 2, Section 2.8.

### 6.5. Results
FA deficits were found in all cerebral lobes in adults with Asperger syndrome. These differences were found in 13 clusters (see Table 6.3 and Fig. 6.1).

6.5.1. Asperger syndrome vs. Controls white matter FA

Bilateral FA reduction in the frontal lobe was found in the corpus callosum (genu), anterior corona radiata and anterior thalamic radiation and uncinate and inferior fronto-occipital fasciculi (Clusters 1 and 2). Also in the frontal lobe, Cluster 8 encompassed the superior longitudinal fasciculus. Similarly, in the left hemisphere, Cluster 11 involved the superior longitudinal fasciculus but additionally included parts of the anterior thalamic radiation and uncinate fasciculus as well as the corpus callosum (splenium). FA deficits were also found in the splenium of corpus callosum in Clusters 7 and 10. In the fronto-parietal lobe, FA deficits were localised to the cingulum and superior longitudinal fasciculus and thalamic radiations (Clusters 12 and 13) as well as the corpus callosum in these same clusters in addition to Cluster 9. In the left hemisphere, three clusters of FA deficits encompassed the retrolenticular part of the internal capsule, optic radiation and corpus callosum (forceps major) and additionally encompassed the inferior longitudinal fasciculus and inferior fronto-occipital fasciculus (Clusters 3, 4 and 6). These same tracts are also implicated contralaterally albeit in a smaller and more dorsally located cluster (Cluster 5). Although there were regions of higher FA found in the Asperger syndrome group relative to controls, these were found in four extremely small clusters of less than 10 voxels each and are therefore not reported (see Chapter 2, Section 2.8.4.).
Fig. 6.1 Reduced FA in Asperger syndrome relative to healthy controls [ascending 2mm transverse sections] where the image is displayed such that the subject's right is to the left and left is right.
### Table 6.3 White matter FA deficits in Asperger syndrome relative to healthy controls controlled for IQ and age (cluster significance threshold for FA maps $p = 0.0025$)

<table>
<thead>
<tr>
<th>Cluster label</th>
<th>Cluster Size (Number of Voxels)</th>
<th>Talairach and Tournoux Coordinates $x$ $y$ $z$</th>
<th>Tract(s) within cluster</th>
<th>Region</th>
<th>Cluster mean FA Asperger syndrome (SD)</th>
<th>Cluster mean FA Control (SD)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cluster 1</td>
<td>79</td>
<td>-14 35 -12</td>
<td>Corpus callosum, inferior fronto-occipital fasciculus, anterior corona radiata</td>
<td>Frontal lobe</td>
<td>0.387 (0.032)</td>
<td>0.414 (0.024)</td>
<td>0.000374</td>
</tr>
<tr>
<td>Cluster 2</td>
<td>296</td>
<td>14 32 -7</td>
<td>Corpus callosum, inferior fronto-occipital fasciculus, anterior thalamic radiation, uncinate fasciculus, anterior corona radiata</td>
<td>Frontal lobe</td>
<td>0.387 (0.039)</td>
<td>0.417 (0.023)</td>
<td>0.000019</td>
</tr>
<tr>
<td>Cluster 3</td>
<td>83</td>
<td>-39 -38 0</td>
<td>Inferior fronto-occipital fasciculus</td>
<td>Temporal lobe</td>
<td>0.459 (0.063)</td>
<td>0.496 (0.030)</td>
<td>0.000248</td>
</tr>
<tr>
<td>Cluster 4</td>
<td>82</td>
<td>-32 -32 2</td>
<td>Inferior fronto-occipital fasciculus, inferior longitudinal fasciculus</td>
<td>Temporal lobe</td>
<td>0.459 (0.057)</td>
<td>0.497 (0.023)</td>
<td>0.000177</td>
</tr>
<tr>
<td>Cluster 5</td>
<td>93</td>
<td>25 -70 7</td>
<td>Inferior fronto-occipital fasciculus, inferior longitudinal fasciculus</td>
<td>Occipital lobe</td>
<td>0.375 (0.046)</td>
<td>0.406 (0.028)</td>
<td>0.000231</td>
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6.6. Discussion

In this study, white matter microstructural integrity in 13 adult males with Asperger syndrome was compared to a healthy control group that was matched for IQ, age and gender. This study represents the first DT-MRI investigation of brain connectivity differences using whole-brain voxel-based analyses of FA maps in a group of adults specifically recruited with Asperger syndrome. Subjects with Asperger syndrome were found to have significant and widespread reduction of FA in a number of white matter tracts. Though this is the first study of adults with Asperger syndrome, the results are consistent with prior whole-brain DT-MRI work in children and even larger populations of mixed groups (children, adolescents and adults) of people with autism spectrum disorder (Barnea-Goraly et al., 2004; Keller et al., 2007).

While studies reporting reduced FA have also been accompanied by reports of higher FA for instance in the right inferior frontal gyrus and left occipital lobe (Cheung et al., 2009) and temporal and frontal lobes (Ke et al., 2009), similarly, regions of increased FA were also found in the present study; however, these were minute and of less than 10 voxels in magnitude. Given that autism spectrum disorder is associated with brain overgrowth during childhood and adolescence, the finding of small regions of increased FA may be indicative of a reversal of this process. Overall, however, the pattern of findings in the current study is highly suggestive of widespread FA deficits in adults with Asperger syndrome.
The findings in the present study are discussed next in the context of constituent lobes and major white matter tracts of the brain while considering previous neuroimaging reports in autism spectrum disorder given the paucity of reports explicitly investigating Asperger syndrome.

6.6.1. Frontal lobe

Significantly reduced FA was found in Asperger syndrome in the current study in a number of white matter tracts of the frontal lobe and included the thalamic radiations; corona radiata; uncinate, inferior fronto-occipital and superior longitudinal fasciculi; corpus callosum and; cingulum. The corpus callosum is discussed in further detail in Section 6.6.2. while the other white matter tracts are discussed below.

Within the literature, differences in frontal lobe anatomy, development and function are frequently reported in autism spectrum disorder (Carper and Courchesne, 2000; Carper et al., 2002; Herbert et al., 2004; Levitt et al., 2003; Luna et al., 2002; Sundaram et al., 2008). Moreover, frontal lobe dysfunction has been linked to specific impairments in autism spectrum disorder. For instance, frontal lobe deficits have been associated with abnormalities in mentalization and Theory of Mind (Castelli et al., 2002; Frith and Frith, 1999; Happe et al., 1996), spatial working memory (Luna et al., 2002), executive function (O’Hearn et al., 2008), joint attention
(Mundy, 2003) and language (Just et al., 2004; Muller et al., 1998). Further, significant differences in the activation of cortical motor areas have been reported (Muller et al., 2001) where motor abnormalities are one of the earliest observable behavioural features described in autism spectrum disorder.

Additionally, in a large DT-MRI study focusing on the frontal lobe of 50 children with heterogeneous autism spectrum diagnoses and mixed gender, ADC was significantly higher for the whole frontal lobe and for long- and short-range association fibres while FA was significantly lower in short- rather than long-range fibres (Sundaram et al., 2008). In another DT-MRI study which compared seven male children and adolescents with autism to nine age-, gender-, and IQ-matched control subjects, widespread FA deficits were found in medial prefrontal, anterior cingulate, corpus callosal and right (pre)motor regions in the group with autism (Barnea-Goraly et al., 2004). Similarly, in a mixed sample of 34 children and adults with HFA, Keller et al. (2007) reported widespread FA reduction in or near the corpus callosum. Moreover, low FA in conjunction with abnormal fMRI-BOLD (blood oxygenation level-dependent) activation in the anterior cingulate cortex has been postulated to account for impairments in response monitoring and repetitive behaviour in a study of 10 subjects with autism spectrum disorder relative to healthy controls (Thakkar et al., 2008).

Although other studies using whole-brain analytical methods with DT-MRI have also reported lower FA in frontal regions, such studies have been conducted in children or mixed samples of children and adults with autism spectrum disorder.
Additional studies support the notion of widespread FA reduction involving other brain regions (Barnea-Goraly et al., 2004; Cheung et al., 2009; Ke et al., 2009). Additionally, while the results from the current study suggest predominantly frontal FA deficits, there is evidence of widespread FA reduction involving other brain regions.

6.6.2. Medial brain regions

There is increasing evidence in the literature of structural and functional deficits involving the corpus callosum in autism spectrum disorder where structural abnormalities reported have included a reduced surface area, size and volume. For instance, the corpus callosum in 16 adolescents and adults with HFA has been reported to demonstrate less white matter concentration than healthy controls which the authors suggested may be accounted by hypoplasia rather than atrophy of this structure (Chung et al., 2004). Other studies have shown a size reduction of the corpus callosum but in inconsistent regions; for example, in a large study of 51 autistic individuals that included both intellectually disabled as well as those lacking an intellectual disability but with a very wide age range (3-42 years), the authors found a reduction in size of the posterior corpus callosum particularly involving fibres projecting to parietal lobe (Egaas et al., 1995) while a study of 22 individuals with HFA found a reduction in size of the anterior regions of the corpus callosum thereby suggesting frontal lobe dysfunction (Hardan et al., 2000). Also, while an assessment conducted in 27 intellectually disabled individuals with autism reported a smaller corpus callosum, size reduction particularly involved the body rather than
either posterior or anterior regions of this structure (Manes et al., 1999).
Additionally, other studies have reported size reduction involving the genu and
splenium (Vidal et al., 2006) as well as volume reduction of the anterior splenium
and isthmus (Waiter et al., 2005) of the corpus callosum.

Moreover, fMRI studies in people with autism spectrum disorder have also reported
significantly lower interhemispheric function and integration during various
language (Just et al., 2004), working memory (Koshino et al., 2005), Theory of Mind
(Mason et al., 2008) and visuospatial imagery tasks (Kana et al., 2006). While
reduced FA has also been reported in the corpus callosum (see Chapter 1, Section
1.8.5.) of mixed child, adolescent and adult cohorts with autism spectrum disorder
(Alexander et al., 2007; Barnea-Goraly et al., 2004; Keller et al., 2007), the
significantly altered microstructural integrity in the anterior and posterior portions of
the corpus callosum of adults specifically with Asperger syndrome in the present
study adds to the literature on deficits encompassing the corpus callosum which may
partially explain some of the clinical features of the disorder.

6.6.3. Temporal and parietal lobes

Structural and functional studies from the literature have confirmed abnormalities in
the temporal lobes of people with autism spectrum disorder (Boddaert et al., 2004;
Gendry Meresse et al., 2005). For example, abnormalities have been found in the
superior temporal region which is important for sentence comprehension/auditory processing (Just et al., 2004; Meyer et al., 2005), cortical integration of both limbic and sensory information (Boddaert et al., 2004) and social perception (Allison et al., 2000).

Damage to the temporal lobe is also thought to contribute to visually-specific semantic, emotional, memory and language deficits due to impaired interaction with occipital, frontal and parietal lobe regions (Catani et al., 2003; Mandonnet et al., 2007; Vigneau et al., 2006). For instance, previous studies in autism spectrum disorder have reported abnormalities in the parietal lobe, such as volume differences (Courchesne et al., 1993; Piven et al., 1996) while abnormal connectivity between temporo-parietal, anterior-posterior and interhemispheric networks have also been reported (Barnea-Goraly et al., 2004; Cherkassky et al., 2006; Just et al., 2007). Further, network impairments between the temporal lobe and medial PFC particularly involving the superior temporal sulcus at the temporo-parietal junction and temporal poles has been suggested to result in abnormal mentalization (Castelli et al., 2002) while abnormalities in interaction between the superior temporal sulcus and the 'mirror neuron system' (consisting of the posterior inferior frontal gyrus, ventral premotor cortex and rostral inferior parietal lobule) results in imitative learning deficits (Iacoboni, 2005).

Prior DT-MRI studies have been conducted in autism spectrum disorder but in cohorts containing mixed groups of children, adolescents and adults (Barnea-Goraly et al., 2004; Conturo et al., 2008; Keller et al., 2007; Lee et al., 2007). Barnea-Goraly
et al. (2004) for example have reported reduced FA bilaterally in the temporo-parietal junctions and occipito-temporal tracts and in white matter adjacent to the superior temporal sulcus and amygdala while Lee et al. (2007) similarly reported FA deficits in the temporal lobe involving the superior temporal gyrus and temporal stem. The authors from the latter report additionally examined the ability of tensor coefficients to correctly discriminate individuals with autism from typically developing individuals in a further study whereby directional diffusion along white matter fibres in the superior temporal gyrus was found to be more coherent in the right hemisphere relative to the left indicating a possible disruption to hemispheric lateralisation and white matter microstructure during development (Lange et al., 2010). In another DT-MRI study but based on tractography, Conturo et al. (2008) investigated temporal lobe white matter pathways in 17 adolescents and adults with HFA; although the hippocampo-fusiform and amygdalo-fusiform pathways were found to be of normal shape and size, these tracts however demonstrated abnormal microstructure.

While significant differences in FA were also found in the current study in parietal lobe regions such as in the superior longitudinal fasciculus, corpus callosum and thalamic radiations, parietal lobe abnormalities were restricted to fewer tracts in comparison to the temporal lobe. Additionally, although people with Asperger syndrome in the present study overall had significant bilateral FA reduction in the temporal lobe and perisylvian areas whereby these deficits were localised to the inferior longitudinal, superior longitudinal and inferior fronto-occipital fasciculi, however, it is likely that an interaction of dysfunctional networks between the
temporal and parietal lobes and with other brain regions contributes to clinical impairment in Asperger syndrome.

6.6.4. Occipital lobe

In a prior fMRI study of adults with Asperger syndrome, reduced visual cortical activity was found in occipital regions to a range of primary facial emotions and intensities (Deeley et al., 2007) underscoring the importance of the occipital region in facial emotion processing. However, in healthy individuals, visual cortical responses to emotionally salient expressions (such as fearful expressions) are modulated by limbic system components such as the amygdala (Vuilleumier et al., 2004). Therefore, impaired connectivity between occipital, limbic and other brain regions may contribute to neural hyporesponsiveness to facial expressions and associated social impairments in people with Asperger syndrome (see also Chapter 5, Section 5.6. for an expanded discussion on facial emotion processing).

White matter regions in Asperger syndrome which differed from controls in the present study included the retrolenticular part of the internal capsule and the inferior fronto-occipital and inferior longitudinal fasciculi in the occipital lobe. As mentioned earlier in Section 6.6.3., prior DT-MRI work by Barnea-Goraly et al. (2004) have also reported FA abnormalities in occipito-temporal tracts while Keller et al. (2007) similarly found FA deficits in the retrolenticular portion of the internal capsule.
Although these authors have reported occipital lobe abnormalities in mixed cohorts involving children, adolescents and adults, it is possible that such deficits persist into adulthood as is the case in the present study.

6.6.5. Cerebellum

In prior studies employing DT-MRI tractography or ROI approaches rather than whole-brain methods, FA deficits have been demonstrated not only in the cerebral lobes but also in the cerebellum. Asperger syndrome has been reported to show significantly lower FA in the right superior cerebellar peduncle (Chapter 1, Section 1.3.1.) and short intracerebellar fibres as compared to controls (Catani et al., 2008). The authors suggested that abnormalities they found in the main cerebellar outflow pathway may inhibit the cerebral cortex from receiving cerebellar modulation necessary for adaptive social behaviour. Although cerebellar deficits were not found in the present study, this may perhaps be accounted for by the older mean age in the present cohort of adults (39 vs. 31 years) with associated slowing of the rate of synaptic pruning and neuronal loss relative to healthy age-matched adults while an alternative explanation is that the current study may not be adequately powered to detect such cerebellar differences given a slightly smaller cohort (15 vs. 13 subjects) in conjunction with the use of conservative permutation-based non-parametric statistics.
6.7. Limitations

This is a preliminary study which has a number of limitations, including the relatively small sample size and a lack of ADI-R/ADOS-G measures in two subjects. Both subjects were neither willing to participate in a formally assessed interactive session nor had a parental informant available/willing to attend with them at clinical sessions which limited the possibility of completing the ADI-R or ADOS-G. The ADI-R was considered the most appropriate assessment tool to complete as it specifically assesses early language development and therefore aids in clinical differentiation between classical autism and Asperger syndrome. Although the two subjects did not have ADI-R or ADOS-G measures, they were subsequently excluded in a further analysis but did not substantially alter findings.

Though children and adolescents were excluded in this study to obtain a more homogeneous adult sample, there is still a wide age range. However, age and IQ were entered as covariates in the statistical model so as to limit their influence on the results. Additionally, while the study was of a cross-sectional design that aimed to capture adults at the peak of their brain maturation trajectory, it is presently unclear whether the white matter abnormalities currently reported represent stable impairments that are present from childhood or are indicative of deficits that occur at the height of brain development. Also, given that brain tissue overgrowth occurs in autism spectrum disorder during childhood and adolescence and may then lead to normal or indeed increased brain compartment volumes during later life, white
matter in these corresponding regions may be abnormal due to underlying
disorganisation. Therefore, where possible, both conventional MRI and DT-MRI
should be used in conjunction to investigate potential brain abnormalities as in
Chapter 3. Moreover, future studies should aim to assess children and adolescents
with Asperger syndrome longitudinally and at multiple sampling time-points so as to
better understand periods of rapid brain expansion which may then be followed by
accelerated pruning of specific brain regions.

With regard to image analysis, although voxel-based analyses may potentially report
false-positive edge effects, and some of the results are indeed located near the
ventricular system, however, edge effects are unlikely to fully account for the results
since the majority of differences encountered were distal to the ventricles. Further,
while neuropsychological tests were not included within the present study, these may
have helped to clarify the neural substrates associated with the social,
communication and behavioural deficits seen in Asperger syndrome.

6.8. Conclusions

Overall, a group of adult males with Asperger syndrome was recruited and compared
with a sample of healthy adults matched for age, gender and IQ. People with
Asperger syndrome were found to have widespread abnormalities in the
microstructural integrity of white matter tracts in all cerebral lobes and particularly
in frontal and sublobar regions and in networks subserving interhemispheric and long-range association connectivity. These findings are reported for the first time in a homogeneous sample encompassing only adults with Asperger syndrome and add to increasing evidence that people with Asperger syndrome have significant differences in brain connectivity in regions responsible for social behaviour, interaction and communication.

While the limitations of each of the experimental studies in Chapters 3-6 have been evaluated in their respective chapters, in the next and concluding chapter, the implications of experimental findings and the limitations of the MRI analytical techniques used in this work are discussed while considering potential future directions.
Chapter 7:

Conclusions and future directions for research

The purpose of this chapter is to provide a summary of findings from the experimental studies conducted in this thesis. Moreover, the psychiatric phenotypes that are examined in this work warrant further assessment and potential future directions are discussed. Finally, the limitations of the methodology used here are discussed in light of newer techniques or technologies that have been made available since commencement of this work and where future studies may wish to consider the utilisation of such approaches.
7.1. Overview of thesis

The primary focus of this thesis is the investigation of psychiatric disorder through *in-vivo* assessment of neural tissue by MRI and the application of whole-brain voxel-based methods whereby these analytical techniques enable the examination of differences in either brain tissue composition or connectivity. In Chapter 1, a background and literature review was provided with particular emphasis on MRI methods whereby these techniques were used in conjunction with voxel-based approaches for analysing structural and DT-MRI data. Additionally, details of cerebral neuroanatomy and major divisions of the brain were provided while highlighting the currently recognised major white matter pathways. Finally the rationale for examining particular psychiatric phenotypes was clarified and was accompanied by a summary of each of these disorders encompassing epidemiology, causation, diagnosis, associated behavioural and psychiatric disorder and prior neuroimaging work.

Subsequently, in Chapter 2, the aims and hypotheses of each experimental study was summarised while clarifying their objectives. Additionally, the specific MRI acquisition protocols and analytical methods used were described in full detail. Distinct psychiatric phenotypes were then examined in each of the following experimental chapters where description of the cohorts recruited and the limitations of each study were evaluated within their respective chapters. The main findings from the experimental studies are provided below while potential directions for the future are considered.
7.2. Current findings and future directions

In Study 1, a cohort of children and adolescents with 22q11DS was compared to healthy matched controls without chromosome 22q11 deletion. Genetic testing was conducted to confirm the presence or absence of a 22q11 deletion and screening of children and adolescents was performed to rule out the presence of comorbid psychiatric disorder. DT-MRI and conventional MRI datasets were acquired in all subjects and were respectively analysed using whole-brain voxel-based analysis and VBM to compare FA and white matter volume of 11 children and adolescents with 22q11DS and 12 healthy matched controls. Also, within 22q11DS, differences in white matter were related to severity of schizotypy, and polymorphism of the COMT gene.

After covarying for IQ, people with 22q11DS were found to have significantly lower FA in interhemispheric and brainstem and frontal, parietal and temporal lobe regions. However, significant white matter volumetric increases were concurrently found in the internal capsule, anterior brainstem and frontal and occipital lobes after controlling for IQ. Further, while there was a significant negative correlation between increased schizotypy scores and reduced white matter FA in the right posterior limb of internal capsule and the right body and left splenium of corpus callosum, the Val allele of COMT was associated with significant reduction in both FA and volume of white matter in the frontal lobes, cingulum and corpus callosum. Overall, young people with 22q11DS were found to have significant differences in
both white matter microstructure and volume and there was also preliminary
evidence that within 22q11DS, some regional differences in FA are associated with
allelic variation in COMT and may also be associated with schizotypy.

Although Study 1 was based on a cross-sectional design, it is part of longitudinal
work to evaluate children and adolescents with 22q11DS at the Institute of
Psychiatry thereby informing on brain maturation throughout their developmental
trajectory. Follow-up work at future sampling points is required to assess further
brain differences and should ideally be based on similar investigative methodology
so as to prevent Type 1 error that may otherwise arise. Also, as the recruitment
strategy in Study 1 attempted to limit assessment to individuals that were
comparatively free from comorbid psychiatric disorder given that psychiatric
disorder is highly prevalent in 22q11DS, however, this led to a relatively reduced
sample size. Future work may therefore wish to recruit larger samples and to also
compare 22q11DS with other developmental disorders that are associated with
similar levels of intellectual impairment (such as populations with Down or Williams
syndrome) in order to match study groups more closely with regard to cognitive
function which would then allow the attribution of brain differences that may be
specific to individuals with 22q11DS.

Further, although the influence of COMT was assessed in Study 1 (as 22q11DS is
associated with chromosomal deletions in the TDR), however, the TDR is rich in
genes coding for brain development. Therefore, other genes that may have a
neuromodulatory effect e.g. PRODH or TBX-1 should be assessed in future
longitudinal neuroimaging work. Also, while schizophrenia-spectrum disorder was
specifically investigated in the present work, additional neuroimaging investigation is required to assess other comorbid disorders such as autism spectrum disorder and ADHD given that 22q11DS is significantly associated with psychiatric disorder.

In Study 2, the first whole-brain computerised statistical analysis of grey and white matter tissue in TLE+psychosis using VBM was undertaken. VBM was considered the most appropriate as it permits a hypothesis-free survey of the entire brain which may then enable future hypotheses generation. The study assessed a population with epilepsy and involved the identification of patients who had a neurological diagnosis consistent with TLE based on seizure semiology, EEG and neuroimaging data. Out of those with a confirmed diagnosis of TLE, patients who developed psychosis and who had suitable neuroimaging data were established and 10 people with TLE+psychosis were matched for age, gender, handedness, epilepsy duration, seizure laterality, severity of epilepsy and antiepileptic medication with 10 comparison participants with TLE only. Significant grey matter reduction was found bilaterally in those with TLE+psychosis in the temporal lobes in the inferior, middle and superior temporal gyri and fusiform gyri, and unilaterally in the left parahippocampal gyrus and hippocampus. Significant extratemporal grey matter reduction was also found bilaterally in the insula, cerebellum, caudate nuclei and in the right cingulum and left inferior parietal lobule.

Additionally, significant white matter reduction in those with TLE+psychosis was found bilaterally in the hippocampus, parahippocampal/fusiform gyri, middle/inferior temporal gyri, cingulum, corpus callosum, posterior thalamic radiation, anterior limb of internal capsule and white matter fibres from the caudate...
nuclei, and unilaterally in the left lingual gyrus and right midbrain and superior temporal gyrus. Overall, significant grey and white matter deficits were found in TLE+psychosis which encompassed the medial temporal lobe structures but also extended to lateral temporal and extratemporal regions where some of these deficits overlap with those also found in schizophrenia.

As Study 2 utilised a retrospective recruitment approach, future work may wish to consider a prospective study design and to utilise objective rating scales in the assessment of comorbid psychosis. However, as a result of the application of strict exclusion criteria and similar to Study 1, the recruitment strategy resulted in a limited number of subjects and therefore future work should attempt to prospectively include larger groups of subjects while acknowledging that such recruitment may require an extended duration of time. Also, healthy comparison subjects were not included in the current study but would however form a useful addition to future work to enable differentiation of brain changes across both healthy and disease groups. Additionally, TLE+psychosis should be compared to subjects with schizophrenia in additional work so as to establish grey and white matter deficits that may be common to both schizophrenia and the psychoses related to TLE and also to delineate brain differences that may be specific to psychosis comorbid with epilepsy. Further, although postictal and interictal psychoses and lesional and non-lesional TLE were studied as combined groups, future studies may wish to characterise neuroanatomical changes in these disorders separately but where such stratification may result in even smaller sample groups.
As regions with significant volumetric deficits were found in Study 2, these may form the basis of future ROIs to be investigated in further studies. Additionally, given the finding of greater white matter content deficits relative to grey matter in TLE+psychosis, assessment of white matter microstructure and connectivity should therefore be considered in future studies using DT-MRI as has been applied to other disorders such as 22q11DS where white matter is preferentially affected. Also, apart from the application of advanced neuroimaging techniques such as DT-MRI, fMRI may be concurrently acquired so as to uncover any associated neuropsychological deficits that are present in TLE+psychosis. Further, studies are beginning to emerge assessing cortical thickness using conventional MRI as has been applied in a recent report of postictal psychosis (DuBois et al., 2011); additional work is required using such grey matter cortical surface reconstruction methods to establish neural endophenotypes in TLE+psychosis. Cortical surface work in conjunction with volumetric assessment may then contribute to the diagnosis of psychiatric disorder in TLE through the use of support vector machine approaches that are derived from computer science and machine learning which enable MRI pattern recognition so as to classify disorders; these techniques have been recently applied to autism and neurodegenerative disorders (Ecker et al., 2010; Kloppel et al., 2008) and add to the MRI methods that may be used in the study of psychiatric disorder.

While Study 2 recruited an adult cohort with TLE, Study 3 recruited instead a cohort of adult males with ASPD from forensic settings who had been assessed with the PCL-R to ascertain severity of psychopathic traits. Additionally, subjects were assessed by clinical interview so as to rule out comorbid psychiatric disorder. Brain analysis was not limited to specific fronto-temporal structures as in previous work.
but rather, involved assessment of the whole brain. Voxel-based analyses were performed on maps of white matter FA acquired with DT-MRI to compare 15 adults with ASPD and healthy age-, handedness-, and IQ-matched controls. Also, within ASPD subjects, differences in FA were related to PCL-R measures. Significant white matter FA reduction was found in ASPD subjects relative to controls whereby FA was bilaterally reduced in the genu of corpus callosum while in the right frontal lobe FA reduction was found in the uncinate fasciculus, inferior fronto-occipital fasciculus, anterior corona radiata and anterior limb and genu of the internal capsule. These differences significantly and inversely correlated with measures of psychopathy. While FA reduction was also found in posterior regions of the left hemisphere, these did not correlates with psychopathy scores. Such FA deficits were found in the temporo-occipital course of the inferior longitudinal fasciculus and inferior fronto-occipital fasciculus, in addition to the retrolenticular section and posterior thalamic radiation of the internal capsule. Overall, FA deficits are reported in tracts involved in interhemispheric and posterior brain networks as well as frontal lobe connectivity. Although Study 3 confirms a previous report of reduced FA in the uncinate fasciculus whereby the latter study was based on tractography of frontal lobe white matter bundles (Craig et al., 2009), however, the current study assessed the whole brain instead of being limited to only frontal white matter pathways.

Also, given that Study 3 is based on a cross-sectional design, future studies should aim to longitudinally assess white matter integrity from childhood onwards. This will help ascertain whether white matter anomalies predate detrimental lifestyle factors such as substance use that frequently coexist in antisocial populations. Additionally, in order to match groups more closely, future studies would benefit
from using non-psychopathic/non-ASPD offenders or patients as controls, rather than the healthy community sample used here as this would minimise potential confounds, including the higher incidence of substance misuse disorders, and differing lifestyle and socio-demographic factors.

While structured interviews were conducted in controls to rule out comorbid psychiatric disorder, future studies should aim to apply objective measures such as the PCL-R or the shorter PCL-SV in healthy controls. Moreover, volumetry and neuropsychology measures were not assessed in the current study and should be considered in future VBM and fMRI work respectively and in conjunction with DT-MRI applied to the same sample (as conducted in Study 1) so as to clarify the functional significance of any deficits found. Overall, while the current study represents the largest cohort of adults with ASPD analysed by DT-MRI to date, prospectively recruiting suitable participants from forensic environments also poses a significant challenge.

For Study 4, a group of adults specifically with Asperger syndrome (as opposed to autism spectrum disorder) was recruited. Diagnosis was based on structured clinical interviews and the application of gold-standard psychological assessment tools either involving subjects themselves or a parent/guardian. Subjects were also screened to exclude psychiatric disorder and haematological/biochemical/chromosomal abnormalities. Whole-brain DT-MRI datasets were acquired and white matter microstructural differences were assessed based on FA maps derived from 13 adults.
with Asperger syndrome who were compared to 13 healthy age-, IQ-, and gender-matched controls using voxel-based analyses.

Adults with Asperger syndrome were found to have significantly lower FA than controls in a number of white matter tracts where these abnormalities were largely bilateral and where FA deficits were found in all cerebral lobes. Further, FA deficits were also found in sublobar regions such as the internal capsule and cingulum in addition to significant FA reduction in the corpus callosum. Overall, the findings suggest that people with Asperger syndrome not only demonstrate impairments in white matter microstructural integrity and connectivity in regions relevant to social skills and behaviour but also have widespread deficits where these abnormalities appear to persist into adulthood.

While these findings are reported for the first time in a homogeneous sample encompassing only adults with Asperger syndrome and add to increasing evidence that people with Asperger syndrome have significant differences in brain connectivity in regions responsible for social behaviour, interaction and communication, Study 4 was based on a cross-sectional design that involved a relatively small sample size and there was a lack of ADI-R/ADOS-G measures in two subjects. Future studies should aim to diagnose Asperger syndrome in childhood while following-up these individuals during their adolescence and adulthood so as to better ascertain brain maturation changes that may be occurring throughout their development as similarly suggested for Study 3. Also, where possible, both VBM and DT-MRI datasets should be acquired in conjunction so as to permit the concurrent investigation of potential volumetric and microstructural brain
abnormalities in addition to fMRI that may simultaneously be acquired in Asperger syndrome so as to clarify the neuropsychological substrates associated with clinical deficits.

Finally, while normal white matter pathways are gradually being established in healthy samples (Thiebaut de Schotten et al., 2011), future work is also required in assessing healthy brain maturation throughout its various developmental phases. Indeed, healthy brain development continues to occur throughout and beyond childhood and adolescence but investigation of such processes are presently lacking (Lebel and Beaulieu, 2011) and future studies are necessary to better inform differences seen in psychiatric, neurological and neurodegenerative disorders.

7.3. Conventional MRI and VBM considerations

In the study of grey and white matter volume differences, VBM has a number of advantages over ROI techniques. These include an increased level of objectivity as it is automated, operator-independent and unbiased. Further, VBM permits investigation of the constituent tissue classes of the brain on a global level rather than a limited number of ROIs as it is not dependent on a priori hypotheses. Moreover, VBM enables the detection of subtle brain abnormalities, is less time-consuming and permits a larger number of individuals to be studied simultaneously. However, VBM is not without its pitfalls and therefore when using VBM, there are several caveats.
For instance, the choice of brain template for registration of subject images may bias results as the templates often used in imaging studies are based on healthy populations. When registering brains with known pathology with a healthy brain template, misalignment may occur and can therefore result in registration biases during the normalisation step. Moreover, the segmentation of brain images usually takes the form of either an initial tissue classification (tissue intensities determine tissue class assignment) or registration with a template (subject brain images are warped to match a standardised brain template); however, when either of these processes are conducted in isolation, these may lead to biases. Therefore, these steps were unified in Studies 1 and 2 into a single generative probabilistic framework through the use of SPM5 in order to overcome brain image alignment, intensity inhomogeneities and segmentation issues (Ashburner and Friston, 2005). However, other issues may potentially occur prior to spatial normalisation.

These include incomplete removal of skull and/or dura during the skull stripping phase which may result in inaccurate classification of tissue. Similarly, diseased brains and intensity variations may contribute to difficulties in tissue segmentation where for example a neoplastic lesion may be mislabelled as being either grey or white matter and therefore the exclusion of such subjects in Study 2 while recognising the contribution of brain tumours to the development of psychoses associated with TLE. During the tissue segmentation step, the accuracy of classification of constituent brain tissue classes may also be dependent on the software and indeed the version of the particular software used e.g. SPM96 vs. SPM2.
In this thesis, SPM2 was only used in the creation of study-specific brain templates derived from all subjects under study and normalisation of their DT-MRI data whereas SPM5 was utilised in the investigation of maps of grey and white matter as it integrates tissue classification, bias correction, and non-linear warping within the same framework (Ashburner and Friston, 2005). A newer version of SPM has since been made available following commencement of this work in the form of SPM8 (http://www.fil.ion.ucl.ac.uk/spm/software/spm8/) with updated algorithms. Further, new toolboxes which aim to enhance registration within SPM5 such as the Diffeomorphic Anatomical Registration Through Exponentiated Lie Algebra (DARTEL, (Ashburner, 2007)) toolbox have also been developed. DARTEL is a high dimensional warping process that aims to increase the precision of inter-subject alignment and localisation of differences while providing better parameterisation of brain shapes. However, some studies have argued that improvements in subject alignment may be due to optimisation of brain morphometry contrasts instead of registration differences while variable velocity diffeomorphic models may be superior to fixed velocity DARTEL registration (Ashburner, 2007; McLaren et al., 2010; Miller et al., 2006; Vaillant et al., 2004). Future studies may wish to consider implementation of SPM8 together with such toolboxes which overall serve to improve localisation and sensitivity of analyses.

The statistical analysis framework used with SPM is based on parametric statistics and where the choice of a Gaussian smoothing kernel size may affect findings. Typically, the smoothing filter needs to correspond to the expected effect size (matched filter). However, Studies 1 and 2 represent novel analyses where the effect sizes are unknown. Also, following image registration and segmentation with SPM5,
non-parametric statistics were used via XBAMM that were not dependent on kernel size. Therefore, a matched filter poses less of an issue and future studies may wish to use similar approaches that exploit the use of non-parametric statistical testing models that overcome distributional assumptions with VBM (Salmond et al., 2002).

7.4. DT-MRI and voxel-based analysis considerations

Given that the experimental studies in this thesis represent preliminary analyses, white matter microstructural integrity was assessed on a whole brain level based on FA abnormalities. In future, in-vivo fibre tractography may be attempted to distinguish between the multiple tracts that run though the regions that were shown to be abnormal. For instance, in the frontal lobe, it can be difficult to demarcate the uncinate fasciculus from fibres of the inferior fronto-occipital fasciculus and corpus callosum. Also, investigation of tract-specific FA values (along with other indices such as MD; see Chapter 1, Section 1.1.3.) over the ROIs defined by multi-fibre tractography algorithms may help elucidate the reasons both for the FA changes seen and for their correlation with behavioural measures. Additionally, the computation algorithms, mathematics and physics underlying MRI need to be considered.

For example, in the field of numerical analysis, the condition number of a function relative to a factor measures the asymptotically worst case of how much the function changes in proportion to small changes in the factor. When using the condition number of the transformation matrix as a measure of noise performance, a DT-MRI
scheme was used in this thesis with a low condition number and therefore high signal to noise ratio (Jones et al., 1999; Jones et al., 2002a; Skare et al., 2000). Also, while k-space samples spatial position, the concept of q-space has been introduced which does not assume a Gaussian shape for the underlying probability density function of molecular diffusion (Callaghan et al., 1999; Cory and Garroway, 1990). Q-space when used with 3-D q-space MRI, DT-MRI and diffusion spectrum imaging allows sampling of the space of the diffusion tensor, spin displacements and dispersion tensor (Basser, 2002; Callaghan, 1991; Wedeen et al., 2005). Indeed, diffusion spectrum imaging is a relatively new technique that samples both k- and q-spaces simultaneously and is a true six-dimensional (6-D) imaging approach that assesses spatial position and displacement but which also permits the resolution of white matter tract intersection (Hagmann et al., 2006; Wedeen et al., 2005) (see Chapter 1, Section 1.1.4.1.) and is of particular relevance to future studies involving \textit{in-vivo} tractography.

Similar to the DT-MRI acquisition scheme used in this thesis (Chapter 2, Table 2.1), tract demarcation with diffusion spectrum imaging is achieved via high angular resolution that is dependent on many image acquisitions at each slice location (usually of the order of 500) but imposes a relatively longer acquisition time in comparison to DT-MRI and therefore potential exposure to artefacts such as movement. However, acquisition and analytical protocols are continuously being optimised to enable evaluation of subjects using MRI scanners with higher field strengths, improved signal to noise ratio, shorter acquisition times and improved spatial and temporal resolution (Hetzer et al., 2011) which may in future enable the application of diffusion spectrum imaging at higher resolutions and with shorter
acquisition sequences. While the acquisition methods used in this work were based on rank two tensors typically used with DT-MRI, they differ from standard DT-MRI protocols (Hagmann et al., 2006) given the large number of diffusion encoding gradients that were applied at each slice location ($64 \text{ vs. } \leq 6$) and are therefore conceptually similar to high angular resolution diffusion imaging (HARDI) methods (Ozarslan and Mareci, 2003) but where HARDI allows for the investigation of greater than two rank tensors.

A further approach that assesses multiple white matter tracts simultaneously while overcoming subject image registration difficulties is tract-based spatial statistics (TBSS) (Smith et al., 2006). The method involves an approximate non-linear initial registration which is then followed by projection onto an alignment-invariant tract representation known as the ‘mean FA skeleton’ which constitutes the centres of all fibre bundles. Although TBSS also overcomes the need for spatial smoothing in image processing, however, it may be insensitive to degeneration or loss of integrity in the periphery of white matter bundles since only maximal FA values from the centres of specific tracts are extracted and analysed. While the methodology used in this thesis is similar to TBSS in that it overcomes subject image registration difficulties and is not dependent on smoothing kernel size, TBSS provides complementary information to SPM/XBAM based methods (Abe et al., 2010; Focke et al., 2008) and therefore their joint use should be considered in future studies.

Further, although studies in this work and TBSS use MNI space for image registration, clusters of significant differences found in this thesis are finally reported
in Talairach space because it is the most commonly used reference grid system for reporting neuroimaging differences in the literature. While the software packages used in this thesis (XBAM and XBAMM for DT-MRI and conventional MRI data respectively) generated coordinates based on MNI space that were then converted to Talairach space, conversion using the Brett transform (Brett et al., 2002) (see Chapter 2, Sections 2.6.2. and 2.8.4.) is commonly utilised by neuroimaging groups while acknowledging that there are brain morphometry differences between these coordinate systems and potentially some inaccuracies with the Brett transform. The Talairach system is based on a single-subject post-mortem brain while MNI space is derived \textit{in-vivo} from an average of 152 normal subjects; future studies may wish to avail of updated standard-space conversion algorithms (Chau and McIntosh, 2005; Lancaster et al., 2007). Finally, development of a standard-space mean FA template image would aid in homogenising analyses not only based on TBSS between research groups but also those utilising SPM/XBAM given that there are currently no standard-space FA registration templates for use with these software packages.

7.5. Final conclusions

As little is currently known regarding aetiology of psychiatric disorder, there is a significant need to establish causative mechanisms. This is in keeping with psychiatry as a specialty moving away from classification of a disorder based on phenomenological or behavioural descriptions alone to defining diseases based on an additional neurobiological substrate. However, psychiatric disorders represent a
heterogeneous group with some being explored more exhaustively than others (as outlined in Chapter 1, Section 1.4.). MRI coupled with newer analytical approaches such as voxel-based methods show significant promise in unravelling the aetiology of psychiatric disorders (Bandettini, 2009), however, such advanced techniques have only been applied on a limited basis in the study of psychiatric disorders. These methods while enabling the potential identification of endophenotypes, also inform on causation. The determination of such neurobiological abnormalities ultimately enhance diagnostic precision which may then aid in the clinical provision of more appropriate and focused treatments.

In Study 1, children and adolescents with 22q11DS demonstrated significant white matter deficits encompassing both FA and volume whereby white matter abnormalities appear to be a cardinal feature of the syndrome. Also, given that 22q11DS is associated with gene haploinsufficiency, COMT polymorphism may contribute to brain maturation differences with Val-COMT associated with reduced brain development in a number of frontal and interhemispheric brain regions. Further, schizotypy in 22q11DS is associated with abnormalities in a number of interhemispheric and posterior brain white matter tracts where these abnormalities may also contribute to schizophrenia spectrum disorder not only seen in 22q11DS but also in the non-deleted population.

Similarly, although Study 2 also assessed a population with psychosis, analyses were based instead on whole-brain assessment of grey and white matter volume in those with an epileptic disorder using VBM. Adults with TLE+psychosis were found to have volume deficits in both grey and white matter tissue; however, white matter
volume was proportionally reduced to a greater extent than grey matter. As volume reduction of both grey and white matter was found in medial and lateral temporal lobe brain regions in addition to extratemporal brain areas where the pattern of volume deficits overlapped with those seen in schizophrenia, therefore, these regional volume deficits may also be relevant to the non-epileptic population with psychosis. Further, given that 22q11DS and TLE are both associated with psychosis and with white matter affected to a greater extent in these disorders, deficits in white matter may particularly underlie psychotic disorders in both genetically deleted and non-deleted populations. Therefore, in future, assessment of white matter integrity and volume may be particularly relevant in diagnosing and addressing psychotic disorder.

White matter FA abnormalities were also found in Study 3 in adults with ASPD. While significant white matter FA deficits were found in frontal, interhemispheric and posterior brain networks, however, only FA deficits in frontal and interhemispheric brain regions correlated negatively with objective psychopathy measures indicating the contribution of these regions to behavioural impairment in ASPD. Given that the diagnosis of personality disorder is currently based on behavioural criteria, the presence of specific regional white matter deficits provides a future substrate for neurobiological diagnosis of ASPD. Diagnosis and subsequent treatment of the disorder may also be enhanced through the application of relevant emotional or cognitive neuropsychological tasks that are mediated by areas that have been shown to be abnormal in Study 3.
Finally, in Study 4, adults with Asperger syndrome also demonstrated significant FA deficits that were widespread and present in all cerebral lobes in conjunction with sublobar and interhemispheric brain regions. Extensive white matter deficits appear to be a hallmark of Asperger syndrome and where future diagnosis of the disorder may therefore be supported by the finding of diffuse white matter FA abnormalities in combination with appropriate neuropsychological, behavioural and developmental investigations.


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Appendix 1: Publications


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BRIEF HISTORICAL PERSPECTIVE

Velocardiofacial syndrome (VCFS) is the most common human genetic deletion syndrome and is associated with deletions in chromosome 22. VCFS was once considered a rare congenital disorder but since the advent of molecular genetics, this view has now been countered. In 1992, a major breakthrough occurred in the study of VCFS and disorders related to chromosome 22 when deletions were specifically localized to the long arm of chromosome 22 (22q11) (Scambler et al., 1992). Subsequently, several reports followed which confirmed the microdeletion in chromosome 22 (Driscoll et al., 1992; Kelly et al., 1993). Tremendous interest in the syndrome still continues today as it is a complex disorder that affects essentially every organ system in the body and psychiatric disturbance is prominent.

Individuals with clinical symptoms of VCFS were described some 50 years ago by Sedlackova (Sedlackova, 1955) in Czechoslovakia and later by Strong (Strong, 1968). However, the earliest use of the term VCFS and categorization as a syndrome did not occur until the 1970s (Shprintzen et al., 1978) and it was not until the early 1990s that research interest in the disorder really emerged. It is possible that VCFS was not formally categorized as a distinct syndrome even earlier because children with the disorder experienced multiple physical complications such as congenital heart defects that resulted in early death. Also, a syndrome is defined as a condition with multiple anomalies, all of which originate from a single cause and it was not until 1992 that the genetic deletion was identified at the 22q11.2 band. Furthermore, individuals with VCFS often presented to a variety of therapeutic disciplines, with each focusing on their own explicit area of expertise rather than the integrated approach that is currently practiced.

Though the syndrome is widely known as VCFS, it is also known as 22q11 deletion syndrome, Sedlackova syndrome, DiGeorge syndrome, Shprintzen syndrome, Cayler syndrome, Takao syndrome and conotruncal anomaly face syndrome amongst others. These nosologic labels represent not only the
extensive variety of academic disciplines involved in the study of VCFS but also the possibility that competing research teams have each advocated the use of their preferred titles. As some of these nosologic labels have been provided by specialists in their own circumscribed field of study, they may not be entirely reflective of the full spectrum of the syndrome. Consequently, researchers and clinicians may have mistakenly believed that these labels represent discrete syndromes that are each caused by the same underlying deletion of chromosome 22 (Robin & Shprintzen, 2005). For the rest of the chapter, the syndrome will be referred to by its current description of 22q11 deletion syndrome (22q11DS).

**CAUSATION**

The majority of cases of 22q11DS are associated with an interstitial deletion of chromosome 22q11 (Carey et al., 1992). However, other genetic abnormalities involving chromosome 22q11, such as balanced translocations, terminal deletions, non-random rearrangements and mosaicism have been reported in a minority to contribute to the disorder. While the majority of deletions occur de novo, 5 to 10 per cent of 22q11DS probands show an autosomal dominant pattern of inheritance for the deletion (Shprintzen et al., 1981; Ryan et al., 1997). There are also rare instances when individuals may possess the typical 22q11DS phenotype but lack chromosome 22 deletions.

22q11DS is regarded as a gene haploinsufficiency syndrome as a result of the deletion being carried in only one arm of chromosome 22 (Lindsay, 2001). Of the 22q11DS cases with chromosome 22 deletion, 90 per cent have a 3 Mb interstitial deletion, known as the typically deleted region, while the remainder has a smaller or 'nested' deletion (1.5 to 2 Mb). Though chromosome 22 is regarded as one of the smallest autosomes in the human genome, the long arm of chromosome 22 contains a large number of genes (of the order of 40 genes). However, no specific gene has been identified as the causative mechanism that may fully explain all the features of the disorder (Scambler, 2000).

The genes in the typically deleted region are thought to play a critical role in neural crest development and migration and therefore...
in the formation of the third and fourth pharyngeal arches/pouches and cardiac outflow tract. Thus, the structures predominantly affected in 22q11DS are to some extent derived from the branchial arch/pharyngeal pouch structures for example the thymus gland, parathyroids and face.

**DIAGNOSTIC CRITERIA**

22q11DS has an extremely broad phenotypic spectrum, with more than 180 clinical characteristics encompassing both physical and behavioral attributes (Robin & Shprintzen, 2005; Shprintzen et al., 2005). Furthermore, as no single clinical feature occurs in all 22q11DS cases and there are no reported cases of the syndrome that have all or even most of the clinical findings, diagnosis through clinical criteria alone has proven unreliable. Therefore, as the syndrome shows marked variability in phenotypic expression, recent advances in molecular genetics have permitted a more robust identification of 22q11DS probands.

As 22q11DS is caused by a microdeletion of chromosome 22 at the q11.2 band, it is possible to detect this genetic deletion through a variety of cytogenetic laboratory techniques. Karyotyping is a method that assesses the physical structure of all chromosomes and has the potential to detect large chromosomal rearrangements or deletions. However, the majority of deletions that cause 22q11DS are too small to be assessed by karyotyping alone. Therefore, fluorescence in situ hybridization (FISH) or multiplex ligation-dependent probe amplification (MLPA) may be better suited for accurate diagnosis. Although FISH is commonly used for diagnosis (Robin & Shprintzen, 2005), chromosomal microarray analysis is gradually replacing FISH as the first line diagnostic tool for genomic disorders in general.

FISH directly assesses the area on chromosome 22 that is related to 22q11DS namely the 3 Mb typically deleted region (TDR). With FISH, chromosome preparations are obtained from peripheral blood samples that have been denatured to allow hybridization of a probe specific to the TDR. Probes are stained with a dye and under proper laboratory conditions these fluorescent probes will bind to the corresponding area of chromosome 22q11. For persons having two normal (non-deleted) chromosome 22s, two stained probes appear with fluorescence microscopy. However, if there is a deletion on one of the chromosome 22s, there is lack of a substrate for the probe to adhere to and therefore only one probe appears (which is the case in 22q11DS).

The decision to perform genomic testing for the diagnosis of 22q11DS depends on clinical suspicion and the degree of clinical experience in developmental disorders. When major congenital heart anomalies are present, especially in the form of conotruncal anomalies and ventricular septal defects (VSDs), the likelihood of diagnosing 22q11DS is much higher.

The large majority of individuals with 22q11DS (90 per cent) have de novo mutations where neither parent is affected. However, if a couple already has one child with 22q11DS or a parent is a known carrier of a 22q11 deletion, they may wish to know if a future pregnancy may be affected. Therefore testing can be performed during the pregnancy to assess for the presence of deletion through chorionic villus sampling (CVS) or amniocentesis. CVS is performed at an earlier stage than amniocentesis and usually at weeks 10 to 12 of gestation. With CVS, a sample of the placenta is taken either through the abdomen or cervix and is associated with approximately one per cent chance of miscarriage. Amniocentesis is a more commonly performed procedure after 15 weeks gestation whereby amniotic fluid is obtained through the abdomen and is associated with a lower miscarriage rate. However, prenatal testing can only distinguish whether a deletion is present or absent and not the degree to which a fetus may be affected. A further option for a suspected pregnancy with
22q11DS is a fetal ultrasound/echocardiogram, which assesses the structure and the circulatory flow of and through the heart. As cardiac defects are found in a substantial proportion of people with 22q11DS (70 to 80 per cent), a fetal echocardiogram can be performed after 18 weeks gestation, when the cardiac structures have been formed.

**CORE CHARACTERISTICS**

**Physical**

The description of the 22q11DS clinical phenotype has undergone an evolution ever since 22q11DS was classified as a syndrome. Currently, 22q11DS is recognized to demonstrate an expansive phenotype with more than 180 clinical features that affect practically every organ and system (Robin & Shprintzen, 2005). As the phenotypic expression shows high variability, some individuals at the mildest end of the spectrum are indistinguishable from normal while the most severe cases can experience life-threatening disorders. The most commonly occurring physical characteristics are palatal anomalies, pharyngeal insufficiency, congenital cardiac defects, vascular abnormalities, immunodeficiency, thymic hypoplasia and renal anomalies. These defects are often associated with small stature, hypotonia, facial dysmorphology and slender hands and digits (Goldberg et al., 1993).

The facial dysmorphology in 22q11DS is typically characterized by a long face with upslanting eyes, widened nasal bridge with a prominent nasal tip, small ears with overfurred helices and a small mouth associated with cleft lip and/or palate (Shprintzen, 2000a). Palatal abnormalities are common and the most frequently occurring are submucous cleft palate and occult submucous cleft palate (Shprintzen, 2000b).

Another striking feature is the presence of cardiac anomalies and after Down syndrome, 22q11DS is the commonest cause of heart defects (Glover, 1995). These are related to the maldevelopment of the conotruncus, embryonic aortic arch structures and the ventricular septum (Goldmuntz et al., 1998) and include such defects as tetralogy of Fallot, interrupted aortic arch, truncus arteriosus, aortic arch anomalies and VSDs. Congenital heart disease is present in approximately 70 per cent of cases and the type of heart anomaly may lead to stronger suspicion of 22q11DS than any other diagnosis (Shprintzen, 2008) as the cardiac phenotypes in 22q11DS are associated with hemizygosity of the gene TBX1 that is expressed on chromosome 22 (Merscher et al., 2001). Approximately a quarter of people with 22q11DS also display abnormalities of the internal carotid arteries such as enlargement, medial displacement or tortuosity (Goldberg et al., 1993).

Immunodeficiency is also very common in 22q11DS (60 to 77 per cent) and was observed as a component of the disorder as early as the 1960s. Although a proportion of children with 22q11DS have an absent thymus, most individuals have thymic tissue located in aberrant locations. This results in a lower number of T cells than normal but not complete aplasia and often leads to an increased prevalence of serious infections such as recurrent bronchitis or sinusitis. Furthermore, 22q11DS was recently found to be significantly associated with eczema and asthma but not with allergic rhinitis (Staple et al., 2005).

Other systems affected in 22q11DS include nephro-urologic, gastrointestinal and ophthalmologic with abnormalities such as multicystic renal dysplasia, renal agenesis, anal stenosis or intestinal malrotation. Tortuosity of the vessels in the retina has also been reported but may not be related to the presence of cardiac anomalies (Abel & O’Leary, 1997).

**Cognition**

General intellectual impairment is common in 22q11DS, with full-scale IQ (FSIQ) scores usually lying within the moderate intellectually disabled to the normal range with a mean of 70 (Swillen et al., 1997). Also, 22q11DS
individuals with a familial deletion are found to have a lower mean FSIQ than individuals with a de novo deletion (Swillen et al., 1997; Gerdes et al., 1999). Cardiac defects, vascular abnormalities and physical comorbidities have been postulated as a causative mechanism for lower IQ in 22q11DS. However, there does not appear to be a well established association between such lesions and intellectual ability (Swillen et al., 1997; De Smedt et al., 2003). There are also reports of a discrepancy between verbal and performance IQ in 22q11DS where verbal IQ (VIQ) exceeds performance IQ (PIQ) and therefore the intellectual disability has been described as a non-verbal learning disability (NVLD) (Swillen et al., 1997; Gerdes et al., 1999; Moss et al., 1999). Such NVLD may include significant deficits in visuospatial skills and non-verbal memory functioning (Lajiness-O'Neill et al., 2006). Some of the visuospatial memory deficits that are described have been in the domains of immediate and delayed memory which contrast with relatively preserved object memory and rote verbal memory (Bearden et al., 2001). Children with 22q11DS are also observed to perform less well in tasks that involve arithmetic in comparison to reading and spelling (Swillen et al., 1999a; Wang et al., 2000). Visual object perception and spatial cognition are other non-verbal abilities that are impaired in 22q11DS and the latter has been found to be related to impaired mathematical abilities.

In one report on educational attainments, Moss and colleagues (1999) excluded individuals with an FSIQ below 70 from the analysis, so as to avoid the potential confounding of intellectual disability. However, the same psych-educational patterns emerged in this more homogenous group where VIQ > PIQ and additionally, reading and spelling > maths abilities suggesting that this profile is indeed characteristic of a 22q11 deletion rather than a consequence of a more general intellectual disability. Following comprehensive neuropsychological assessments of 22q11DS adults with schizophrenia and those without the psychiatric disorder, van Amelsvoort et al. (2004a) suggested that developmental brain abnormalities, for instance in the frontal lobe, may predispose to the development of schizophrenia in 22q11DS individuals.

As the frontal lobe is extensively involved in executive function (the coordination of cognitive processes involved in the execution of complex tasks), it has been proposed that abnormalities in the frontal lobe lead to difficulties in, for example, inhibiting inappropriate responses, generating novel responses or devising problem-solving strategies. In 22q11DS, deficits have also been found in planning that have been associated with increased impulsivity and poorer problem-solving skills (Henry et al., 2002). Attention, concentration and tracking deficits have also been implicated in the behavioral profile of individuals with 22q11DS in a number of studies (Gerdes et al., 1999; Woodin et al., 2001). In particular, the behavioral pattern of children and adolescents is suggestive of impaired attentional networks. It is possible that significant deficits affecting these attentional networks may consequently lead to marked behavioral problems. However, most studies so far have used different tasks to assess attentional abilities, which makes it difficult to ascertain the exact nature of these impairments.

There are, however, also areas of relative strength in the 22q11DS cognitive profile. For example, auditory/verbal rote memory skills are usually relatively preserved in 22q11DS (Wang et al., 2000; Bearden et al., 2001). Other domains that are relatively preserved are reading (decoding), spelling and phonological processing skills (Moss et al., 1999; Woodin et al., 2001). Overall, an understanding of the neurocognitive processes characterizing 22q11DS and the associated behavioral outcomes is essential in order for clinicians to implement early, appropriate and targeted interventions.

**Linguistic**

Delayed speech and language development is characteristic of 22q11DS. Receptive
language ability often exceeds expressive language ability and pose a significant concern for parents (Golding-Kushner et al., 1985; Gerdes et al., 1999). Although early development in 22q11DS is often characterized by mild delay in most areas, expressive language is often specifically delayed compared with other milestones. Speech impairments are very likely multifactorial in origin and linked to factors such as velopharyngeal insufficiency and developmental delay. Children with 22q11DS also demonstrate associated speech abnormalities such as hoarseness, compensatory articulation errors and a high-pitched voice.

Furthermore, language development in 22q11DS is thought to follow a different developmental trajectory compared with other groups of children. For instance, when 22q11DS children are compared to children with cleft lip/palate, increasing severity of impairments can be seen up to three years of age (Scherer et al., 1999) and these children can be non-verbal up to 30 months of age. Subsequently, 22q11DS children often show dramatic improvement between three and four years of age (Solot et al., 2001) and by school age, expressive language and speech improve (perhaps as a result of intervention) although specific language impairment persists. In addition, higher-order receptive language skills involving abstract thinking remain poorly developed, thereby affecting both communication and academic skills.

The process by which one's voice is produced is termed phonation and also appears to be abnormal in 22q11DS. Phonation is normally characterized by volume, quality and pitch and is dependent on the articulatory apparatus and respiration. In 22q11DS there is a larger prevalence of laryngeal webs, which shortens the length of the vibratory component of the vocal cords and impairs movement of the vocal folds and thereby results in hoarseness and an increased vocal pitch. A large majority of 22q11DS individuals (75 per cent) are also hypernasal owing to velopharyngeal insufficiency despite palatal repair, which may be perhaps accounted for by shrunken or absent adenoids (Havkin et al., 2000).

**Behavioral and psychiatric disorder**

Although the variety of psychiatric presentations in 22q11DS is heterogeneous, high rates of psychiatric morbidity have been reported ever since it was described as a genetic disorder (Shprintzen et al., 1992). Early studies of children with 22q11DS reported a characteristic personality with poor social interaction, both quantitatively and qualitatively, that was associated with a bland affect with minimal facial expression. It was also reported that children often exhibited extremes of behavior: notably, behavior that was disinhibited/impulsive or serious/shy (Golding-Kushner et al., 1985; Swillen et al., 1997). Parents of 22q11DS children up to the age of three years have often reported that their children present with somatic complaints, for example eating difficulties or withdrawn behavior. This social withdrawal often persists into adolescence and is compounded by attentional deficits and poor social skills, which may be accounted for by impaired communication abilities. Additional temperamental features described in children and adolescents with 22q11DS include an exaggerated response to threatening stimuli, and an enduring fearfulness of painful situations (Golding-Kushner et al., 1985). While only a limited number of studies have assessed the psychiatric profile in 22q11DS children, these have reported high rates of comorbid psychiatric disorder (Arnold et al., 2001; Feinstein et al., 2002; Baker & Skuse, 2005).

Children with 22q11DS are reported to have high levels of anxiety and depression (Golding-Kushner et al., 1985; Swillen et al., 1997) and to display a diverse range of psychiatric symptoms, the most prevalent of which are mood disruptions, attention deficits and psychotic phenomena that appear to be independent of intellectual impairment (Baker & Skuse, 2005). To exclude the
potential confounding effects of intellectual disability on mood and anxiety measures, primary school children with 22q11DS were compared to a group of children matched for intellectual ability and speech and language impairment (children with IQ < 70 were excluded from both groups) (Swillen et al., 2001). While both groups behaved similarly with respect to problematic social interaction, poor attention and anxiety, 22q11DS children were found to be more withdrawn and less aggressive compared to controls. This finding may suggest that 22q11DS children are more likely to show internalizing behaviors when compared to children at a similar developmental level but without the 22q11 deletion.

Mood disorder is common in 22q11DS children and adults and rates between 12 and 47 per cent have been reported in the literature (Papolos et al., 1996; Carlson et al., 1997; Murphy et al., 1999; Arnold et al., 2001). Although an increased rate of bipolar affective disorder (BPAD) was initially reported (Papolos et al., 1996), subsequent studies have described an unstable mood disorder instead of BPAD (Vogels et al., 2002). It is possible that pubertal changes, increased social demands and genetic predisposition may place 22q11DS individuals at a heightened risk of developing anxiety and depressive disorders (Swillen et al., 1999b).

Attention-deficit hyperactivity disorder (ADHD) is common, with rates between 35 and 46 per cent in children and young adults (Niklasson et al., 2001; Feinstein et al., 2002; Gothelf et al., 2004a) and perhaps is the most common psychiatric disorder in 22q11DS (Antshel et al., 2006; Zagursky et al., 2006). This is in marked contrast to an estimated prevalence of between three and seven per cent for non-deleted school-aged children (Polanczyk et al., 2007).

Rates of psychotic disorders such as schizophrenia and schizotypy are also significantly increased, at between 20 and 30 per cent in 22q11DS, a rate approximately 25 times greater than that in the general population (Pulver et al., 1994; Murphy et al., 1999). Deletion of 22q11.2 is the third highest risk factor for the development of schizophrenia, with only a greater risk conferred by being the child of two parents with schizophrenia or the monozygotic co-twin of an affected individual. More recently, a report suggested that the development of psychotic disorders in 22q11DS is a gradual process, with an initial presentation of sub-threshold psychotic symptoms in childhood (Gothelf et al., 2007a). Furthermore, 22q11DS children display more serious psychiatric symptoms as they go through adolescence compared to children with idiopathic learning disability.

Higher rates of autism spectrum disorder in 22q11DS have also been reported at between 14 and 50 per cent (Niklasson et al., 2001; Fine et al., 2005; Vorstman et al., 2006). Vorstman and colleagues suggest that autistic and psychotic disorders are major features of the behavioral phenotype in children with 22q11DS. The authors also suggested that the autistic symptoms identified in their study may be a reflection of the neurodevelopmental abnormalities in individuals with schizophrenia where autistic symptoms may represent prodromal features of psychosis rather than exclusively an autism spectrum disorder. Finally, other disorders, such as obsessive-compulsive disorder (OCD), have been described in several studies, with rates ranging from 8 to 33 per cent (Papolos et al., 1996; Gothelf et al., 2004b).

DIFFERENTIAL DIAGNOSIS AND ASSOCIATIONS

Although 22q11DS shows broad phenotypic expression, some clinicians adopt a minimal diagnostic criteria or a probability approach to diagnosing genetic syndromes (Shprintzen, 2008). If this approach is employed in the absence of genetic confirmation, it is possible to diagnose someone who has cleft palate, intellectual disability, congenital heart defects, immunodeficiency, hypotonia,
developmental delay, and small ears with overfurled helices with 22q11DS while these clinical criteria may be equally applicable to trisomy 21/Down syndrome (see Chapter XXXX).

Some patients with 22q11DS may also be incorrectly diagnosed as having the CHARGE syndrome (i.e. coloboma of the eye, heart defects, atresia of the nasal choanae, retardation of growth and/or development, genital and/or urinary abnormalities, and ear abnormalities and deafness). Although CHARGE is related to a mutation or deletion in the chromodomain helicase DNA-binding protein-7 (CHD7) gene located on chromosome 8 (Vissers et al., 2004) and genetic testing available for this disorder, it is still a largely clinical diagnosis as only 60 per cent of patients tested have the CHD7 mutation (Lalani et al., 2006).

A further differential diagnosis to consider is another chromosome 22q deletion syndrome, i.e. the 22q13.3 syndrome (Phelan-McDermid syndrome or deletion 22q13 syndrome). This syndrome is a chromosome microdeletion syndrome, also on the long arm of chromosome 22 but in the 13.3 band. It is characterized by neonatal hypotonia, global developmental delay, normal to accelerated growth, absent to severely delayed speech and minor dysmorphic features. Although this syndrome is not routinely tested for, it can be confirmed via FISH or array comparative genomic hybridization (CGH) and should be considered in all cases of hypotonia of unknown etiology and in individuals with absent speech (Phelan, 2008).

Partial monosomy of chromosome 10p is a rare genetic disorder where a significant proportion of individuals with the disorder demonstrate features of 22q11DS. Its main characteristics are hypoparathyroidism, deafness and renal dysplasia; hence, it is also known as the HDR syndrome (Lichtner et al., 2000). However, those with HDR lack the cardiac, palatal and T cell abnormalities that are present in 22q11DS.

An environmental disorder which causes impairment in neural crest development and branchial arch abnormalities is isotretinoin treatment for acne. As a result of increased use of isotretinoin, there has been a concomitant increase of associated birth defects. Isotretinoin embryopathy produces a phenotype of 22q11DS (Coberly et al., 1996; Aggarwal & Morrow, 2008) and may be related to downregulation of TBX1 (Zhang et al., 2005).

**CURRENT RESEARCH**

**Genetic**

The chromosome 22q11.2 locus is rich in genes including those that are involved in neurotransmission and neurodevelopment. As the large majority of individuals with 22q11DS have approximately 40 genes deleted in the TDR, the process of determining the specific factors involved in the causation of both physical and psychiatric/behavioral disorders is currently underway.

The phenotype of 22q11DS is highly variable and there is no evidence presently to suggest that the size of the underlying deletion has any influence on the physical or behavioral phenotype. However, given that 22q11DS is a genetic disorder with a high prevalence of psychiatric disorders, identification of susceptibility genes that may contribute to psychiatric disorders in individuals with 22q11DS, may be relevant to the non-deleted general population (Murphy & Owen, 2001; Prasad et al., 2008).

Although there is also accumulating evidence that the genetic basis of psychiatric disorders is heterogeneous, several promising genes that may have implications for the general population have been highlighted in recent studies.

**Catechol-O-methyltransferase (COMT)**

The gene for catechol-O-methyltransferase (COMT), an enzyme that degrades dopamine, and COMT deletion can be mapped to the 22q11 region (Grossman et al., 1992). COMT
also appears to be a candidate gene that accounts for the higher levels of psychiatric morbidity seen in 22q11DS (Dunham et al., 1992; Craddock et al., 2006).

The gene coding for COMT encodes two distinct COMT isoenzymes: the membrane bound COMT (MB-COMT) and soluble COMT (S-COMT) (Bertocci et al., 1991). MB-COMT is primarily found in the brain, whereas S-COMT is found predominantly in peripheral tissue (Chen et al., 2004). The MB-COMT gene undergoes a naturally occurring polymorphism which has been reported to affect dopamine regulation (Akil et al., 2003). The polymorphism leads to an amino acid substitution (valine(Val) to methionine(Met)) at codon 158, whereas S-COMT has the same polymorphism at codon 108 (Lachman et al., 1996) and results in decreased thermostability and variable enzymatic activity. The Met allele of COMT has a lower activity compared with the Val allele, which results in the Met/Met variant of COMT displaying approximately 40 per cent less enzymatic activity than the Val homozygote, whereas heterozygotes possess intermediate levels of activity (Chen et al., 2004). As a consequence of chromosomal deletion, 22q11DS individuals possess only one working copy of the COMT gene (i.e. they are haploinsufficient), which results in either a high-activity Val or low-activity Met isoform of the COMT enzyme.

Dopamine degradation in the prefrontal cortex (PFC) is mediated primarily through COMT activity (Tunbridge et al., 2004) while monoamine oxidase (MAO) and the dopamine transporter (DAT) are largely responsible for dopamine degradation in brain regions elsewhere (Chen et al., 2004). COMT activity attains maximal levels in early adulthood, especially in the PFC region, and COMT is also thought to modulate brain development and function (Hoglinger et al., 2004; Zinkstok et al., 2006). Indeed, disrupted dopaminergic neurotransmission has been reported in adults with 22q11DS but without a psychiatric history (Boot et al., 2008) and this may explain their vulnerability for psychiatric disorders.

However, recent evidence suggests that the association between COMT and the risk of schizophrenia may be more complex than Met/Val polymorphism alone and may involve an association with other loci within COMT, a gene-gene interaction or an environmental interaction (Caspi et al., 2005; Williams et al., 2007).

**TBX1**

The **TBX1** gene is a member of the T-box gene family of transcription factors with more than 20 genes identified in humans (Packham & Brook, 2003). It maps to chromosome 22q11.2 and is recognized to contribute to regulation of developmental processes and has a role in modulating defects arising from the pharyngeal apparatus (Stoller & Epstein, 2005; Aggarwal & Morrow, 2008).

In murine studies, **TBX1** mutations can cause gene-dosage-dependent pharyngeal arch and pouch abnormalities, particularly affecting the cardiac and vascular structures (Vitelli et al., 2002). **TBX1** may also contribute to brain development as it has been found to be expressed in the mouse brain, although this expression is limited to the vasculature in term embryos and adult mice (Paylor et al., 2006). This study further reported that **TBX1** also contributes to prepulse inhibition deficits in 22q11DS, which is considered an endophenotype of schizophrenia (Braff & Light, 2005).

**Proline Dehydrogenase**

**PRODH** is a mitochondrial membrane enzyme that catalyses the first step in the proline degradation pathway (Bender et al., 2005). Proline is a non-essential amino-acid and may have a modulatory role in both glutaminergic and acetylcholinergic activity (Delwing et al., 2003). Hyperprolinemia has not only been documented in individuals with 22q11DS (McDermid & Morrow, 2002) but also in some individuals with schizophrenia (Liu et al., 2002). As discussed above, in 22q11DS, there is an increased risk for psychosis and learning
disability and recently, it has been shown in a hyperprolinemic mouse model, that an interaction between proline dehydrogenase and COMT could be involved in this phenotype (Raux et al., 2007). The authors characterized eight children with type I hyperproline-mia (HPI), an autosomal recessive disorder associated with reduced proline dehydrogenase activity and as a result, raised plasma proline levels. The children under investigation presented with learning disability and epilepsy and, in some cases, psychiatric features. Within the same study, the authors subsequently examined a cohort of 92 adults and adolescents with 22q11DS, of whom a subset had severe hyperprolinemia and demonstrated a phenotype distinguishable from that of other 22q11DS individuals and reminiscent of HPI. They conducted a forward stepwise multiple regression analysis and selected hyperprolinemia, psychosis and COMT genotype as independent variables influencing IQ; they found an inverse correlation between plasma proline level and IQ in the 22q11DS sample.

Neuropsychological

There is much inter-subject variability in 22q11DS cognitive functioning and most studies performed so far have been cross-sectional (Murphy & Scambler, 2005). Currently, work is underway to understand the process of cognitive development within the syndrome longitudinally. With regard to IQ, there is a discrepancy between VIQ and PIQ whereby VIQ exceeds the latter (Swilen et al., 1997). However, the degree of the discrepancy may not only predispose to learning difficulty but also to the development of psychosis. It has been reported that 22q11DS children and adolescents with significantly reduced adaptive skills and decreased VIQ are at higher risk for psychosis compared to their counterparts without psychotic symptoms (Debbane et al., 2006). Additionally, in the same study, psychotic probands were perceived by their parents as being more withdrawn and anxious/depressed, and psychotic manifestations were postulated to present earlier than typically reported in the literature.

School-aged children with 22q11DS have also been reported to demonstrate marked deficits in mathematics while showing normal scores for reading (decoding) and spelling (Moss et al., 1999; Woodin et al., 2001) and a similar profile has also been reported, as already established, in pre-school children (De Smedt et al., 2003). A more recent study (De Smedt et al., 2007) suggested that children with 22q11DS had preserved number reading abilities and retrieval of arithmetic facts, which, in combination, indicate that the verbal subsystem is not impaired in 22q11DS. It was further reported that 22q11DS children, by contrast, showed difficulties in number comparison, execution of a calculation strategy and word problem solving which all involve the semantic manipulation of quantities. The authors concluded that there is evidence for a specific deficit in the quantity subsystem in children with 22q11DS, which neuroanatomically suggests underlying abnormalities in the intraparietal sulcus.

Although reading decoding abilities are preserved in 22q11DS, reading comprehension skills are far less well developed (Antshel et al., 2005a; Antshel et al., 2008). Overall, mathematical abilities and reading comprehension and visuospatial, attention and executive function skills have all been reported to be well below average for age (Antshel et al., 2008).

There have been no studies comparing the cognitive and neuroanatomical characteristics of 22q11DS with other syndromes to determine if the cognitive strengths and difficulties and neuroanatomical differences associated with 22q11DS are specific to the syndrome. In view of this, we recently compared cognition and brain anatomy in 12 children with 22q11DS to 12 age-, gender- and FSIQ-matched children with Williams syndrome in order to investigate which cognitive and neuroanatomical features are
specific to 22q11DS (Campbell et al., 2009). Williams syndrome was chosen as a comparator group since the literature suggests that both groups have areas of physical/cognitive/behavioral overlap but there has yet to be any direct comparison between the two groups. Despite being matched on FSIQ, the 22q11DS group showed significantly less impairment than those with Williams syndrome on tests of PIQ, while performing significantly worse on tasks measuring verbal, social and facial processing skills. Therefore, different neuropsychological profiles need to be considered when designing educational frameworks for working with such children.

**Neuroimaging**

The very high prevalence of cognitive, behavioral and psychiatric disorders in people with 22q11DS is likely to be caused by haploinsufficiency of one or more genes deleted on chromosome 22q11.2 and subsequent differences in brain maturation and neurotransmitter systems. Thus, 22q11DS provides a unique neurobiological template for understanding the evolution of such disorders through the assessment of influences on brain anatomy (Murphy & Owen, 2001).

Early neuroimaging studies in subjects with 22q11DS were mostly qualitative, and differences reported include an increased incidence of midline abnormalities such as white matter (WM) hyperintensities and septum pellucidum defects (Mitnick et al., 1994; van Amelsvoort et al., 2001) and there were additional reports of severe cerebral malformation (Kraynack et al., 1999; Bolland et al., 2000). Cortical malformations manifesting with polymicrogyria have also been found in individuals with 22q11DS (Robin et al., 2006) that is characterized by a thick cortex in association with shallow sulci, which is usually caused by ischemic injury during a critical period of embryonic brain maturation (Barkovich et al., 1995). Qualitative studies were an important first step and subsequently led to quantitative studies that examined both gross anatomical changes and subtle cerebral anatomy differences.

Quantitative studies have found a significant reduction in total brain volume in children and adolescents with 22q11DS relative to normally developing subjects (of the order of 8.5 to 11 per cent). The posterior brain structures in particular, such as the cerebellum, temporal and parietal lobes, are the main structures affected, with volume reduction being largely accounted for by decreased WM volume, with relatively preserved or enlarged frontal lobe tissue (Eliez et al., 2000; Kates et al., 2001; van Amelsvoort et al., 2001; van Amelsvoort et al., 2004b; Bish et al., 2006). In addition, in a study of 22q11DS children using a combination of automated voxel-based morphometry (VBM) and manual (hand tracing) magnetic resonance imaging (MRI) analytical techniques (Campbell et al., 2006), specific vulnerability of the cerebellar-cortical and fronto-striatal networks was reported.

Region-of-interest (ROI) studies have further supported a rostro-caudal gradient in volume reduction, whereby anterior regions are relatively preserved/enlarged while the structures in more posterior regions undergo volumetric reduction. Several structures exhibit such a gradient and include both WM and grey matter areas such as the caudate nucleus (Eliez et al., 2002; Kates et al., 2004; Campbell et al., 2006), corpus callosum (Antshel et al., 2005b; Machado et al., 2007), thalamus (Bish et al., 2004) and fusiform gyrus (Glaser et al., 2007).

In order to investigate neuroanatomical features that may be specific to 22q11DS, we recently compared the brain anatomy of 12 children with 22q11DS to 12 age-, gender- and FSIQ-matched children with Williams syndrome (Campbell et al., 2009). Despite similar overall brain volumes, there were significant differences in 22q11DS brain anatomy as reflected by regional differences where for instance, increased striatal volumes and reduced cerebellar volumes that may be specific to 22q11DS. Although
quantitative neuroimaging studies have been carried out to identify cerebral volume alterations underlying the cognitive, behavioral, and psychiatric impairments associated with 22q11DS, only a limited number have focused on functional MRI (fMRI).

fMRI studies so far have targeted four areas recognized to be impaired in 22q11DS: arithmetic processing, face processing, executive function and working memory (Gothelf et al., 2008). In an fMRI study exploring the neural substrate underlying deficiencies in arithmetic reasoning using mathematical tasks of increasing difficulty (Eliez et al., 2001), the authors reported that children with 22q11DS showed more intensive and diffuse activity in the inferior parietal regions only during performance of difficult three-operator equations, whereas controls showed a similar pattern of parietal activation both during easy and difficult tasks. With regard to facial processing, two fMRI reports have been completed so far, with one study reporting less activation in both the right insula and frontal regions while increased activation was found in occipital regions (van Amelsvoort et al., 2006). However, when corrected for multiple comparisons, these results did not remain significant. A further study on facial processing which also assessed emotional processing in 22q11DS relative to healthy controls reported impaired face selectivity in the fusiform gyrus in the 22q11DS group but responses were intact to houses in both groups, with preserved selectivity in the parahippocampal gyrus (Andersson et al., 2008). The study also reported an abnormal repetition-suppression effect for fearful faces in the right amygdala, suggesting a lack of amygdala modulation by fear expression in 22q11DS.

In an fMRI study which assessed response inhibition with a Go/NoGo task, adolescents with 22q11DS were able to perform as well as typically developing controls and matched controls with developmental disability, although individuals with 22q11DS demonstrated more activation of the left inferior parietal regions (Gothelf et al., 2007b). Based on their findings, the authors suggested that adolescents with 22q11DS compensate for executive dysfunction via recruitment of parietal regions.

Although there is increasing evidence that people with 22q11DS have regionally specific differences in brain anatomy, supra-regional brain systems often share common developmental influences (Cheverud, 1984) and perhaps especially affecting WM. If so, this suggests that people with 22q11DS may have differences in both brain 'connectivity' and in the microstructure of WM (Kiehl et al., 2009). Hence, interest has recently turned from assessing simple 'lesion of a region' to complementary approaches that investigate abnormalities in the 'connectivity' of neural systems using proxy measures of microstructural integrity acquired using diffusion tensor MRI (DT-MRI) (Basser et al., 1994a).

DT-MRI permits assessment of WM through a directional dependence of diffusion of water molecules termed anisotropy and is usually quantified through the calculation of fractional anisotropy (FA) (Pierpaoli & Basser, 1996). In the first DT-MRI study of people with 22q11DS (Barnea-Goraly et al., 2003), significantly reduced FA of WM was reported in frontal, parietal and temporal regions; and in WM tracts connecting the frontal and temporal lobes. This study was a valuable first step. However, both adults and children were combined as one group, and most of the cerebellum and brainstem was excluded from the analysis. A subsequent DT-MRI study of 22q11DS children and adolescents (Simon et al., 2005) replicated the earlier findings but additionally reported that the corpus callosum was posteriorly displaced in young people with 22q11DS. However, a healthy pediatric brain template was used for spatial normalization in this study which may present a potential cause of misregistration. Given that the cerebellum and posterior brain structures have been previously implicated in volumetric MRI studies of 22q11DS, and that age and choice of brain template may confound measures of brain anatomy, it is important to examine WM
anatomy in a relatively homogeneous group of children and adolescents with no clinically detectable comorbid psychiatric disorder and to include the posterior brain structures. Therefore, we recently examined the DT-MRI data of children and adolescents with 22q11DS but without psychiatric disorder relative to healthy controls. A customized brain template derived from all participants in the study was used for registration of FA maps and included the brainstem and cerebellum; after controlling for IQ, significantly lower FA was localized to interhemispheric and brainstem areas in addition to FA deficits in frontal, parietal and temporal lobe regions (Sundram et al., 2010).

In summary, while there is mounting evidence that people with 22q11DS have significant differences in the development of certain brain regions, ‘connectivity’ between specific brain regions may have a significant contribution to the evolution of the behavioral, psychiatric and cognitive deficits seen in 22q11DS; however, relatively few studies have directly examined the anatomy and microstructural integrity of WM.

TREATMENT AND INTERVENTION

There is currently no genetic cure for 22q11DS and treatment will be dependent on the underlying impairments or associated symptoms. As 22q11DS individuals have a variety of abnormalities in many organ systems, a multidisciplinary approach is required. A range of clinical assessments is required to identify areas of disability and subsequently, a treatment plan formulated to address these needs within the framework of a multidisciplinary team. Early identification of the syndrome (ideally genetic testing) is crucial to the institution of appropriate intervention.

A range of clinical specialties including psychiatry, psychology, speech and language therapy, cardiology, immunology, otolaryngology and clinical genetics should be involved in assessment and treatment (Murphy, 2005). As a minimum, all suspected 22q11DS individuals should have detailed genetic, neuropsychological, psychiatric and speech and language assessments. Furthermore, educational provision is essential not only for the parents of children with 22q11DS but also mental health professionals working with such children, and children with the syndrome.

Physical

As cardiac abnormalities are common in 22q11DS, newborns will need to be evaluated by a cardiologist in the immediate postnatal phase. Newborns with the deletion may sometimes require early cardiac intervention for congenital heart anomalies due to the potentially life-threatening nature of such defects. Subsequently, a plastic surgeon and a speech therapist will need to evaluate cleft lip and/or palate and children will benefit from early intervention to aid with muscle strength and articulatory difficulties.

Additionally, it is vital that problems with the immune system are identified early so that special precautions may be taken with regard to blood transfusions or immunization with live vaccines. Thymus transplantation may also be considered to address absence of the thymus gland while bacterial infections are treated with antibiotics. In severe cases where immune system function is absent, bone marrow transplantation may be required. In the setting of hypoparathyroidism, which causes hypocalcaemia, this often requires lifelong vitamin D and calcium supplements.

Cognitive, linguistic, behavioral and psychiatric disorder

Given that the understanding of neuropsychological abnormalities in children with 22q11DS is gradually improving and that children with the syndrome continue to develop over the course of their lifetime, it is important that a
dynamic approach is utilized to assess their abilities longitudinally. Some of the cognitive difficulties evident in 22q11DS include poor attentional and executive functioning, academic achievement and visuospatial skills which may manifest in disturbed behavior at home or at the school setting.

Difficult behaviors seen in children with 22q11DS (e.g. aggression, poor sleep patterns, temper outbursts) can be addressed with behavioral modification techniques, including the use of token economies and reward schemes (Murphy, 2005). Additionally, anger management programs and social skills training may also be effective. The majority of children with 22q11DS will be able to attend mainstream school, with varying degrees of additional intervention in the classroom. However, depending on their level of intellectual ability, a minority of children with 22q11DS will benefit by attending a school specializing in education for children with special needs, both in terms of their academic needs and their behavioral difficulties.

22q11DS is associated with high comorbidity with psychiatric disorders that are responsive to standard treatment protocols. Schizophrenia can be treated using conventional antipsychotic medication (Murphy et al., 1999; Bassett et al., 2003) while ADHD can be addressed by the use of psychostimulant medication such as methylphenidate. In future, larger controlled clinical trials are required to determine the relative efficacies of both pharmacological and psychological interventions in the treatment of schizophrenia, ADHD and other psychiatric disorders associated with 22q11DS.

CONCLUSIONS

The key challenges for the future include identification of the neuroanatomical and genetic basis underlying the cognitive, psychiatric and behavioral disorders associated with 22q11DS. Furthermore, identification of definitive predictive precursors to the subsequent development of prominent psychiatric disorders such as psychosis will help ensure the implementation of effective early interventions in such individuals.

Currently there is an increasing number of studies that have investigated the behavioral and psychiatric morbidity in either children or adults with 22q11DS. However, recruiting suitable control groups has always posed a challenge for researchers investigating syndromes associated with intellectual disability and developmental delays. Many studies have used normally developing children as control samples and, more recently, attempts have been made to use comparison samples that are functioning in the same general range of cognitive ability; however, even the latter practice may confound results, as the etiology of the intellectual disability may have an influence, e.g. fetal alcohol syndrome versus more appropriate control samples such as Williams or Turner syndrome (Murphy et al., 2006).

Moreover, some of the completed studies have been hampered by methodological constraints such as sample heterogeneity, limited sample sizes and the lack of operationalized criteria for psychiatric diagnoses. Also, most of the research conducted in 22q11DS so far has relied on cross-sectional samples. The cross-sectional design of such studies may not have been able to detect within-individual change and thus may have inaccurately reported a more temporally stable developmental trajectory than is really occurring (Antshel et al., 2008). Therefore, future research will need to adopt a longitudinal design to assess not only variability within but also between subjects.

REFERENCES


VELO-CARDIO-FACIAL SYNDROME/22Q11 DELETION SYNDROME


Lalani, S.R., Safiullah, A.M., Fernbach, S.D., Harutyunyan, K.G., Thaller, C., Peterson, L.E.,


22q11.2 deletion syndrome (22q11.2DS), also known as velocardiofacial syndrome, is the most frequent known interstitial deletion found in humans. It is associated with high rates of psychiatric disorder and, in particular, schizophrenia (approximately 30%) and schizophrenia-spectrum disorders. The 22q11.2 region is rich in genes coding for brain development, and consequently, deletion of 22q11.2 provides a useful neurodevelopmental model for understanding the evolution of psychotic disorders in both 22q11.2DS and the nondeleted general population. 22q11.2DS is also associated with significant structural and functional neuroanatomical abnormalities, which underlie the high rates of psychosis seen in affected individuals. Further research is currently underway to determine the cognitive, genetic and neuroanatomical correlates of psychosis seen in the disorder.

Introduction

22q11.2 deletion syndrome (22q11.2DS) is the most frequent human genetic deletion syndrome and is associated with an interstitial deletion that occurs with an estimated prevalence of 1 in 2000–4000 live births (Botto et al., 2003; Oskarsdottir et al., 2004; Robin and Shprintzen, 2005; Kobrinski and Sullivan, 2007). The syndrome is also known as velocardiofacial syndrome (VCFS) or DiGeorge syndrome (Karayiorgou et al., 2010). The majority of microdeletions (approximately 85%) are 3 Mb in size and include some 60 genes (Figure 1). A smaller number of microdeletions (approximately 8%) involve the same proximal region but have a different distal breakpoint that results in a smaller 1.5 Mb deletion that includes approximately 30 genes, the majority of which are expressed in the brain. In addition, some atypical deletions and point mutations have also been described (Urban et al., 2006). The 22q11.2DS phenotype is complex, with multiple congenital abnormalities affecting a number of tissues and organs, many of which are embryologically derived from neural crest cells (Shprintzen et al., 2005). Although considerable phenotypic variability occurs where severity is unrelated to the size of the deletion (Carlson et al., 1997), common clinical features include characteristic dysmorphism (Figure 2), cleft palate, congenital heart defects, borderline learning disability and high rates of psychiatric disorder, particularly schizophrenia-spectrum disorders. See also: 22q11 Deletion Syndrome: A Role for Tbx1 in Pharynx and Cardiovascular Development

Elevated Rates of Schizophrenia in 22q11.2DS

In 1992, Shprintzen and colleagues first reported psychotic symptoms, which they described as resembling 'chronic paranoid schizophrenia' in 12 of 90 children with 22q11.2DS (Shprintzen et al., 1992). Subsequently, several studies have reported the presence of transient or subthreshold psychotic symptoms in children and adolescents with 22q11.2DS (Feinstein et al., 2002; Baker and Skuse, 2005; Debbane et al., 2006; Gothelf et al., 2007a). This is supported by robust evidence from numerous studies that approximately 30% of adults with 22q11.2DS have schizophrenia (Pulver et al., 1994; Murphy et al., 1999; Bassett et al., 2003, 2007; Green et al., 2009). It is likely that a variety of genetic, cognitive and psychiatric factors contribute to the development of psychosis in 22q11.2DS. However, whether there are differences in the clinical phenotype between 22q11.2DS and nondeleted schizophrenia remains unclear as data on clinical features and treatment response have been conflicting.

Additionally, higher rates of autism spectrum disorder in 22q11.2DS have also been reported at 14—50% (Niklasson...
Schizophrenia and 22q11.2 Deletion Syndrome

et al., 2001; Fine et al., 2005; Vorstman et al., 2006). Indeed, Vorstman and colleagues suggest that psychotic and autistic disorders are major features of the behavioural phenotype in children with 22q11DS. However, they suggest that the autistic symptoms identified in their study may also be a reflection of the neurodevelopmental abnormalities present in individuals with schizophrenia where autistic symptoms may be representing prodromal features of psychosis rather than being specific for an autism spectrum disorder.

Chromosome 22 and Schizophrenia

Evidence from family, twin and adoption studies demonstrates a major genetic contribution to the aetiology of schizophrenia. The high rates of schizophrenia in 22q11.2DS suggest that, with the exception of being the offspring of a dual mating or the monozygotic (MZ) cotwin of an affected individual, deletion of chromosome 22q11 represents the highest known risk factor for the development of schizophrenia identified to date. See also: Schizophrenia: Molecular Genetics

There are also several other lines of evidence to suggest that a locus conferring susceptibility to schizophrenia resides on chromosome 22q. Studies have reported that the prevalence of 22q11.2 deletions in populations of people with schizophrenia is approximately 1% (Karayiorgou et al., 1995; Horowitz et al., 2005), and 22q11.2 microdeletion, including copy number variation, is associated with the development of schizophrenia (Karayiorgou et al., 1995, 2010; Xu et al., 2008). Results from linkage studies of individuals with schizophrenia also provide supportive evidence for a susceptibility locus for schizophrenia mapping to 22q. Whereas no single study has demonstrated genome-wide significance, several meta-analyses suggest that a broad region of 22q is implicated in schizophrenia susceptibility (Badner and Gershon, 2002; Lewis et al., 2003). In addition, a recent genome-wide association study reported a significant association with 22q11 in a sample of 3322 individuals with schizophrenia (Purcell et al., 2009).

Several genes that map to the 22q11 deleted region have been examined as candidates for schizophrenia both in 22q11DS and in nondeleted schizophrenia. In a review of candidate gene association studies involving 22q11 deletion, Arinami concluded based on the evidence from linkage studies that mutations of genes on 22q11 are unlikely to contribute to familial aggregation of schizophrenia in the general population (Arinami, 2006). Furthermore, he concluded that association studies of six genes (COMT (catechol-O-methyltransferase), PRODH (proline dehydrogenase), ZDHHC8 (zinc finger, DHHC domain containing 8), CLDN5 (claudin-5), DGCR14 (DiGeorge syndrome critical region gene 14) and DGCR2 (DiGeorge syndrome critical region gene 2)) in nondeleted schizophrenia were inclusive or inconsistent.

More recent studies have also examined the role of three additional genes mapping to 22q11 (TBX1 (T-box 1), GNB1L (guanine nucleotide binding protein (G protein), beta polypeptide 1-like) and PIK4CA (phosphatidylinositol

Figure 1 Chromosome 22q11 microdeletion identified by fluorescence in situ hybridisation (FISH).

Figure 2 Characteristic facial dysmorphism in 22q11.2 deletion syndrome.
4-kinase, catalytic, alpha)). Whereas genetic variation in TBX1 has been shown to play a pivotal role in the development of congenital heart defects in 22q11DS (Merscher et al., 2001; Yagi et al., 2003), Funke et al. (2007) reported no association with TBX1 in a general population of non-deleted schizophrenia (Funke et al., 2007). Williams et al. (2008) reported evidence for a male-specific genotypic association for TBX1, GNB1L in three independent case-control samples of people with schizophrenia, and a significant allelic association was also found with psychosis in males with 22q11DS (Williams et al., 2008). However, these findings await replication by other groups. Furthermore, Jungerius et al. (2008) reported an association between PIK4CA and schizophrenia in a Dutch population of non-deleted schizophrenia (Jungerius et al., 2008). Whereas Vorstman et al. (2009) reported that variation in PIK4CA was associated with the presence of schizophrenia in a sample of 79 adults with 22q11DS (Vorstman et al., 2009), Ikeda et al. (2010) failed to replicate this finding (Ikeda et al., 2010).

Neurocognition

People with 22q11.2DS are known to have characteristic cognitive deficits including borderline to mild intellectual disability and impairments in visuospatial and planning, abstract thinking, mathematical attainment and executive functioning (Henry et al., 2002; Antshel et al., 2008). Additionally, some studies report that mean full-scale intelligence quotient (IQ) is similar in the presence or absence of schizophrenia in people with 22q11DS (Murphy et al., 1999; van Amelsvoort et al., 2004a; Chow et al., 2006), whereas others report that the mean full-scale IQ is lower in deleted individuals with psychosis compared to nonpsychotic individuals (Raux et al., 2007). In a recent study of 172 children and adults with 22q11DS, Green et al. (2009) reported that while performance IQ scores did not differ significantly between psychotic and nonpsychotic subjects, verbal IQ scores of young adults and adults (age > 18 years) with psychotic disorders were significantly lower than deleted young adults and adults without psychotic disorders, with specific reductions in the vocabulary and similarities subscale scores (Green et al., 2009).

Several studies have also reported on other aspects of the cognitive profile in people with 22q11.2DS and schizophrenia. van Amelsvoort et al. (2004a) reported that, compared to the nonpsychotic group matched for age, gender and IQ, people with 22q11DS and schizophrenia had specific impairments of strategy formation, spatial working memory, visual recognition and attention (van Amelsvoort et al., 2004a). Another study reported that people with 22q11DS and schizophrenia performed significantly worse in tests of motor skills, verbal learning and social cognition relative to those without schizophrenia (Chow et al., 2006). These results are in agreement with the neurocognitive studies of nondeleted schizophrenia in the general population and suggest that the cognitive profile is similar in 22q11DS-related schizophrenia and nondeleted schizophrenia in the wider population.

Neuroimaging

Numerous magnetic resonance imaging studies of people with 22q11DS have reported global brain volumetric reductions affecting both grey and white matter with specific reductions of the cerebellum, hippocampus and temporal and parietal lobes with most of the volumetric loss being accounted for by reduction of white matter with relatively preserved or enlarged frontal lobe volume (Kates et al., 2001; van Amelsvoort et al., 2001) and concomitant increased corpus calloidal volume (Tan et al., 2009). van Amelsvoort et al. (2004b) reported that adults with 22q11DS and schizophrenia, compared with both controls and nonschizophrenic subjects with 22q11DS, had a significant reduction in volume of whole-brain (grey and white) matter and whole-brain white matter and an increase in total and sulcal cerebrospinal fluid volume (van Amelsvoort et al., 2004b). Their results also suggested that reduction in volumes of the frontal and temporal lobes may be associated with the presence of psychotic symptoms. Although Goihelf and colleagues (2007b) reported no differences in brain volume between psychotic and nonpsychotic children with 22q11DS, their sample had a mean age of 13.1 years suggesting that subjects remain at risk for the subsequent development of schizophrenia (Goihelf et al., 2007b). These findings are in agreement with neuroimaging studies of nondeleted schizophrenia in the general population and suggest a unifying neuroanatomical substrate underlying schizophrenia in 22q11DS and in the wider nondeleted population.

Overall, there is increasing evidence that people with 22q11.2DS have regionally specific differences in brain anatomy. However, although white matter is preferentially affected in 22q11.2DS, the underlying brain 'connectivity' or microstructure of white matter has been less extensively investigated. Such abnormalities in the 'connectivity' of neural systems can be undertaken using proxy measures of microstructural integrity acquired by diffusion tensor magnetic resonance imaging (DT-MRI; Basser et al., 1994) and is usually quantified through the calculation of fractional anisotropy (FA).

In the first ever DT-MRI study of people with 22q11.2DS (Barnes-Goral et al., 2003), significantly reduced FA of white matter was reported in frontal, parietal and temporal regions and in white matter tracts connecting the frontal and temporal lobes. Although this study was a valuable first step, however, both adults and children were combined as a single group, and most of the cerebellum and brainstem was excluded from analysis. A subsequent DT-MRI report of 22q11.2DS children and adolescents (Simon et al., 2005) replicated findings from the earlier study, but additionally reported a posteriorly displaced corpus callosum in young people with 22q11.
2DS. However, a healthy paediatric brain template was used for spatial normalisation in this study. Recently, the DT-MRI data of children and adolescents with 22q11.2DS but without psychiatric disorder relative to healthy controls was examined. A customised brain template derived from all participants in the study was used for registration of FA maps and included the brainstem and cerebellum; after controlling for IQ, significantly lower FA was localised to interhemispheric and brainstem areas in addition to FA deficits in frontal, parietal and temporal lobe regions (Sundram et al., 2010).

Risk Factors for the Development of Psychosis in 22q11.2DS

As approximately 30% of people with 22q11DS develop psychosis, one of the main challenges for clinicians and researchers is to identify those susceptible children and adolescents who subsequently develop psychosis in adulthood. Findings from the first prospective longitudinal study of people with 22q11.2DS reported that the catechol-O-methyltransferase low-activity allele (COMT<sup>−/−</sup>) is a risk factor for decline in prefrontal cortical volume, IQ and the subsequent development of psychosis in people with 22q11DS (Gothelf et al., 2005). In a further study, Gothelf et al. (2007a) reported that baseline subthreshold psychotic symptoms interacted with both COMT genotype and with baseline symptoms of anxiety or depression to predict 61% of the variance in severity of psychosis at follow-up assessment. Lower baseline verbal IQ was also associated with more severe psychotic symptoms at follow-up. They suggested that early intervention in the subgroup of children with subthreshold signs of psychosis and internalising symptoms (especially anxiety symptoms) may reduce the risk of developing psychotic disorders during adolescence although randomised controlled trials are necessary to test this hypothesis. In a more recent study, Schuer et al. (2009) reported significant differences in cortical thickness in people with 22q11DS and schizophrenia and suggested that deviant trajectories of cortical thickness from childhood to adulthood may underpin the subsequent development of psychosis in affected individuals (Schuer et al., 2009).

Conclusions

Apart from the offspring of a dual mating or the MZ co-twin of an affected individual, the presence of a chromosome 22q11 deletion represents the highest known risk factor for the development of schizophrenia identified to date. It is likely that haploinsufficiency of a neurodevelopmental gene or genes mapping to chromosome 22q11, leading to disturbed neural cell migration, underlies susceptibility to psychosis in 22q11.2DS. See also: Schizophrenia: Molecular Genetics

While deletion of chromosome 22q11 may only account for a small proportion of risk to the development of schizophrenia in the general population, nondeletion mutations or polymorphisms in genes within the 22q11 region may make a more general and widespread contribution to susceptibility to schizophrenia in the wider population. Experience with other complex diseases (e.g. Alzheimer disease, diabetes mellitus and breast cancer) suggests that unravelling the molecular basis for uncommon subtypes with high penetrance to be the most successful approach to understanding the genetics and underlying pathophysiology of complex diseases. As the entire sequence of chromosome 22 has now been determined, the future identification of the genetic determinants of the psychosis in 22q11.2DS individuals will have profound implications for our understanding of the molecular genetics and pathogenesis of psychosis in the wider nondeleted population. See also: 22q11 Deletion Syndrome: A Role for Tbx1 in Pharynx and Cardiovascular Development

References


Schizophrenia and 22q11.2 Deletion Syndrome


Further Reading


White matter microstructure in 22q11 deletion syndrome: a pilot diffusion tensor imaging and voxel-based morphometry study of children and adolescents

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Abstract Young people with 22q11 Deletion Syndrome (22q11DS) are at substantial risk for developing psychosis and have significant differences in white matter (WM) volume. However, there are few in vivo studies of both WM microstructural integrity (as measured using Diffusion Tensor (DT)-MRI) and WM volume in the same individual. We used DT-MRI and structural MRI (sMRI) with voxel based morphometry (VBM) to compare, respectively, the fractional anisotropy (FA) and WM volume of 11 children and adolescents with 22q11DS and 12 controls. Also, within 22q11DS we related differences in WM to severity of schizotypy, and polymorphism of the catechol-O-methyltransferase (COMT) gene. People with 22q11DS had significantly lower FA in inter-hemispheric and brainstem and frontal, parietal and temporal lobe regions after covarying for IQ. Significant WM volumetric increases were found in the internal capsule, anterior brainstem and frontal and occipital lobes. There was a significant negative correlation between increased schizotypy scores and reduced WM FA in the right posterior limb of internal capsule and the right body and left splenium of corpus callosum. Finally, the Val allele of COMT was associated with a significant reduction in both FA and volume of WM in the frontal lobes, cingulum and corpus callosum. Young people with 22q11DS have significant differences in both WM microstructure and volume. Also, there is preliminary evidence that within 22q11DS, some regional differences in FA are associated with allelic variation in COMT and may perhaps also be associated with schizotypy.

Keywords 22q11DS • VCFS • COMT • DTI • VBM • Psychosis

Introduction

22q11 Deletion Syndrome (22q11DS) is the most frequent human genetic deletion syndrome (Gothelf and Lombroso 2001) with an approximate incidence of 1 per 4,000 live births (Oskarsdottir et al. 2004). People with 22q11DS have...
a deletion at chromosome 22q11.2 which is associated with a phenotype that includes physical, behavioural, psychiatric and neuropsychological anomalies (Swillen et al. 1999; Feinstein and Eliez 2000). Characteristic physical features of 22q11DS include facial dysmorphology and palatal defects, cardiac anomalies and thyMIC hypoplasia (Goodman 2003). People with 22q11DS are also reported to have an increased incidence of neuroanatomical abnormalities.

Such neuroanatomical differences include midline anomalies (e.g., white matter (WM) hyperintensities and septum pellucidum defects (Minnick et al. 1994; van Amelsvoort et al. 2001; Campbell et al. 2006) and cortical dysgenesis such as polymicrogyria (Robin et al. 2006). In children and adolescents with the disorder, a significant reduction in volume of posterior brain structures (especially in the cerebellum, temporal and parietal lobes) is largely accounted for by decreased WM volume (Eliez et al. 2000; Kates et al. 2001; Campbell et al. 2006). 22q11DS children are also reported to have a significant reduction in WM content of the frontal lobe, cerebellum and internal capsule as compared to their healthy sibling controls (Campbell et al. 2006).

Thus, there is increasing evidence that children and adolescents with 22q11DS have regionally specific differences in brain anatomy, and perhaps especially affecting WM. If so, this suggests that differences in both brain ‘connectivity’ and in the microstructure of WM occurs in 22q11DS (Kielhl et al. 2008). Hence interest has recently turned to investigating abnormalities in the ‘connectivity’ of neural systems using proxy measures of microstructural integrity acquired using Diffusion Tensor MRI (DT-MRI) (Basser et al. 1994a).

DT-MRI permits assessment of WM through a directional dependence of diffusion of water molecules termed anisotropy, which is usually quantified through the calculation of fractional anisotropy (FA) (Pierpaoli and Basser 1996). In the first DT-MRI study of 22q11DS (Barnes-Goraly et al. 2003), significantly reduced FA of WM was reported in frontal, parietal and temporal regions of the 22q11DS subject group which included children, adolescents and adults with the disorder. Additionally, FA deficits were also reported in WM tracts connecting the frontal and temporal lobes of the same group. This study was a valuable first step. However, both adults and children were combined as one group, and most of the cerebellum and brainstem was excluded from analysis.

A subsequent study using DT-MRI only involving 22q11DS children and adolescents (Simon et al. 2005) reported decreased FA in the corpus callosum of 22q11DS subjects but increased FA in the cingulum and right inferior parietal lobule of the same group. The authors postulated a posteriorly displaced corpus callosum in young people with 22q11DS to account for their findings; however, a healthy paediatric brain template was used for spatial normalisation in this study which is a potential cause of mis-registration. Given that the cerebellum and posterior brain structures have been previously implicated in volumetric MRI studies of 22q11DS, and that age and choice of brain template may affect measures of brain anatomy, we wished to examine WM anatomy in a relatively homogeneous group of children and adolescents with no clinically detectable comorbid psychiatric disorder, to include the cerebellum and brainstem, and to determine if differences in WM FA overlap with those in WM volume as assessed by VBM.

As supra-regional brain systems often share common developmental influences (Cheverud 1984), 22q11DS also allows us to examine the modulatory effect of a number of candidate genes located at 22q11.2. One of these is the gene for catechol-o-methyltransferase (COMT), an enzyme that degrades dopamine, which in turn modulates brain development and function (Halginger et al. 2004; Zinkstok et al. 2006). The COMT gene undergoes a naturally occurring polymorphism which has been reported to affect dopamine regulation (Akil et al. 2003); the polymorphism leads to an amino acid substitution (Valine[Val] to Methionine[Met]) and results in decreased thermostability and variable enzymatic activity. The Met/Met variant of COMT displays approximately 40% less enzymatic activity than Val/Val (Chen et al. 2004). COMT may influence WM integrity through the regulation of dopamine levels, which in turn can further modulate the proliferation and differentiation of oligodendrocytes and thus affect the formation of myelin (Bongarzone et al. 1998). More recently, COMT Val/158-Met polymorphism has been found to affect the association between IQ and white matter architecture in the prefrontal lobe and hippocampal formation (Li et al. 2009). However, as a consequence of chromosomal deletions, 22q11DS individuals possess only one working copy of the COMT gene (i.e. they are haploinsufficient) which results in either a high activity Val (4COMT) or low activity Met (4COMT) isoform of the COMT enzyme. Disrupted dopaminergic modulation and neurotransmission may occur as a result of haploinsufficiency and has been reported in adults with 22q11DS without a psychiatric history (Boo et al. 2008) which may explain their vulnerability for the development of psychiatric disorders.

The very high prevalence of psychiatric disorders in 22q11DS is likely to be caused by haploinsufficiency of one or more genes deleted on 22q11.2 and subsequent differences in brain structure, cerebral maturation and neurotransmitter systems. Schizophrenia spectrum disorders are especially prevalent in young adults with 22q11DS (Murphy et al. 1999; Murphy 2002) and through the assessment of schizophrenia and related disorders such as schizotypy, 22q11DS provides a unique neurobiological template for understanding the evolution of psychosis.
Schizotypy is significantly increased in both 22q11DS children and adults (Murphy et al. 1999; Baker and Skuse 2005) and when present in childhood (both deleted and non-deleted populations), it confers greater susceptibility to the development of psychosis in later life (Chapman et al. 1994; Poulton et al. 2000; Siever and Davis 2004). Given that WM structure is significantly affected in 22q11DS and WM integrity abnormalities have been related to schizotypy in the non-deleted population (Nakamura et al. 2005), we wished to examine for the first time if similar disruption occurs in 22q11DS.

In summary, there is mounting evidence that children and adolescents with 22q11DS may have significant differences in the development and 'connectivity' of specific brain regions; however relatively few studies have directly examined the anatomy and microstructural integrity of WM in a young cohort with the disorder. Further, COMT polymorphism may modulate brain maturation through dopamine metabolism but its effect on WM integrity has been relatively understudied in children and adolescents with 22q11DS. Also, given the high rates of schizophrenia spectrum disorders reported in 22qDS adults and schizotypy being a risk factor in children for the future development of psychosis, we wished to assess WM changes associated with schizotypy in a young 22q11DS population at risk of developing psychosis. Therefore we tested the main hypothesis that children and adolescents with 22q11DS have significant differences from controls in WM microstructural integrity and WM volume. Also, we carried out preliminary post hoc investigations to test additional hypotheses that, within young people with 22q11DS, (1) severity of schizotypy is correlated with differences in WM integrity; and (2) polymorphism of the COMT gene is associated with differences in WM volume and coherence.

**Method**

**Subjects**

As part of our group’s longitudinal work into 22q11DS, we have recruited subjects from the 22qDS support group (UK) and the Behavioural Genetics Clinic at the South London and Maudsley NHS Foundation Trust. Thirty-nine children and adolescents with 22q11DS have previously had a structural MRI scan (Campbell et al. 2006) of which, 12 successfully underwent DT-MRI at the same scanning session which forms the focus of the current study.

All participants were medication free, had English as their first language, and were right handed as assessed by the Annett Handedness Questionnaire. The study was approved by the Ethics Committee at the Institute of Psychiatry, London (IoP). Written informed consent was obtained from carers, and assent from children, after full description of the study.

As a DT-MRI scan for one 22q11DS subject demonstrated excessive motion artefact, his scans were excluded from further analysis. We therefore included 11 young people (five male and six female) with clinical features of 22q11DS and an established genetic 22q11.2 deletion (mean age: 12 years, SD±2.2, range 9–17 years; mean FSIQ: 66, SD±8.0, range 56–84). Exclusion criteria were a 22q11DS clinical phenotype but without the large 3 Mb 22q11.2 deletion, a clinically detectable medical disorder known to affect brain structure (e.g. epilepsy), a history of head injury or contraindications to MRI scanning.

22q11DS cases were compared to 12 healthy controls (eight male and four female) with a non-deleted 22q11.2 region (mean age: 13 years, SD±3, range 9–17 years; mean FSIQ: 116, SD±16, range 90–141). In contrast to our previous structural MRI work using only sibling controls (Campbell et al. 2006), controls in this study were a combination of sibling (n=7) and non-sibling (n=5) controls. All healthy controls were free from genetic and physical disorders affecting brain anatomy or function. See Table 1.

**Medical and psychiatric disorders**

Children and parents were examined through semi-structured interview to evaluate past medical history and all subjects had a detailed physical examination. We obtained copies of past assessments from their local health services. Controls were free from medical illness while 22q11DS subjects (as expected) had a range of palatal (n=9) and cardiac/vascular anomalies (n=6).

The Wechsler Intelligence Scale for Children-III (WISC-III) (Wechsler 1991) was used to assess general intellectual functioning while the strengths and difficulties questionnaire (SDQ) (Goodman et al. 2000) measured emotional symptoms, hyperactivity/inattention, conduct problems, peer problems and pro-social behaviour in all subjects. The autism screening questionnaire (ASQ) (short-version) (Berument et al. 1999) was used to measure autistic symptoms with a cut-off score of 7 for individuals who may have autism.

We used a previously published schizotypy scale designed for young people with 22q11DS (Campbell et al. 2006) and all behavioural assessments were completed by a primary caregiver or parents. Also, due to the high incidence of co-morbid neuropsychiatric disorder in 22q11DS and the potential for non-homogeneity, we only recruited subjects that were free (as far as we could best determine) from Axis One psychiatric disorder.
Table 1: Demographic, genetic and behavioural data and global volume

<table>
<thead>
<tr>
<th></th>
<th>22q11DS (n=11)</th>
<th>Controls (n=12)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender male/female</strong></td>
<td>5/6</td>
<td>8/4</td>
<td>1.0, 0.39</td>
</tr>
<tr>
<td><strong>Mean Age</strong></td>
<td>12 (SD±2.2; Range: 9–17)</td>
<td>13 (SD±2.5; Range: 9–17)</td>
<td>0.83</td>
</tr>
<tr>
<td><strong>Mean FSIQ</strong></td>
<td>66 (SD±8.0; Range: 56–84)</td>
<td>116 (SD±15.9; Range: 90–141)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Handedness</strong></td>
<td>11 Right</td>
<td>12 Right</td>
<td></td>
</tr>
<tr>
<td><strong>Strengths and Difficulties Questionnaire mean total score</strong></td>
<td>15.2 (SD±7.1; Range: 6–25)</td>
<td>4.9 (SD±3.4; Range: 0–11)</td>
<td>0.002</td>
</tr>
<tr>
<td><strong>Schizotypy mean score</strong></td>
<td>2.0 (SD±2.3; Range: 0–7)</td>
<td>0 (SD±0; Range: 0)</td>
<td>0.034</td>
</tr>
<tr>
<td><strong>Autism Screening Questionnaire mean score</strong></td>
<td>6.8 (SD±4.3; Range: 0–13)</td>
<td>2 (SD±4.0; Range: 0–10)</td>
<td>0.044</td>
</tr>
<tr>
<td><strong>COMT status</strong></td>
<td>7 &quot;V&quot; vs. 4 &quot;M&quot;</td>
<td></td>
<td>0.549</td>
</tr>
<tr>
<td><strong>Mean global grey matter volume (ml)</strong></td>
<td>738 (SD±69.1; Range: 649–814)</td>
<td>790 (SD±59.0; Range: 692–896)</td>
<td>0.067</td>
</tr>
<tr>
<td><strong>Mean global white matter volume (ml)</strong></td>
<td>385 (SD±36.5; Range: 321–436)</td>
<td>418 (SD±62.2; Range: 327–512)</td>
<td>0.138</td>
</tr>
<tr>
<td><strong>Mean total brain volume (ml)</strong></td>
<td>1122 (SD±97.8; Range: 975–1234)</td>
<td>1207 (SD±108.3; Range: 1018–1408)</td>
<td>0.062</td>
</tr>
</tbody>
</table>

**Genetics**

DNA was extracted from blood samples collected on all subjects. Fluorescence in situ hybridisation (FISH) (Oncor Inc, Gaithersburg, MD, USA) confirmed 3 Mb 22q11.2 deletion in 22q11DS cases while chromosome 22q11.2 deletion was excluded in all controls. The COMT Val<sup>158</sup>Met polymorphism was genotyped using the SNaPshot technique of single base extension (Applied Biosystems, Foster City, CA, USA). The initial PCR reaction was performed using a Touchdown-PCR-protocol, with the following primers: forward: 5'-ACTGTGGCTACTCAGCTGTG-3' and reverse: 5'-cctTTTTCCAGGTCTGACAA-3'. The allele at the single nucleotide polymorphism (SNP) position was determined by use of a 30 bp extension primer (5'-ATCACC CAGCGGATGGTGGATTTCGCTGGC-3'). All alleles were resolved on an ABI 3100 sequencer (Applied Biosystems, Foster City, CA, USA).

**MRI acquisition protocol**

Data were acquired using a 1.5 T GE Signa LX system (General Electric, Milwaukee, WI, USA), with actively shielded magnetic field gradients (maximum amplitude 40 mT m<sup>-1</sup>). A standard quadrature birdcage head coil was used for both RF transmission and signal reception. Each DT-MRI volume was acquired using a multi-slice peripherally-gated echo-planar imaging (EPI) sequence, optimised for precise measurement of the diffusion tensor in brain parenchyma, from 60 contiguous near-axial slice locations. Images were acquired with isotropic (2.5×2.5×2.5 mm) voxels, reconstructed to 1.875×1.875×2.5 mm. Image acquisition was synchronised to the cardiac cycle using a peripheral gating device placed on the subject’s forehead. Echo time was 107 ms while the effective repetition time was 15 R-R intervals. Duration of the diffusion encoding gradients was 17.3 ms giving a maximum diffusion-weighting of 1,300 s mm<sup>-2</sup>. At each slice location, seven images were acquired with no diffusion gradients applied (b=0), together with 64 diffusion-weighted images in which gradient directions were uniformly distributed in space.

Following correction of the diffusion-weighted images for image distortions introduced by the diffusion-weighting gradients, in-house software was used to 1) remove non-brain tissue and 2) determine the diffusion tensor in each voxel (Basser et al. 1994a, b). Images of 1) mean T<sub>2</sub>-weighted intensity (with no diffusion gradients applied) and 2) FA were computed for each subject. Full details are given elsewhere (Jones et al. 2002a). FA which is an intravoxel measure of the degree to which there is a preferential direction of diffusion of water molecules within tissue has values ranging from 0–1 (perfectly isotropic to perfectly anisotropic diffusion) and provides a measure of “tissue integrity” (Horsfield and Jones 2002; Jones et al. 2002b; Mori and Zhang 2006).

A 3D inversion recovery prepared spoiled gradient recalled acquisition in the steady state (IR-SPGR) scan [repetition time (TR)=11.9 ms, echo time (TE)=5.2 ms, inversion time (TI)=450 ms] was also acquired in the same scanning session. Images were acquired with a 256×192 matrix over a 200×160 mm field of view, and reconstructed to a 256×256 matrix, giving a final in plane pixel size of 0.78125 mm. Data were collected, in a coronal orientation, from 124 1.5 mm thick sections.

**Pre-processing DT-MRI data**

Any scans demonstrating image corruption or motion artefacts were excluded. As mentioned earlier, a DT-MRI scan for one 22q11DS subject demonstrated excessive motion artefact and as a result, both structural and DT-MRI scans for this individual were excluded from the imaging pipeline. To facilitate a voxel-based comparison of
FA between subjects, images were preprocessed using SPM2 (Wellcome Department of Imaging Neuroscience, University College London) within MATLAB 6.5.2 (The MathWorks, Natick, MA, USA) and used a two stage approach. DT-MRI group mapping techniques (derived from Voxel-Based Morphometry (VBM) analysis methods developed for structural T1 and/or T2-weighted images) aligned DT-MRI data into standard space.

**Normalisation**

We performed a two stage normalisation. The first step was to align all the scans into standard Montreal Neurological Institute (MNI) space using the T2-weighted (non-diffusion-weighted) images of the DT-MRI data. We then used parameters from this registration to map all the FA images into MNI space, and average them to generate a customised FA template. Finally, we re-registered all the original FA images to this new FA template. Use of such a study specific, "half way", template reduces the bias that would otherwise exist due to the larger degree of warping needed to match the 22q11DS brains than the control brains to a standard (control subject based) template.

**Smoothing**

Scans need to be smoothed in order to reduce confounds due to individual variation in WM anatomy. Smoothing the data in order to coerce it into the appropriate statistical distribution is also a prerequisite for some analysis approaches, but is not necessary for our non-parametric approach (see below). The degree of smoothing to apply is still a subject of much discussion as different smoothing levels result in varying results (Jones et al. 2005); in the absence of a specific hypothesis about the spatial extent of any abnormalities, we applied a smoothing filter (Gaussian, 5 mm full-width at half maximum) to aid between-subject anatomical matching and improve the signal-to-noise ratio.

**Segmentation and masking**

The registered FA images were segmented to give maps of the probability of a tissue being either white or grey matter, and these segmented images were thresholded at a low level (10%) to provide a binary mask of WM; visual inspection of example datasets confirmed that masking at this threshold produced maps which included all major WM areas, and did not suffer from any unexpected 'holes' (e.g. in low FA regions caused by crossing fibres). An accurate segmentation was not essential, and a relatively liberal threshold was used deliberately, in order to create a slightly 'over inclusive' mask.

**Statistics**

**Demographic, genetic and behavioural data**

Statistical analysis was performed in SPSS (SPSS 14.0 for Windows, SPSS Inc, Chicago, IL, USA). Student's t-test ($p<0.05$, two-tailed) for independent samples was used to examine between-group differences on age and IQ and ASQ, SDQ and schizotypy scores while the binomial test assessed COMT and gender distribution ($p<0.05$, two-tailed).

**DT-MRI group mapping**

We examined the statistical significance of between group differences in FA using a non-parametric permutation-based method. Locally developed software, XBAM (version 3.4) (IoP, http://www.brainmap.co.uk/) measured between group differences at each intracerebral voxel in standard space by fitting an analysis of covariance model (ANCOVA) where FA was the dependent variable and group classification as the key predictor variable. In additional analyses, IQ and gender were also used as covariates in between-group comparisons.

Given that brain changes are likely to extend over a number of contiguous voxels, test statistics incorporating spatial information such as 3D cluster mass (the sum of suprathreshold voxel statistics), are generally more powerful than other possible test statistics, which are informed only by data at a single voxel (Bullmore et al. 1999). The voxelwise statistic images were therefore thresholded at a relatively lenient level of $p<0.05$, and voxels that were spatially contiguous in three dimensions in the thresholded maps were assigned to the same cluster. The sum of voxel statistics within each cluster was computed for each randomisation to form a distribution of cluster mass under the null hypothesis.

As no parametric distribution is known for cluster mass, permutation testing was used to assess statistical significance; the mass of each cluster in the observed data was compared to this randomised distribution, and significant clusters were defined as those that had a greater cluster mass than the randomised distribution at a particular significance level. Such a non-parametric approach also overcomes the assumption that parametric methods adopt that the residuals of the model tested will follow a Gaussian distribution (which has been shown to not always be true for DT-MRI data, even after extensive smoothing) (Jones et al. 2005).

At this stage, we considered only those voxels at which all subjects contribute data which, along with the masking procedure above, restricted the analysis to core WM regions. This reduced the search volume (and thus the number of comparisons made) and also avoided testing at the grey/white interfaces, where the high grey/white contrast of FA images exacerbates any edge effects.
Overall, analyses are reported at a stringent adaptive cluster level threshold where the expected number of false-positive clusters is less than one per analysis. XBAM reported coordinates of the centre of mass of clusters in MNI space which is used by SPM; MNI coordinates were subsequently converted to Talairach space via a non-linear linear transformation (Brett et al. 2002) (details at http://imaging.mrc-cbu.cam.ac.uk/imaging/MniTalairach) and these were interpreted with the aid of known neuroanatomical pathways (Talairach and Tournoux 1988; Mori et al. 2005).

Voxel-based morphometry (VBM)

The pre-processing and processing steps of our structural MRI data have been published previously (Ashbumer and Friston 2000). In brief, we used SPM5 (Wellcome Department of Imaging Neuroscience, University College London, UK) which integrates initial tissue classification and registration into a single model and also includes correction of the effects of image intensity non-uniformity often termed the ‘bias field’ (Ashbumer and Friston 2005). WM was extracted from the normalised images and “modulated” to compensate for the effects of spatial normalisation. Modulation consists of multiplying each voxel value by its relative volume before and after warping, in order to compensate for the fact that spatial normalisation expands/contracts some brain regions. After modulation, the total amount of white (or grey) matter is the same as in the original images. Good and colleagues (Good et al. 2001) note that “in effect, an analysis of modulated data tests for regional differences in the absolute amount (volume) of grey matter.” Images were then smoothed by convolution with a 5 mm Gaussian kernel. The maps so produced are referred to as images of “grey (or white) matter volume” to distinguish them from the images of “concentration” or “density” which result if the modulation stage is omitted. Additionally, global WM volumes were extracted via SPM5 and between group differences were compared using Student’s t-tests. If global volumes significantly differ, these are then entered as further covariates in between-group analyses.

As with DT-MRI, structural brain changes are likely to extend over a number of contiguous voxels and thus permutation testing was also used to assess statistical significance, in this case using the XBAMMM programme; details of which can be found at http://www-bmu.psychiatry.cam.ac.uk/software/docs/xbamm/. IQ was also used as a covariate in additional between-group analysis.

Post hoc analysis of Schizotypy scores

We carried out a preliminary (post hoc) analysis to determine if significant differences in WM FA were associated with behavioural variation within people with 22q11DS. To do this, we extracted mean FA values from each of the clusters that were significantly different from healthy controls. We then related these values (using Pearson product-moment correlation coefficients) to severity of schizotypy (Campbell et al. 2006). A Bonferroni adjusted alpha of 0.025 was subsequently applied.

Post hoc analysis of COMT status

Finally, we carried out a second preliminary investigation within people with 22q11DS by comparing the WM FA and volume of those with vCOMT to their counterparts with MCOMT.

## Results

### Demographic, genetic and behavioural data

The groups did not differ significantly in age, or gender distribution. However, as expected, the mean FSIQ of those with 22q11DS was significantly lower; also mean SDQ, ASQ and schizotypy scores were higher in VCFS individuals than controls. Six 22q11DS subjects and one healthy subject scored above the cut-off of 7 in the ASQ. With regard to COMT status, seven subjects with 22q11DS had vCOMT while four had MCOMT (Table 1).

### Group contrasts of FA using DT-MRI group mapping

#### 22q11DS vs. controls FA (not controlled for IQ)

Relative to controls, WM FA in 22q11DS subjects was significantly reduced in the frontal, parietal and temporal lobes of the left hemisphere. The centre of the most significant cluster was localised to the WM of the superior thalamic radiation and the cluster encompassed the: 1) projections from the thalamus to the parietal lobe via the posterior limb of the internal capsule; 2) projections from the motor cortex of the frontal lobe to the posterior limb of the internal capsule via the superior region of the corona radiata; 3) tapetum (Dejerine 1895; Crosby et al. 1962) lateral to the posterior horn of the lateral ventricle; 4) posterior thalamic radiation; and 5) the fronto-parietal course of the arcuate fasciculus (AF) (Fig. 1, Table 2).

In contrast, people with 22q11DS had a significantly increased FA relative to controls (again, exclusively in the left hemisphere) in regions that were anatomically more anterior and inferior to the FA decreases described above. The most significant clusters of FA increases included the WM of the genu/anterior limb of the internal capsule together with the anterior and superior portions of the corona radiata.
Fig. 1 Fractional anisotropy (FA) in 22q11DS subject group vs. control group (not controlled for IQ). Higher FA in controls than in 22q11DS subjects (orange). Higher FA in 22q11DS subjects than in controls (blue). Ascending 2 mm transverse sections; (reversed where L = R, R = L).

Table 2 Cluster localisation for significant differences in white matter fractional anisotropy (FA)

DT-MRI group mapping of white matter FA of 22q11DS subjects vs. controls (cluster significance threshold \( p \geq 0.0005 \))

<table>
<thead>
<tr>
<th>Not controlled for IQ</th>
<th>White matter FA deficits in 22q11DS subjects</th>
<th>White matter FA excesses in 22q11DS subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cluster size (number of voxels)</td>
<td>Talairach and Toumouex coordinates</td>
<td>Region/tract</td>
</tr>
<tr>
<td>378</td>
<td>(-23)</td>
<td>(-22)</td>
</tr>
</tbody>
</table>

**White matter FA excesses in 22q11DS subjects**

- 463 | \(-13\) | 5 | 0 | Genu and anterior limb of internal capsule/anterior and superior portions of the corona radiata | Left |

**Controlled for IQ**

<table>
<thead>
<tr>
<th>White matter FA deficits in 22q11DS subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cluster size (number of voxels)</td>
</tr>
<tr>
<td>201</td>
</tr>
<tr>
<td>280</td>
</tr>
<tr>
<td>180</td>
</tr>
<tr>
<td>699</td>
</tr>
<tr>
<td>752</td>
</tr>
<tr>
<td>150</td>
</tr>
</tbody>
</table>
When gender was added as a covariate, a small cluster was localised to the left frontal lobe but subsequently did not reach statistical significance when correction for type I error was applied.

22q11DS vs. controls FA (controlled for IQ)

Significant widespread FA deficits were found in the 22q11DS group relative to healthy controls that extended from the brainstem to more superior brain sections. These deficits were mainly bilateral and found in structures close to the mid-line such as the corpus callosum, internal capsule and cingulum but also more laterally in temporal and parietal lobe regions. There were no regions of increased FA in the 22q11DS group relative to controls (Fig. 2, Table 2).

FA deficits in the brainstem encompassed the right corticopontine, corticospinal and middle cerebellar peduncle while the corpus callosum was found to have reduced FA especially in the splenium, tapetum and body and to a lesser extent in the genu. FA deficits were found in the cingulum adjacent to the body of the corpus callosum and in the uncinate fasciculus in the temporal lobe. The hippocampi and inferior longitudinal and inferior fronto-occipital fasciculi in the temporal lobes were found to have reduced FA bilaterally while the bilateral corona radiata in the occipital and parietal lobes showed FA deficits.

Within 22q11DS, a preliminary analysis of Schizotypy and differences in FA

There was a significant negative correlation between high schizotypy scores and decreased WM FA in the right posterior limb of internal capsule ($r=−0.822$, $p=0.023$), right body of corpus callosum ($r=−0.827$, $p=0.022$) and left splenium of corpus callosum ($r=−0.851$, $p=0.015$).

Within 22q11DS, a preliminary analysis of COMT variation

22q11DS individuals with $^v$COMT had significantly lower FA than their counterparts with $^m$COMT. The most signi-
significant differences were found bilaterally in the: 1) anterior cingulum; 2) frontal lobe; and 3) corpus callosum. However, 22q11DS people with VCOMT also had a unilateral FA reduction in right hemisphere regions incorporating the: 1) forceps minor; 2) inferior fronto-occipital and uncinate fasciculi; and 3) anterior/superior corona radiata (Fig. 3, Table 3).

Group contrasts of WM volume using VBM

22q11DS vs. controls WM volume (not controlled for IQ)

Mean global WM volume did not differ significantly between young people with 22q11DS and controls (mean=385 ml, SD±36.5 ml vs. mean=418 ml, SD±62.2 ml respectively; p=0.138) (refer to Table 1 for details of global volumes). However, there were significant differences in the regional distribution of WM (Fig. 4, Table 4).

Young people with 22q11DS, compared to controls, had a significant reduction bilaterally in the 1) middle cerebellar peduncle of the cerebellum and brainstem; 2) optic radiation and lingual, middle and inferior occipital gyri; 3) cuneus and precuneus; 4) posterior thalamic radiation; 5) body, genu and tapetum of the corpus callosum; 6) hippocampus; and 7) paracentral lobule. In contrast, 22q11DS people had a significantly greater WM volume bilaterally in the 1) anterior limb and genu of the internal capsule; 2) WM tracts from the basal ganglia; 3) medial frontal gyrus and cingulum; and 4) body and splenium of the corpus callosum.
Table 3 Genetic influence of COMT allele variation on white matter fractional anisotropy (FA) and volume

White matter FA reduction of 22q11DS subjects with mCOMT vs. MCOMT (cluster significance threshold \( p=0.00175 \))

<table>
<thead>
<tr>
<th>Cluster size (number of voxels)</th>
<th>Talairach and Toumoux coordinates</th>
<th>Region/tract</th>
<th>Hemisphere</th>
</tr>
</thead>
<tbody>
<tr>
<td>2721</td>
<td>-7 -6 26</td>
<td>Anterior cingulum/frontal lobe/corpus callosum forceps minor/inferior fronto-occipital and uncinate fasciculi/anterior and superior corona radiata</td>
<td>Bilateral</td>
</tr>
</tbody>
</table>

White matter volume reduction of 22q11DS subjects with mCOMT vs. MCOMT (cluster significance threshold \( p=0.005 \))

<table>
<thead>
<tr>
<th>Cluster size (number of voxels)</th>
<th>Talairach and Toumoux Coordinates</th>
<th>Region</th>
<th>Hemisphere</th>
</tr>
</thead>
<tbody>
<tr>
<td>212</td>
<td>-36 -35 -2</td>
<td>Hippocampus and superior/middle/inferior temporal gyri</td>
<td>Left</td>
</tr>
<tr>
<td>374</td>
<td>7 20 6</td>
<td>Anterior limb of internal capsule</td>
<td>Right</td>
</tr>
<tr>
<td>329</td>
<td>25 -2 11</td>
<td>Retrolenticular limb of internal capsule</td>
<td>Right</td>
</tr>
<tr>
<td>4924</td>
<td>8 -14 28</td>
<td>Cingulum and corpus callosum</td>
<td>Bilateral</td>
</tr>
<tr>
<td>213</td>
<td>30 -36 31</td>
<td>Supramarginal gyrus</td>
<td>Right</td>
</tr>
</tbody>
</table>

22q11DS vs. controls WM volume (controlled for IQ)

WM volume was found to be significantly increased relative to healthy controls. These regional increases were mainly bilateral and found in the brainstem, the internal capsule (posterior limb and genu) and superior corona radiata while unilateral increases were found in the posterior corona radiata in the right occipital lobe and in the left pre-central gyrus. There were no regions of WM volume reduction in 22q11DS (Fig. 5, Table 4).

Within 22q11DS, a preliminary analysis on the effect of COMT genotype

mCOMT 22q11DS individuals had a significantly decreased WM volume relative to those with MCOMT bilaterally in the 1) frontal lobes; 2) cingulum; 3) corpus callosum; 4) internal capsule; 5) hippocampus; and 6) superior and middle temporal gyri; and unilaterally in the right inferior temporal and supramarginal gyrus (Fig. 6, Table 3).

Discussion

In this cross sectional study we compared measures of WM microstructural integrity and volume using DT-MRI group mapping and VBM respectively in young people with 22q11DS and healthy controls. Also, within 22q11DS, we carried out a preliminary analysis of the relationship between WM abnormalities and schizotypy, and with variation in COMT genotype.

In young people with 22q11DS, we found reduced FA in numerous brain regions as compared to controls. However, and consistent with previous DT-MRI studies (Barnea-Goraly et al. 2003; Simon et al. 2005), these predominantly affected the parietal lobe. When IQ was included as a covariant in the analysis, FA deficits in the corpus callosum, internal capsule, AF and thalamic radiations remained significant. It has been suggested that a reduction in FA is caused by damage to highly-aligned axonal structures or replacement of axonal fibres with less tightly organised cells (Horsfield and Jones 2002). Therefore these differences in FA may be associated with impaired 'connectivity' in a number of neural systems, and confirm earlier reports of widespread deficits in microstructural integrity in 22q11DS.

We also found higher FA in 22q11DS relative to controls that were localised to regions distinct from the FA reductions described above. These occurred exclusively in the left hemisphere and particularly in the left internal capsule. Higher FA has previously been reported (Barnea-Goraly et al. 2003; Simon et al. 2005) but it has been suggested that higher FA reported by others in 22q11DS may be artefactual—because 22q11DS brains are abnormally shaped and so may not register perfectly to the brain template that is typically used in imaging studies (e.g. healthy child or adult templates) during the normalisation step in SPM. As noted in our methods, we addressed this...
potential confound by employing a customised FA brain template derived from all the subjects under study, aiming to minimise both the degree of warping needed for any individual subject, and bias in such warping between the groups. Increased FA did not remain significant in 22q11DS when we included IQ as a covariate, although this may simply reflect a loss of sensitivity (i.e. a type II error) due to the inclusion of two strongly correlated variables (IQ and group) in the analysis (Miller and Chapman 2001). Compared to previous DTI reports in 22q11DS, our study is the first to covary for IQ, and this combined with template considerations (i.e. our use of a study specific template), may have resulted in our findings differing from other work.

In our VBM analyses of WM proportion, regional increases and decreases of volume were found within young people with 22q11DS. A bilateral reduction in WM was found (consistent with prior reports (Eliez et al. 2000; Kates et al. 2001; van Amelsvoort et al. 2001, 2004; Campbell et al. 2006)) in regions such as the cerebellum, corpus callosum, and occipital and medial temporal lobes. Although, these findings did not remain significant when covaried for IQ, increased WM proportion bilaterally in regions close to the mid-line cerebral structures did; and particularly in the posterior limb of the internal capsule and superior corona radiata. Unilateral increases were also found in the right posterior corona radiata and left pre-central gyrus. Our finding of increased WM volume in the frontal lobe contrasts with a previous study which concurrently examined WM FA and volume in 22q11DS (Simon et al. 2005) that reported right middle frontal gyrus volume reduction but supports the finding of relatively increased frontal WM
Table 4  Cluster localisation for significant differences in white matter volume

VBM analysis of white matter volume of 22q11DS subjects vs. controls (cluster significance threshold \( p = 0.006 \))

<table>
<thead>
<tr>
<th>Not controlled for IQ</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>White matter volume deficits in 22q11DS subjects</strong></td>
</tr>
<tr>
<td>Cluster size (number of voxels)</td>
</tr>
<tr>
<td>4218</td>
</tr>
<tr>
<td>149</td>
</tr>
<tr>
<td>77</td>
</tr>
<tr>
<td>533</td>
</tr>
<tr>
<td>688</td>
</tr>
<tr>
<td>489</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>White matter volume excesses in 22q11DS subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>133</td>
</tr>
<tr>
<td>459</td>
</tr>
<tr>
<td>396</td>
</tr>
<tr>
<td>341</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Controlled for IQ</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>White matter volume excesses in 22q11DS subjects</strong></td>
</tr>
<tr>
<td>108</td>
</tr>
<tr>
<td>602</td>
</tr>
<tr>
<td>771</td>
</tr>
<tr>
<td>210</td>
</tr>
<tr>
<td>85</td>
</tr>
</tbody>
</table>

volume in other studies of 22q11DS (Eliez et al. 2000; Antshel et al. 2008).

Overall, a number of findings for FA and volume were not co-located after IQ was covaried for e.g. reduced FA in the corpus callosum where reduction in WM volume was absent. Therefore in such regions, the differences in FA cannot be accounted for simply by differences in WM volume (and vice versa). However, there were also regions where reduced FA but increased volume both occurred for instance, in the frontal lobes. It is possible that given 22q11DS is associated with cortical dysgenesis, an increased number of ectopic neurones in the WM may account for a reduced FA in association with a relatively increased volume that may be attributed to a loss of directional organisation with a relative preservation or increase of cell density (Wiesmann et al. 1999; Trivedi et al. 2006). Therefore, (wherever possible) both sMRI and DT-MRI should be used together to investigate WM pathology given that the latter may be able to detect abnormalities in normal appearing WM on conventional MRI (Rugg-Gunn et al. 2001; Makki et al. 2007).

As some of the differences we found in WM microstructural integrity may affect behaviour and/or risk for developing psychosis, we investigated for the first time, the relationship between schizotypy and WM FA in brain regions which differed significantly between young people with 22q11DS and controls. We found a significant negative correlation between increased schizotypy scores and reduced FA in the clusters that encompassed WM of the right posterior limb of internal capsule and the right body and left splenium of corpus callosum within young people with 22q11DS. This may be relevant to the non-deleted general population as the internal capsule contains reciprocal WM fibres from the thalamus to the cerebral cortex and reduced FA of the corpus callosum and internal capsule has been previously reported in schizophrenia (Buchsbaum et al. 2006; Mitelman et al. 2007). Our pilot evidence provides tentative support for the suggestion that microstructural abnormalities in these WM tracts may partially explain some schizotypic behaviours in 22q11DS but these results however require replication in future studies.
We conducted a further preliminary investigation on the effect of COMT polymorphism within 22q11DS youngsters where vCOMT was found to be associated with significant reduction in local WM volume; and perhaps especially in the frontal lobes, cingulum and corpus callosum. Similarly, we also found preliminary evidence for decreased FA in these same regions. As COMT activity is largely responsible for dopamine modulation in the pre-frontal cortex (PFC) (Tunbridge et al. 2004), haploinsufficiency of the COMT gene means that individuals with 22q11DS are exposed to high levels of prefrontal dopamine (Gothelf et al. 2008). COMT polymorphism is known to affect global brain development in both healthy adults (Zinkstok et al. 2006) and adults with 22q11DS (van Amelsvoort et al. 2008) where in the latter whole-brain investigation of 22q11DS adults by our group, we reported that variation in COMT activity not only affects the anatomy of the frontal lobes but also a number of non-frontal regions. Most studies so far in children and adolescents have only assessed the PFC (Gothelf et al. 2005; Kates et al. 2006) and the results from our current study involving children and adolescents with 22q11DS demonstrate the widespread effects of COMT polymorphism that affects both the volume and microstructural integrity of WM which extends beyond frontal lobe anatomy.

Our study has a number of methodological considerations including, the sample size, cross-sectional design, lack of an FSIQ-matched control group, and the multiple comparisons we carried out (and therefore the increased risk for Type 1 error). However, we deliberately did not include a learning disabled control group as our study was

Fig. 5 White Matter volume in 22q11DS subjects vs. controls (controlled for IQ). Significant volumetric excesses in 22q11DS (red/yellow). Ascending transverse sections; (reversed where L = R, R = L)
designed to address the question how people with 22q11DS differ from those with healthy brain development. It would be virtually impossible to recruit a ‘perfect’ control group of non-22q11DS controls with the same degree of learning disability as the 22q11DS probands. With regard to genetics, we only investigated COMT (because it modulates both brain maturation and psychosis) although a number of other important genes that impact on brain development are also deleted in the 22q11.2 region e.g. TBX-1 and PRODH. Our study does however have some strengths, for instance a customised FA brain template was used in order to reduce registration errors, and the level of significance was adapted in order to yield less than one false-positive cluster over the entire imaging volume investigated. Therefore, we feel Type 1 errors are unlikely to fully explain our results.

In conclusion, our study demonstrates that people with 22q11DS have widespread differences in both the microstructural integrity and volume of WM that are largely independent. Although WM volume may appear relatively normal as measured using standard MRI techniques, this does not necessarily indicate that the underlying WM microstructural integrity is also unaffected. Further studies on the relationship of WM differences to the behavioural phenotype of 22q11DS and to genetic variation are required.

Acknowledgements We thank all our patients and the control subjects for participating in this study.

We are grateful to the radiographers at the Centre for Neuroimaging Sciences for their help at scanning sessions.

Conflict of interest None.
References


Neuroanatomical correlates of psychosis in temporal lobe epilepsy: voxel-based morphometry study

Frederick Sundram, Mary Cannon, Colin P. Doherty, Gareth J. Barker, Mary Fitzsimons, Norman Delanty and David Cotter

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Neuroanatomical correlates of psychosis in temporal lobe epilepsy: voxel-based morphometry study

Frederick Sundram, Mary Cannon, Colin P. Doherty, Gareth J. Barker, Mary Fitzsimons, Norman Delanty and David Cotter

Background
Temporal lobe epilepsy is associated with a significant risk of psychosis but there are only limited studies investigating the underlying neurobiology.

Aims
To characterise neuroanatomical changes in temporal lobe epilepsy and comorbid psychosis.

Method
The study population comprised all individuals with temporal lobe epilepsy on the epilepsy database at the National Centre for Epilepsy and Epilepsy Neurosurgery in Ireland (Beaumont Hospital) between 2002 and 2006. Ten people with temporal lobe epilepsy with psychosis were matched for age, gender, handedness, epilepsy duration, seizure laterality, severity of epilepsy and anti-epileptic medication with ten comparison participants with temporal lobe epilepsy only. Participants received a magnetic resonance imaging scan and voxel-based morphometry analyses were applied to grey and white matter anatomy.

Results
Significant grey matter reduction was found bilaterally in those with temporal lobe epilepsy with psychosis in the temporal lobes in the inferior, middle and superior temporal gyri and fusiform gyri, and unilaterally in the left parahippocampal gyrus and hippocampus. Significant extra-temporal grey matter reduction was found bilaterally in the insula, cerebellum, caudate nucleus and in the right cingulum and left inferior parietal lobule. Significant white matter reduction in those with temporal lobe epilepsy with psychosis was found bilaterally in the hippocampus, parahippocampal/fusiform gyrus, middle/inferior temporal gyr, cingulum, corpus callosum, posterior thalamic radiation, anterior limb of internal capsule and white matter fibres from the caudate nuclei, and unilaterally in the left lingual gyrus and right midbrain and superior temporal gyrus.

Conclusions
Significant grey and white matter deficits occur in temporal lobe epilepsy with psychosis. These encompass the medial temporal lobe structures but also extend to lateral temporal and extratemporal regions. Some of these deficits overlap with those found in schizophrenia.

Declaration of interest
C.P.D. is on the advisory boards for Eisai and UCB Pharma and has received educational funding from Janssen-Cilag. G.J.B. has received honoraria for lecturing for GE Healthcare.

Epilepsy is one of the most common and debilitating neurological disorders, with a prevalence of 5–10 per 1000 population. The lifetime risk of psychiatric disorder in temporal lobe epilepsy is estimated to be as high as 60%. Specifically, temporal lobe epilepsy carries a substantial risk of psychosis at a prevalence rate of 2–7%, which is several times greater than that seen in the general population. Although temporal lobe epilepsy represents one of the highest known risk factors for the development of psychosis, very little is known about the neurobiology of temporal lobe epilepsy and associated psychosis.

The relationship between the temporal lobe and psychosis was first reported in 1963 by Slater and colleagues and some forms of temporal lobe epilepsy with psychosis have been noted to closely resemble schizophrenia. Temporal lobe epilepsy with psychosis has also been postulated to arise from abnormalities in fetal brain development, and to represent a model or ‘mock-up’ of schizophrenia. It is recognised that these conditions possibly share common genetic or environmental causes, and recently the mean interval between the onset of epilepsy and that of psychosis has been reported to be approximately 14.4 years.

Temporal lobe epilepsy has been associated with a variety of environmental insults such as birth injury, febrile convulsions, head trauma or central nervous system infection and where associated with genetic vulnerability may lead to acquired changes in brain anatomy. Up until the 1980s such changes in neuroanatomy were assessed through post-mortem neuropathological studies; however, with the advent of magnetic resonance imaging (MRI), most of such changes have been readily imaged in vivo and have been quantified using manual and computerised statistical methods. These methods provide an opportunity to improve our understanding of the pathogenesis of not only the psychosis associated with temporal lobe epilepsy but the causative mechanisms of psychosis in general.

So far, there have only been a limited number of MRI studies that have assessed regional volumetric differences in people with temporal lobe epilepsy and psychosis specifically. Three previous studies used manual region of interest (ROI) volumetry to compare participants with temporal lobe epilepsy only versus those with temporal lobe epilepsy with psychosis. Findings have included reduction of volume in the temporal, frontal and parietal lobes and superior temporal gyrus and left hippocampus grey matter volumes and also bilateral amygdala enlargement. Overall, however, the findings from manual ROI studies have not been consistent. A more recent study attempted to overcome the difficulties of reproducibility introduced by manual volumetry by using automated whole brain voxel-based morphometry (VBM). This study limited the assessment to grey matter and observed no cortical differences between groups. As this was not consistent with neuroimaging findings in schizophrenia, the authors suggested that temporal lobe epilepsy with psychosis may be a distinct entity to schizophrenia.
As reports thus far have inconsistently reported neuroanatomical correlates in several brain regions and the application of ROI volumetry to investigate structural abnormalities in people with temporal lobe epilepsy is difficult to reproduce and time consuming, we undertook the first VBM study of temporal lobe epilepsy with psychosis using unbiased, automated, quantitative voxel-based techniques to encompass both grey and white matter measures at a whole brain level. As this is the first study of its kind, the VBM approach was considered the most appropriate because it allows a hypothesis-free survey of the entire brain. This may subsequently permit hypotheses generation and areas to be investigated in future projects or the further application of advanced neuroimaging techniques.

Given the previous findings using manual volumetry, in the current study we hypothesised that individuals with temporal lobe epilepsy with psychosis when compared to those with temporal lobe epilepsy without psychiatric disorder would demonstrate: reduction in total brain and grey and white matter content; grey and white matter reduction in the temporal lobe; and structural differences that overlap with those found in schizophrenia.

**Method**

**Study population**

Our study was carried out at the National Centre for Epilepsy and Epilepsy Neurosurgery, Beaumont Hospital, Dublin, Ireland. We used a retrospective approach to identify people with temporal lobe epilepsy and ethics approval was provided by the Beaumont Hospital Medical Research Ethics Committee (reference number: 04/55).

Our study population included all individuals on our hospital’s research and clinical epilepsy database. The database represents ongoing efforts to develop a register of patients with epilepsy within Ireland (more details available at www.epilepsyprogamme.ie). We assessed individuals on the database attending the in-patient and out-patient neurology service at Beaumont Hospital between 2002 and 2006 (n = 860). As our centre has an epilepsy surgery programme where appropriate candidates receive presurgical evaluation, the epilepsy population under study represents a combination of medically managed and those with medically intractable or surgically remediable epilepsies.

Two epileptologists (C.R.D. and N.D.) examined a combination of seizure semiology, electroencephalogram (EEG), video-EEG telemetry and neuroimaging data. A diagnosis of epilepsy was based on the International League Against Epilepsy classification system, and our study participants had already been examined in prior studies and had consented to further assessment. The epilepsy service routinely refers people with epilepsy and ethics approval was provided by the Beaumont Hospital Medical Research Ethics Committee (reference number: 04/55).

Exclusion criteria for participation were a clinically detectable medical disorder known to affect gross brain structure (e.g. tumour, haemorrhage), pervasive developmental disorders (e.g. autism-spectrum disorder), individuals with an extratemporal epileptic focus, generalised or unclassified seizures, an IQ < 70 on the WAIS-R,26 age < 18 years, previous neurosurgery, non-right-handedness and individuals with contraindications to MRI scan or no suitable MRI scan. As the software on our MRI scanner was upgraded in 2002, people scanned prior to this date were excluded to ensure homogeneity of scan parameters.

Participants with temporal lobe epilepsy with psychosis

The clinical syndrome we were interested in was defined as complex partial seizures with clinical findings and investigations (EEG and MRI) compatible with temporal lobe epilepsy. Further, the presence of delusions and/or hallucinations resulting in an ICD–10 diagnosis for psychosis was essential. Acute confusional states or depressive symptomatology alone were not deemed sufficient. Individuals with a drug-induced psychosis or episodes of psychosis provoked by excessive alcohol consumption or those representing complex partial status were also excluded.

Of the 860 individuals with epilepsy identified on the database, 280 had a diagnosis of temporal lobe epilepsy of whom 26 had been diagnosed with psychosis by the neuropsychiatric service. However, of these 26 with temporal lobe epilepsy with psychosis, 7 had previous neurosurgery for medically intractable seizures, 2 had an existing tumour, 1 was left-handed, 4 had an MRI scan at another institution either in Ireland or abroad and 2 had incomplete MRI scans. Consequently, ten individuals with temporal lobe epilepsy with psychosis (the epilepsy+psychosis group) were considered suitable for participation in our study (Fig. 1).

**Comparison group**

Using the Beaumont Hospital research and clinical epilepsy database, each epilepsy+psychosis participant included in the study was then matched for age (s.d. 5 years), gender, handedness, epilepsy duration, seizure laterality, severity of epilepsy and anti-epileptic medication with an individual with temporal lobe epilepsy but no psychosis who was attending the same neurology service. None of the comparison group (epilepsy-only group) had a lifetime history of prior psychosis and all were free of comorbid psychiatric disorder in the preceding year.

**Chart review**

Chart reviews were conducted for both the epilepsy+psychosis group and epilepsy-only group. The clinical assessments of the epilepsy+psychosis group included: total number of seizures, seizure laterality, seizure frequency, seizure semiology, presence of complex partial seizures, EEG and MRI results, results of sleep studies and neuropsychiatric assessments (psychometric test battery, psychopathology rating scale).

**Fig. 1 Summary of participant selection. MRI, magnetic resonance imaging.**
epilepsy + psychosis group completed by the specialised neuro-psychiatry service were objectively assessed using the Operational Criteria Checklist for Psychotic Illness (OPCRIT).27 This was used as it offers a polydiagnostic classification system that yields operationally defined psychiatric diagnoses with good reliability.28

All the epilepsy + psychosis group had to fulfil ICD-10 criteria for psychotic disorder that encompassed schizophrenia, persistent delusional disorder or other non-organic psychotic disorder; affective psychoses were excluded. With regard to timing of psychoses relative to seizure events, post-ictal psychoses were included where MRI and neuropsychiatric assessments were performed within a week of psychotic episodes, whereas inter-ictal psychoses were included where the same assessments were completed within a month of commencement of psychosis in the absence of antecedent seizure activity. Neither the epilepsy + psychosis group nor the epilepsy-only group had features of intellectual disability, non-epileptic seizures or history of poor adherence with their anti-epileptic medication. For a summary of participant characteristics see Tables 1 and 2.

Table 1 Characteristics of cohort: epilepsy + psychosis v. epilepsy-only group

<table>
<thead>
<tr>
<th></th>
<th>Epilepsy+psychosis group (n = 10)</th>
<th>Epilepsy-only group (n = 10)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age: years, mean (s.d.)</td>
<td>35 (5.2)</td>
<td>33 (6.1)</td>
<td>0.36</td>
</tr>
<tr>
<td>Male/female, n</td>
<td>7/3</td>
<td>7/3</td>
<td></td>
</tr>
<tr>
<td>Right-handed, n</td>
<td>10</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Age at diagnosis of epilepsy, mean (s.d.)</td>
<td>12 (11.0)</td>
<td>16 (8.2)</td>
<td>0.41</td>
</tr>
<tr>
<td>Epilepsy duration: years, mean (s.d.)</td>
<td>23 (12.4)</td>
<td>17 (8.6)</td>
<td>0.20</td>
</tr>
<tr>
<td>Site of epileptic focus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right-sided</td>
<td>6</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Left-sided</td>
<td>4</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Lesional vs. non-lesional</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lesional</td>
<td>6 (4 right and 2 left)</td>
<td>5 (4 right and 1 left)</td>
<td></td>
</tr>
<tr>
<td>Non-lesional</td>
<td>4</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Age at first psychotic event: years, mean (s.d.)</td>
<td>30 (5.6)</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Duration of epilepsy at time of first psychotic event: years, mean (s.d.)</td>
<td>18 (9.9)</td>
<td>N/A</td>
<td></td>
</tr>
</tbody>
</table>

N/A, not applicable.
a. All values represent t-tests.

Table 2 Clinical characteristics and prescribed antipsychotic and anti-epileptic medication in epilepsy + psychosis group and anti-epileptic medication in matched epilepsy-only group

<table>
<thead>
<tr>
<th>Participant</th>
<th>Timing of psychosis relative to seizure event</th>
<th>Antipsychotic medication</th>
<th>Daily dosage</th>
<th>Seizure Frequency</th>
<th>Aura</th>
<th>CPS</th>
<th>SG</th>
<th>Refract</th>
<th>Epilepsy clinical data</th>
<th>Epilepsy+psychosis group anti-epileptic medication</th>
<th>Matched epilepsy-only group anti-epileptic medication</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Inter-ictal</td>
<td>Haloperidol</td>
<td>20 mg</td>
<td>1/week</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
<td>N</td>
<td>Levetiracetam</td>
<td>Valproate</td>
<td>Valproate</td>
</tr>
<tr>
<td>2</td>
<td>Post-ictal</td>
<td>Amisulpride</td>
<td>800 mg</td>
<td>1/month</td>
<td>N</td>
<td>Y</td>
<td>N</td>
<td>N</td>
<td>Valproate</td>
<td>Levetiracetam</td>
<td>Valproate</td>
</tr>
<tr>
<td>3</td>
<td>Inter-ictal</td>
<td>Risperidone</td>
<td>6 mg</td>
<td>1/week</td>
<td>N</td>
<td>Y</td>
<td>N</td>
<td>N</td>
<td>Leviracetam</td>
<td>Valproate</td>
<td>Valproate</td>
</tr>
<tr>
<td>4</td>
<td>Inter-ictal</td>
<td>Haloperidol</td>
<td>2 mg</td>
<td>2/day</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Carbamazepine</td>
<td>Carbamazepine</td>
<td>Carbamazepine</td>
</tr>
<tr>
<td>5</td>
<td>Post-ictal</td>
<td>Olanzapine</td>
<td>5 mg</td>
<td>1/day</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Levetiracetam</td>
<td>Levetiracetam</td>
<td>Carbamazepine</td>
</tr>
<tr>
<td>6</td>
<td>Post-ictal</td>
<td>Thioridazine/</td>
<td>400 mg/30 mg</td>
<td>1/day</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Carbamazepine</td>
<td>Carbamazepine</td>
<td>Carbamazepine</td>
</tr>
<tr>
<td>7</td>
<td>Post-ictal</td>
<td>Haloperidol/</td>
<td>5 mg/20 mg</td>
<td>1/2 weeks</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
<td>N</td>
<td>Carbamazepine</td>
<td>Carbamazepine</td>
<td>Carbamazepine</td>
</tr>
<tr>
<td>8</td>
<td>Post-ictal</td>
<td>Olanzapine</td>
<td>20 mg</td>
<td>3-4/week</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Topiramate</td>
<td>Carbamazepine</td>
<td>Carbamazepine</td>
</tr>
<tr>
<td>9</td>
<td>Inter-ictal</td>
<td>Olanzapine</td>
<td>20 mg</td>
<td>1/month</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
<td>N</td>
<td>Carbamazepine</td>
<td>Carbamazepine</td>
<td>Carbamazepine</td>
</tr>
<tr>
<td>10</td>
<td>Inter-ictal</td>
<td>Olanzapine</td>
<td>12.5 mg</td>
<td>2-3/week</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Carbamazepine</td>
<td>Carbamazepine</td>
<td>Carbamazepine</td>
</tr>
</tbody>
</table>

CPS, complex partial seizures; SG, secondary generalisation; Refract, refractory to medical treatment.
MRI brain image acquisition

All participants had a volumetric spoiled gradient recall (SPGR) acquisition in the steady-state MRI brain scan at Beaumont Hospital using a 1.5 T scanner (GE Signa Systems, Paris). Coronal thin-cut 1.5 mm slices were obtained via a three-dimensional (3D)-volume gradient echo-pulse sequence that was radio-frequency spoiled. A sagittal localiser was first acquired and the volume of interest was then arranged to include the whole brain. The 3D-SPGR sequence was acquired over a period of 14 min with the following MRI parameters: repetition time (TR) = 35 ms, echo time (TE) = 15 ms, readout bandwidth of 16 kHz and excitation flip angle of 35°. The data were collected with an in-plane image matrix of 256 x 256 pixels over a field of view (FOV) of 24 x 24 cm, leading to a voxel size of 0.09375 x 0.09375 cm. In total, 128 partitions were collected, of which 4 were discarded during reconstruction to minimise wraparound artefacts, resulting in a final 124 1.5 mm slices covering a field of view of 18.6 cm.

Image analysis

Investigators were masked to participant group and each scan was checked for movement artefact and corruption prior to inclusion in the image-processing pipeline. In brief we used VBM that offers an unbiased and fully automated whole brain measurement technique that normalises all the images to the same stereotactic space and subsequently segments, modulates and smooths images (as described in more detail below); a statistical analysis is finally performed on the smoothed images to localise and make inferences about group differences. Registration into standard space, segmentation, modulation and smoothing was performed using Statistical Parametric Mapping software (SPM5, Wellcome Department of Imaging Neurosciences, University College London, UK; www.fil.ion.ucl.ac.uk/spm) within Matlab 7.0 (The MathWorks, Natick, Massachusetts, USA; www.mathworks.com/products/matlab) run on UNIX and statistical analyses were applied in the Brain Activation and Morphological Mapping (BAMM; www-bmu.psychiatry.cam.ac.uk/BAMM/index.html) package.

With previous versions of SPM, a set of processing steps commonly known as ‘optimised VBM’ was needed to ensure high-quality segmentations. However, the methodology was inherently circular as the registration required an initial tissue classification, and the tissue classification requires an initial registration. In SPM5 both components are integrated into a single model and it also includes correction of the effects of image intensity non-uniformity termed ‘the bias field’. Grey and white matter were extracted from the normalised images and ‘modulated’ to compensate for the effects of spatial normalisation. This is achieved by multiplying each voxel value by its relative volume before and after warping, in order to compensate for the fact that spatial normalisation expands/contracts some brain regions. After modulation, the total amount of grey (or white) matter is the same as in the original images. Good and colleagues note that ‘in effect, an analysis of modulated data tests for regional volume before and after warping, in order to compensate for omitted. In this study, however, in order to avoid potential confusion with manual volumetry measures such as stereology, we refer to them as ‘maps of grey (or white) matter content’, or simply ‘grey (or white) matter maps’.

Scans need to be smoothed in order to reduce confounds as a result of individual variation in neuroanatomy. Smoothing the data in order to coerce it into the appropriate statistical distribution is also a prerequisite for some analytical approaches, but is not necessary for our non-parametric approach (see below). The degree of smoothing to apply is still a subject of much discussion as different smoothing levels result in varying results. We applied a smoothing filter (Gaussian, 8 mm full-width at half maximum) as the literature suggests that this would aid the detection of potentially widespread changes in the neocortex and changes in smaller subcortical structures, between-participant anatomical matching and to improve the signal-to-noise ratio. Additionally, total global, grey matter and white matter volumes were extracted via SPm5 and between-group differences were compared using non-parametric Mann–Whitney U-tests. If these volumes were to significantly differ, they are then entered as covariates in between-group analyses. Similarly, characteristics such as epilepsy duration or age are entered as covariates in the analytical model should they significantly differ between groups.

Brain activation and morphological mapping

As structural brain changes are likely to extend over a number of contiguous voxels, test statistics incorporating spatial information such as 3D cluster mass (the sum of suprathreshold voxel statistics), are generally more powerful than other possible test statistics, which are informed only by data at a single voxel. Given that no parametric distribution is known for cluster mass, permutation-based testing that is implemented in the BAMM package (a joint development of the Brain Mapping Unit, Department of Psychiatry, University of Cambridge and The Institute of Psychiatry, London, UK) was used to assess statistical significance at both the voxel and cluster levels.

Between-group differences in grey and white matter volume were estimated by fitting an analysis of covariance (ANCOVA) model at each intracerebral voxel in standard space where proportional volume for each tissue class (grey or white matter) was the dependent variable and group classification as the key predictor variable. Instead of setting a single a priori P-value below which we regard findings as significant at the cluster level, we calculated, for a range of P-values, the number of clusters that would be expected by chance alone. We started by setting a relatively lenient P (P \leq 0.05) to detect voxels putatively demonstrating differences between groups; subsequently, we searched for spatial clusters of such voxels and tested the 'mass' of each cluster (the sum of suprathreshold voxel statistics it comprises) for significance.

We then set the statistical threshold for cluster significance such that the expected number of false positive clusters arising by chance alone would be less than one over the whole imaging volume. As SPM was used initially for segmentation, BAMM yielded coordinates of clusters in Montreal Neurological Institute (MNI) space; MNI coordinates were subsequently converted to Talairach space via a non-linear transformation (further details can be found at http://imaging.mrc-cbu.cam.ac.uk/imaging/MniTalairach) and interpreted with the aid of widely accepted atlases.

Our non-parametric or distribution-free hypothesis testing procedure allows us to use cluster-level statistics even if their distribution is non-Gaussian (even after smoothing); further, there is significant evidence in the literature that cluster-level statistics incorporating information about the spatial neighbourhood of each voxel may be more sensitive than voxel test statistics. As voxel-level statistics are associated with multiple comparisons and thus increased risk of type I error, cluster-level statistics reduce such error due to the performance of fewer comparisons of several orders of magnitude. Finally, although variance of the data may differ with brain area, our clusters are evaluated over the whole brain and we also inspected
the voxel-level maps, in order to check for any gross discrepancies between cluster and voxel level. More details can be found at www-bmu.psychiatry.cam.ac.uk/software/docs/xbamm/.

Results

Total tissue volumes

There were no significant differences in median total global, grey or white matter volumes between the groups at the $P = 0.05$ level, although there was an approximate reduction of 6, 5 and 7% respectively in these tissue classes in the epilepsy+psychosis group (Table 3).

VBM of grey and white matter content

We found regional deficits affecting both grey and white matter but these changes were confined to the epilepsy+psychosis group. We found significant regional grey matter reduction unilaterally in the medial temporal lobe structures such as the left parahippocampal gyrus and hippocampus. Deficits also extended to the lateral temporal lobes encompassing the bilateral inferior, middle and superior temporal gyri and fusiform gyri. However, grey matter reduction was not limited to the temporal lobe structures but also extended to extratemporal regions. The most significant extratemporal deficits were distributed bilaterally and included the insula, cerebellum and caudate nuclei, and unilaterally in the right cingulum and left inferior parietal lobe (Fig. 2).

We also found significant regional white matter reduction in the epilepsy+psychosis group. Within the medial temporal lobe, these deficits were distributed bilaterally in the hippocampus and parahippocampal gyrus. White matter deficits were also found in the lateral temporal lobes bilaterally in the middle and inferior temporal gyri and fusiform gyri, whereas unilateral deficits were found in the right superior temporal gyrus. Reduction of white matter extended beyond the boundaries of the temporal lobes and bilaterally involved the cingulum, corpus callosum (genu, splenium and tapetum), anterior limb of internal capsule, posterior thalamic radiation and white matter fibres from the caudate nuclei, whereas unilateral deficits were found in the left lingual gyrus and right midbrain (Fig. 3). Tables 4 and 5 provide a summary of the anatomical locations of grey and white matter deficits.

Discussion

Main findings

Although Slater and colleagues8 observed the occurrence of schizophrenia-like psychoses in association with epilepsy some 50 years ago, very few MRI studies have been undertaken since then and even fewer have attempted to accurately quantify in vivo the brain changes seen in the psychoses related to temporal lobe epilepsy. In this study we conducted the first ever comparison of the brain changes seen in the psychoses related to temporal lobe epilepsy only, whereas unilateral deficits were mainly localised to the temporal lobe. For example, significant grey matter deficits were found in the medial temporal lobes in the left hippocampus and parahippocampal gyrus. These findings are compatible with the neuroanatomical and neuropsychological deficits described in schizophrenia.18,40,41 However, we also found evidence of deficits bilaterally in the lateral temporal lobes and in extratemporal regions perhaps suggesting that the psychosis seen in temporal lobe epilepsy is the result of more widespread abnormalities.

Strengthening our argument that psychosis in temporal lobe epilepsy may be a more widely distributed disorder is the finding of grey matter deficits in the cingulum, insula and cerebellum; grey matter reduction in the insula and cingulum have been associated with psychosis45,46 and the cerebellum is emerging as an organ not only involved in motor coordination but also in higher cortical functions. Abnormalities in the cerebellum have previously been postulated to result in a "cognitive dysmetria"42 characterised by impairments in coordination of the perception, encoding, retrieval and prioritisation of experience and information and may arise from a defect in circuitry connecting the thalamus, frontal cortex and cerebellum. Although cerebellar grey matter deficits have been previously reported in people with temporal lobe epilepsy without comorbid psychiatric disorder,43 given that there are reciprocal neural pathways between the hippocampus and cerebellum, the cerebellum in those with psychosis may perhaps be particularly vulnerable to excitotoxic damage as a result of its connectivity with a pathological hippocampus. Currently, cerebellar abnormalities are also recognised to contribute to the development of schizophrenia45,46 and although we found evidence of grey matter reduction in the cerebellum, these were not specifically quantified and should be thus considered preliminary.

Pronounced white matter deficits were also found in our study in regions that included the corpus callosum, hippocampi, cingulum and parahippocampal gyri. Impaired intra- and inter-hemispheric connectivity has been suggested to play a major role in the development of schizophrenia45,46 and currently there is strong evidence of widespread altered cortico-cortical and intrahemispheric white matter integrity in schizophrenia.

<table>
<thead>
<tr>
<th>Table 3: Median total volume of tissue classes in epilepsy+psychosis vs. epilepsy-only group</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Epilepsy+psychosis group, median (s.d.)</strong></td>
</tr>
<tr>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>Total brain volume, ml</td>
</tr>
<tr>
<td>Total grey matter volume, ml</td>
</tr>
<tr>
<td>Total white matter volume, ml</td>
</tr>
</tbody>
</table>

| a. All values represent asymptotic significance (2-tailed) Mann-Whitney U-tests. |
transcallosal connections between homologous brain regions in schizophrenia.46 Abnormalities in such tracts may also contribute to the development of psychosis seen in temporal lobe epilepsy. Given our finding of greater white matter content deficits relative to grey matter in temporal lobe epilepsy with psychosis, assessment of white matter microstructure and connectivity should therefore be considered in future studies using a combination of VBM and advanced neuroimaging techniques such as diffusion tensor imaging as has been applied in other disorders where white matter is preferentially affected.47

Findings from other studies

Although the left temporal lobe has been associated with temporal lobe epilepsy with psychosis in older reports,48 the literature is not entirely consistent.49 Suboptimal matching may have contributed to this inconsistency. Furthermore, the majority of studies have employed manual volumetric region of interest techniques for example hand-tracing or stereology that may not be easily reproducible. One such manual volumetry study that compared temporal lobe epilepsy, temporal lobe epilepsy with psychosis and schizophrenia with healthy controls via hand-tracing methods reported ventricular enlargement and smaller temporal, frontal and parietal lobes and superior temporal gyrus grey matter volumes in all groups with the most pronounced differences being found in the temporal lobe epilepsy with psychosis group.14 Based on their findings, the authors concluded that cortical grey matter deficits in temporal lobe epilepsy with psychosis and schizophrenia predispose to chronic psychosis.

In another manual volumetry study, participants with temporal lobe epilepsy with psychosis were reported to have smaller total brain volumes than either individuals with temporal lobe epilepsy alone or healthy volunteers. No group differences were observed in hippocampal volumes, although bilateral amygdala enlargement of the order of 16-18% was reported in those with temporal lobe epilepsy with psychosis.15 However, as some of the participants with temporal lobe epilepsy only were dysthymic, this may have confounded findings. Hippocampal volume deficit was found in a further manual volumetry study of temporal lobe epilepsy with psychosis relative to healthy controls where the left hippocampus was reported as significantly smaller than the right.16

Overall, manual volumetry-based studies of temporal lobe epilepsy with psychosis have not reported consistent findings. Keller and colleagues50 argue that there are inherent difficulties
Fig. 3 Ascending transverse sections demonstrating regional white matter reduction (blue) in participants with temporal lobe epilepsy with psychosis. (Image is flipped so left is right and right is left.)

with manual volumetry (for instance stereology) that may account for such inconsistent evidence. For example, there is subjectivity associated with point counting on MRIs and in judgement of boundaries of structures under investigation (e.g. delineation between hippocampus, white matter and cerebrospinal fluid). These differences in judgement may lead to differing volume estimates between raters. Additionally, the calibration of MRIs with settings such as image brightness may influence the perception of brain tissue contrasts. Despite the inconsistencies reported with manual volumetry, automated techniques have only been applied on a limited basis in temporal lobe epilepsy with psychosis.

In one such automated computerised statistical study, the authors retrospectively explored cortical grey matter differences between 26 participants with temporal lobe epilepsy with psychosis, 24 with temporal lobe epilepsy only and 20 healthy comparisons. This was the same cohort as previously examined by Tebartz Van Elst and colleagues; VBM based on SPM99 was used to assess for morphometric differences and no significant cortical grey matter differences between the temporal lobe epilepsy with psychosis and the temporal lobe epilepsy only groups were found. However, the temporal lobe epilepsy only group showed a significant increase in grey matter concentration in the right temporal lobe relative to healthy controls. The authors concluded that since they observed no differences between temporal lobe epilepsy with psychosis and the temporal lobe epilepsy only groups, and since cortical pathology is prominent in schizophrenia, temporal lobe epilepsy with psychosis may represent a clinically distinct entity from schizophrenia.

In another VBM analysis, 20 people with temporal lobe epilepsy with psychosis were compared with 20 participants with temporal lobe epilepsy without psychosis where they were matched with respect to conventional MRI findings. Global and hippocampal volumes were assessed. No significant differences were found between those with and without psychosis but significant reductions of magnetisation transfer ratio (an index of signal loss derived from magnetisation transfer imaging) in the absence of atrophy was found in the left superior and middle temporal gyri in participants with psychosis.

Our finding of cortical grey matter abnormalities are in keeping with those of Flugel and colleagues but contrast with those of Rusch et al. Although our sample size was smaller than that of Rusch et al (26 v. 10 with temporal lobe epilepsy with psychosis), the participants in our study were more tightly
matched for psychiatric disorder and handedness. Furthermore, Rusch and colleagues utilised a neuroimaging protocol involving a brain template derived from healthy participants. Given that individual variability of brains in the study population is substantially increased by injury or disease, achieving satisfactory alignment across individual brains may have resulted in registration difficulties. Additionally, we report widespread grey and white matter changes not confined to the temporal lobes where our findings may contrast with previous automated statistical methods as our approach utilised instead unified segmentation that combined tissue classification, bias correction and non-linear warping within the same framework and our

### Table 4 Significant grey matter deficits in epilepsy+psychosis group v. epilepsy-only group

<table>
<thead>
<tr>
<th>Cluster size: voxels, n</th>
<th>Talairach and Tournoux coordinates</th>
<th>Region</th>
<th>Hemisphere</th>
</tr>
</thead>
<tbody>
<tr>
<td>1770</td>
<td>-25  -6  -33</td>
<td>Inferior temporal gyrus</td>
<td>Left</td>
</tr>
<tr>
<td></td>
<td>-40  -65  -27</td>
<td>Posterior lobe of cerebellum</td>
<td>Left</td>
</tr>
<tr>
<td></td>
<td>-21  -10  -20</td>
<td>Parahippocampal gyrus</td>
<td>Left</td>
</tr>
<tr>
<td></td>
<td>-37  -34  -15</td>
<td>Fusiform gyrus</td>
<td>Left</td>
</tr>
<tr>
<td></td>
<td>-39  -3  -13</td>
<td>Hippocampus</td>
<td>Left</td>
</tr>
<tr>
<td></td>
<td>-56  -17  -9</td>
<td>Middle temporal gyrus</td>
<td>Left</td>
</tr>
<tr>
<td>475</td>
<td>49  -13  -33</td>
<td>Inferior temporal gyrus</td>
<td>Right</td>
</tr>
<tr>
<td></td>
<td>44  -30  -21</td>
<td>Fusiform gyrus</td>
<td>Right</td>
</tr>
<tr>
<td></td>
<td>41  1  -14</td>
<td>Superior temporal gyrus</td>
<td>Right</td>
</tr>
<tr>
<td></td>
<td>52  12  8</td>
<td>Superior temporal gyrus</td>
<td>Right</td>
</tr>
<tr>
<td>354</td>
<td>1  2  -10</td>
<td>Anterior cingulum</td>
<td>Right</td>
</tr>
<tr>
<td></td>
<td>1  -1  1</td>
<td>Caudate</td>
<td>Right</td>
</tr>
<tr>
<td></td>
<td>-9  6  14</td>
<td>Caudate</td>
<td>Left</td>
</tr>
<tr>
<td>597</td>
<td>-43  -16  10</td>
<td>Insula</td>
<td>Left</td>
</tr>
<tr>
<td></td>
<td>-52  -33  24</td>
<td>Inferior parietal lobule</td>
<td>Left</td>
</tr>
<tr>
<td>632</td>
<td>43  -12  -6</td>
<td>Insula</td>
<td>Right</td>
</tr>
<tr>
<td></td>
<td>39  -19  19</td>
<td>Insula</td>
<td>Right</td>
</tr>
</tbody>
</table>

a. Cluster significance threshold P=0.002.

### Table 5 Significant white matter deficits in epilepsy+psychosis group v. epilepsy-only group

<table>
<thead>
<tr>
<th>Cluster size: voxels, n</th>
<th>Talairach and Tournoux coordinates</th>
<th>Region</th>
<th>Hemisphere</th>
</tr>
</thead>
<tbody>
<tr>
<td>1733</td>
<td>-41  -8  -27</td>
<td>Fusiform gyrus</td>
<td>Left</td>
</tr>
<tr>
<td></td>
<td>-36  -6  -23</td>
<td>Inferior temporal gyrus</td>
<td>Left</td>
</tr>
<tr>
<td></td>
<td>-36  -8  -20</td>
<td>Parahippocampal gyrus</td>
<td>Left</td>
</tr>
<tr>
<td></td>
<td>-36  -18  -13</td>
<td>Hippocampus</td>
<td>Left</td>
</tr>
<tr>
<td></td>
<td>-44  -7  -10</td>
<td>Middle temporal gyrus</td>
<td>Left</td>
</tr>
<tr>
<td></td>
<td>-29  -50  3</td>
<td>Lingual gyrus</td>
<td>Left</td>
</tr>
<tr>
<td></td>
<td>-13  16  3</td>
<td>Anterior limb of internal capsule</td>
<td>Left</td>
</tr>
<tr>
<td></td>
<td>-33  -43  6</td>
<td>Posterior thalamic radiation</td>
<td>Left</td>
</tr>
<tr>
<td></td>
<td>-14  22  8</td>
<td>Genu of corpus callosum</td>
<td>Left</td>
</tr>
<tr>
<td></td>
<td>-14  16  10</td>
<td>Caudate</td>
<td>Left</td>
</tr>
<tr>
<td></td>
<td>-21  41  17</td>
<td>Splenium of corpus callosum</td>
<td>Left</td>
</tr>
<tr>
<td></td>
<td>-14  -15  27</td>
<td>Cingulum</td>
<td>Left</td>
</tr>
<tr>
<td>1106</td>
<td>35  -4  -23</td>
<td>Inferior temporal gyrus</td>
<td>Right</td>
</tr>
<tr>
<td></td>
<td>49  -24  -16</td>
<td>Fusiform gyrus</td>
<td>Right</td>
</tr>
<tr>
<td></td>
<td>14  -15  -13</td>
<td>Midbrain</td>
<td>Right</td>
</tr>
<tr>
<td></td>
<td>32  -16  -9</td>
<td>Hippocampus</td>
<td>Right</td>
</tr>
<tr>
<td></td>
<td>31  -18  -6</td>
<td>Parahippocampal gyrus</td>
<td>Right</td>
</tr>
<tr>
<td></td>
<td>41  -27  2</td>
<td>Posterior thalamic radiation</td>
<td>Right</td>
</tr>
<tr>
<td></td>
<td>45  -27  5</td>
<td>Superior temporal gyrus</td>
<td>Right</td>
</tr>
<tr>
<td></td>
<td>22  18  6</td>
<td>Anterior limb of internal capsule</td>
<td>Right</td>
</tr>
<tr>
<td></td>
<td>21  20  14</td>
<td>Genu of corpus callosum</td>
<td>Right</td>
</tr>
<tr>
<td></td>
<td>28  -36  20</td>
<td>Splenium of corpus callosum</td>
<td>Right</td>
</tr>
<tr>
<td></td>
<td>18  -1  26</td>
<td>Cingulum</td>
<td>Right</td>
</tr>
</tbody>
</table>

a. Cluster significance threshold P=0.006.
non-parametric or distribution-free hypothesis testing procedure permits us to use cluster-level statistics even if their distribution is non-Gaussian (even after smoothing).

Limitations

There are other methodological considerations and limitations to our study. We only used MRI data-sets from 2002 onwards so as to ensure homogeneity of scans while applying strict exclusion criteria for recruitment into the study; although this may have limited the number of participants and affected the power of our study, there are several other studies that have applied VBM in schizophrenia where significant differences in grey and white matter have been reported using similar sample sizes. Therefore it is unlikely that type I error fully accounts for our findings.

The clinical psychiatric diagnoses that were obtained through neuropsychiatric assessment were not achieved through formal structured clinical interviews but they contained comprehensive clinical information that could be retrospectively evaluated through OPCRIT. Future studies investigating temporal lobe epilepsy with psychosis may wish to consider a methodological design that prospectively examines individuals with temporal lobe epilepsy through structured clinical interviews, for example the Structured Clinical Interview for DSM–IV, and further, to utilise objective rating scales for psychosis, for example the Brief Psychiatric Rating Scale or the Positive and Negative Syndrome Scale. However, to recruit reasonable numbers prospectively would take many years; despite our retrospective recruitment, finding appropriate participants once exclusion criteria have been applied (surgery, tumours, etc.) left us with ten participants in a 4-year period; thus recruitment is a problem in the study of temporal lobe epilepsy with psychosis.

Moreover, in a recent study, although widespread neocortical abnormalities were found in both temporal lobe epilepsy with and without mesial sclerosis, the pattern of thinning in the former contrasted with the latter, which led the authors to suggest that these might constitute two distinct temporal lobe epilepsy syndromes. Future studies may wish to separately characterise the neurobiology of these disorders. Similarly, the assessment of post-ictal and inter-ictal forms of psychoses related to temporal lobe epilepsy should perhaps be investigated separately in future studies. However, when these factors are taken together, they may restrict further the overall number of study participants and given that it is not uncommon for the post-ictal form to progress to the inter-ictal variant, assessing both forms of psychoses in the same study represents a valid approach.

We did not include normal controls in the study as we were primarily interested in the brain changes in temporal lobe epilepsy associated specifically with psychosis rather than the effects of epilepsy. Further, the absence of a control group is because this was a study based on an epilepsy patient database and therefore no individuals without epilepsy were recruited. However, the lack of a healthy control group limits the extent to which we can interpret our findings in the context of the general population. The current literature would suggest that people with temporal lobe epilepsy will have some regional volume loss compared with healthy controls, usually ipsilaterally and predominantly in temporal lobe regions. Undoubtedly, the recruitment of healthy controls poses an ongoing need and future studies at our centre should involve a healthy comparator group so as to enable meaningful differentiation of brain changes across both healthy and disease groups.

Tissue loss commonly affects disease groups, and tissue gain is less likely (apart from for instance a developmental disorder that might be accounted for by ‘differential pruning’). In our study, however, clearly both groups are ‘disease groups’ with tissue loss most likely occurring in both. Even if the differences we are seeing are being driven by the non-psychosis group (i.e. grey matter increase), this still implies that these regions are somehow affected. It may also reflect that the psychosis group could be lacking a potential ‘neuroprotective response’ that is preventing the controls with temporal lobe epilepsy only from developing psychosis. Although this is one possibility, another is that SPM99 has been used in previous reports in temporal lobe epilepsy, whereas we have used SPM5 with better algorithms for tissue classification and bias correction. Indeed, Keller and colleagues suggest that the grey matter concentration excesses they found using SPM99 in their study reflects diminished grey–white matter demarcation, underlying white matter atrophy, or structural displacement as a result of cerebrospinal fluid expansion. This may also have accounted for an apparent increase in grey matter concentration in temporal lobe epilepsy relative to healthy controls in the report by Rusch and colleagues.

Implications

Overall, our study recruited a population of people with temporal lobe epilepsy with strong diagnostic validity (both neurological and psychiatric) and through VBM, permitted an unbiased computerised statistical analysis of grey and white matter measures. The findings show that participants with temporal lobe epilepsy with psychosis have marked cortical, subcortical and extratemporal grey and white matter deficits compared with those with temporal lobe epilepsy alone and thus provide support for the psychosis literature that also shows this pattern of change. Our study has provided evidence allowing specific hypotheses to be tested in future studies, although recruitment of suitable individuals to investigate may prove to be an issue. Owing to such difficulties, it is important to conduct ‘pilot’ work such as ours to determine very specific hypotheses before attempts are made to recruit even larger cohorts.

Funding

M.C. was funded by a Clinician Scientist Award (CSA/2004/1) from the Health Research Board (Ireland) and an Essel Foundation Independent Investigator award from NARSAD (USA).

Acknowledgements

We thank all those who participated in this study. We are grateful to the radiographers at the Beaumont Hospital MRI scanner for their help at scanning sessions.
Hannah: a case of infertility and depression

George Stein

Childlessness was a tragedy for a woman in the ancient near East and the barren wife was likely to be despised by her husband, family and society at large. In the Old Testament, three childless women are described whose infertility was relieved by divine intervention, resulting in the birth of important patriarchal leaders.

Sarah, the mother of Isaac, was 90 years old when she heard she was to have a baby.

Gen. 18:13 'And the Lord said to Abraham, why did Sarah laugh and say shall I indeed bear a child now that I am old'.

The only other recorded emotional reaction Sarah has to her infertility is that she was harsh to Hagai, Abraham's other wife and the mother of Ishmael - then Sarah dealt harshly with her (Hagai) and she ran away from her'.

We know even less about the infertility of the mother of Samson. Her story is described in the book of Judges and she is known only as the wife of Manoah.

Judg. 13:13 'And the angel of the Lord appeared to the woman and said to her "although you are barren, having no children you shall conceive and bear a son."' in due course she gave birth to Samson. Her existence in the Bible relates solely to her maternal function and the text says nothing about her personal feelings, her relationships, or even her name as she is identified only through her husband, as 'the wife of Manoah'.

The third woman, Hannah, is also childless but eventually gives birth to another important biblical prophet, Samuel. However, her story is very different from the other two women's stories and the Bible gives a very personal account of her feelings, her sadness at the infertility, her relationship with her husband, Elkanah, the difficulties and envy of living with Elkanah's other wife, Peninnah, who did have children, as well as her misery, described in sufficient detail to make a diagnosis of depression likely.

1 Sam. 1:2 'Ho (Elkanah) had two wives: the name of one Hannah, and the name of the other Peninnah. Peninnah had children, but Hannah had none... 15 Her rival (Peninnah) used to provoke her severely, to irritate her because the Lord had closed her womb. 7 Therefore Hannah wept and would not eat. 8 Her husband said to her 'Hannah why do you weep? Why do you not eat? Why is your heart so sad?... 10 She was deeply distressed and prayed to the Lord and wept bitterly. 11 She made this vow "Oh Lord of hosts, if only you will look on the misery of your servant and remember me and not forget your servant but will give to your servant a male child then I will set him before you as a Nazirite until the day of his death."' 12 As she continued praying before the Lord, Eli the priest observed her mouth. 13 Hannah was praying silently, but her voice was not heard; therefore Eli thought she was drunk. 14 So Eli said to her "How long will you make a drunken spectacle of yourself?" But Hannah answered "No my Lord, I am a woman deeply troubled, I have drunk neither wine nor strong drink, but I have been pouring out my soul before the Lord. 16 Do not regard your servant as a worthless woman, for I have been speaking out of my great anxiety and vexation all the time." 17 Then Eli answered 'Go in Peace; the God of Israel will grant the petition you have made to him"... 18 Then the woman went to her quarters, ate and drank with her husband and her countenance was no longer sad... 19 And then they went back to their house at Ramah. Elkanah knew his wife Hannah and the Lord remembered her. in due time Hannah conceived and bore a son. She named him Samuel for she said "I have asked him of the Lord"'.

In this account we learn of Hannah's great distress because of her infertility and a sufficient number of symptoms are mentioned to make a diagnosis of depression. Thus, Hannah is weeping, feels bitterness, misery and sadness, but after her prayer her countenance is no longer sad (possible depressive facies which Is then relieved). Further, there is irritability, especially with Peninnah, loss of appetite, general distress, vexation and anxiety. There is also a curious loss of voice while she prays. This could be no more than silent prayer, but as she is mouthing the words, the high priest Eli believes her to be intoxicated. This picture is typical of agraphia, a hysterical conversion symptom commonly associated with depression.

The biblical feminist literature attributes great significance to Hannah's tale. It is the story of a woman with infertility and her psychological reactions, her relationships with her husband and God, her feelings towards her husband's other wife and the depression associated with her infertility. It stands in stark contrast to the earlier impersonal accounts of the childlessness of Sarah and the wife of Manoah where only their impaired maternal function is reported. Hannah's tale is also of great importance to psychiatry, particularly to the history of psychiatry. There are very few accounts of depression in the literature of the ancient world, and they appertain only to men, even though depression is more common in women. Hence it is likely that Hannah's story represents the first recognisable case of liaison psychiatry.

Hannah is thought to have lived in the 11th century BCE, but the books of I and II Samuel were probably compiled sometime between the 7th and the 9th century BCE, more than two millennia before the more definitive accounts of depression written by Burtons in the 17th century AD. Liaison psychiatry concerns the association between a psychiatric disorder and a general medical condition. Infertility is a recognised gynaecological disorder and so Hannah's tale represents the first recognisable case of liaison psychiatry.
Antisocial personality disorder (ASPD) and psychopathy involve significant interpersonal and behavioural impairments. However, little is known about their underlying neurobiology and in particular, abnormalities in white matter (WM) microstructure. A preliminary diffusion tensor magnetic resonance imaging (DT-MRI) study of adult psychopaths employing tractography revealed abnormalities in the right uncinate fasciculus (UF) (Craig et al., 2009), indicating fronto-limbic disconnectivity. However, it is not clear whether WM abnormalities are restricted to this tract or are more widespread, including other tracts which are involved in connectivity with the frontal lobe.

We performed whole brain voxel-based analyses on WM fractional anisotropy (FA) and mean diffusivity (MD) maps acquired with DT-MRI to compare 15 adults with ASPD and healthy age, handedness and IQ-matched controls. Also, within ASPD subjects we related differences in FA and MD to measures of psychopathy.

Significant WM FA reduction and MD increases were found respectively in ASPD subjects relative to controls. FA was bilaterally reduced in the genu of corpus callosum while in the right frontal lobe FA reduction was found in the UF, inferior fronto-occipital fasciculus (IFOF), anterior corona radiata and anterior limb and genu of the internal capsule. These differences negatively correlated with measures of psychopathy. Also in the right frontal lobe, increased MD was found in the IFOF and UF, and the corpus callosum and anterior corona radiata. There was a significant positive correlation between MD and psychopathy scores.
Conclusions: The present study confirms a previous report of reduced FA in the UF. Additionally, we report for the first time, FA deficits in tracts involved in interhemispheric as well as frontal lobe connectivity in conjunction with MD increases in the frontal lobe. Hence, we provide evidence of significant WM microstructural abnormalities in frontal brain regions in ASPD and psychopathy.

1. Introduction

1.1. The frontal lobe theory of antisocial personality disorder (ASPD) and psychopathy

The importance of the frontal lobes to social behaviour was first recognised in the 19th century following the case of Phineas Gage, in whom frontal lobe damage resulted in profound personality change associated with markedly inappropriate social behaviour [Harlow, 1993 (1869)]. A ‘frontal lobe’ syndrome was subsequently delineated based on clinical observations of the behaviour of patients with frontal lobe damage (Lishman, 1998) where symptoms included apathy, emotional lability, a lack of social awareness, unconcern for social rules, impulsivity, and reactive aggression.

It is currently recognised that there is much overlap between frontal lobe syndrome and ‘functional’ or ‘non-organic’ personality disorders (PDs), particularly ASPD and psychopathy (Damasio, 2000). The definition of psychopathy has changed little since Hervey Cleckley published The Mask of Sanity in 1941 where he described the psychopath as a charming, callous, superficial individual, lacking conscience and genuine emotion (Cleckley, 1941). The Psychopathy Checklist (PCL, Hare, 1980) and the later Psychopathy Checklist — Revised (PCL-R, Hare, 1991) were designed to operationalise Cleckley’s concept of psychopathy as a basis for diagnosing the disorder. The PCL-R consists of 20 items characterised broadly by two dimensions: Factor 1 items are primarily interpersonal or emotional traits such as remorselessness, deception, shallow affect and callowness, whereas Factor 2 items assess behavioural symptoms such as violence, criminality, and dysfunctional lifestyle. For a diagnosis of psychopathy, attributes from both of these factors need to be present. While PCL-R scores ≥30 have traditionally been used to classify an individual as having psychopathy (Hare, 2003), more recent studies have argued for a score of ≥25 as sufficient for diagnosis (Edens and Petralia, 2006; Edens et al., 2010; Rutherford et al., 1999).

While the related construct of ASPD in DSM-IV-TR (Diagnostic and Statistical Manual Fourth Edition — Text Revision, American Psychiatric Association, 2000) includes several traits present in psychopathy (e.g., lack of guilt/remorse, and impulsivity), diagnostic criteria can be met based entirely on antisocial behaviours (e.g., violation of social norms, irresponsibility, and criminality). Hence, the emotional deficits fundamental to psychopathy are not necessary for a diagnosis of ASPD, even if these are present in some cases. Estimates of the prevalence of the two disorders also differ, suggesting that these are non-equivalent diagnoses. While most adult psychopathic offenders meet criteria for ASPD, only approximately one third of those with ASPD are psychopathic (Hart and Hare, 1997). Psychopathy has therefore been postulated to be a particularly severe subtype of ASPD (Dolan and Doyle, 2007). Psychopathy and ASPD are however distinguished from behaviours secondary to frontal lobe lesions by high levels of both reactive (elicited by frustration) and instrumental (goal-directed) violence (Blair, 2001; Glenn and Raine, 2009). Nevertheless, overlaps between traits of both psychopathy and ASPD, and frontal lobe syndrome, have led to the suggestion that both PDs may result from frontal lobe abnormality (Damasio, 2000). Neuroimaging studies of both people with ASPD, and of individuals with psychopathy, have provided evidence of abnormalities of frontal lobe structure and function relative to control populations, together with deficits in temporal, limbic, and other brain regions (see Table 1).

For example, neuroimaging studies of adult psychopaths examining the frontal cortex have reported reduced grey matter volume in conjunction with reduction in the superior temporal gyrus (Muller et al., 2006), and in the prefrontal cortex (PFC) of ‘unsuccessful’ (caught) psychopaths, versus healthy controls (Yang et al., 2005). Furthermore, higher total and subfactor PCL-R scores (arrogant/deceptive, affective, and impulsive/unstable) were associated with reduced prefrontal grey matter volume (ibid.). Similarly, prefrontal and temporal cortical grey matter thinning was found in psychopathic individuals, with right hemisphere reductions related to elevated PCL-R Factor 1 ‘Affective’ facet scores (Yang et al., 2009b).

Other studies have identified associations between psychopathic traits and specific subregions of the PFC. In particular, the association found in brain injured patients between ventromedial PFC (vmPFC) damage and reactive aggression (Blair and Cipolotti, 2000; Grafman et al., 1996) is mirrored by vmPFC structural and functional impairments in psychopaths (Tilhonen et al., 2008). Functional neuroimaging studies of people with psychopathy have also provided evidence of abnormal frontal lobe perfusion and abnormalities of task-related activation in prefrontal and other brain regions on reversal learning paradigms (Table 1). Individuals with ASPD have shown similar structural and functional prefrontal abnormalities to psychopaths — for instance reduced prefrontal grey matter volume has been found in both antisocial adults (Raine et al., 2000) and conduct disordered children (Bluehner et al., 2008), compared with healthy controls. Also, cortical thinning of the medial frontal lobe has been found in ASPD (Narayan et al., 2007).

Nevertheless, structural and functional abnormalities in people with psychopathy and ASPD are not restricted to the frontal lobe. For example, abnormal amygdala structure and function have each been found to correlate with the emotion processing deficits observed in ASPD and psychopathic individuals (Gordon et al., 2004; Kleih et al., 2001; Yang et al., 2009a), as well as in regions (such as fusiform—extrastriate cortices) known to be modulated by the amygdala (Deeley...
### Table 1 – Summary of volumetry, functional and DT-MRI findings implicating fronto-limbic and other brain region abnormalities in antisocial populations.

<table>
<thead>
<tr>
<th>Author</th>
<th>Method</th>
<th>Population</th>
<th>Comparison group</th>
<th>Finding</th>
<th>Region</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Barkataki et al., 2006)</td>
<td>MRI</td>
<td>ASPD</td>
<td>Violent and non-violent schizophrenia</td>
<td>Reduced volume</td>
<td>Whole brain, bilateral temporal lobe</td>
</tr>
<tr>
<td>(Laakso et al., 2000)</td>
<td>MRI</td>
<td>ASPD with alcoholism</td>
<td>Healthy controls</td>
<td>Increased volume</td>
<td>Putamen</td>
</tr>
<tr>
<td>(Laakso et al., 2001)</td>
<td>MRI</td>
<td>ASPD with alcoholism</td>
<td>Psychiatric patients</td>
<td>Reduced volume</td>
<td>Right hippocampus, posterior hippocampus</td>
</tr>
<tr>
<td>(Narayan et al., 2007)</td>
<td>MRI</td>
<td>ASPD</td>
<td>Violent and non-violent schizophrenia and healthy controls</td>
<td>Volume inversely related to PCL score</td>
<td>Bilateral posterior hippocampus</td>
</tr>
<tr>
<td>(de Oliveira-Souza et al., 2008)</td>
<td>MRI</td>
<td>ASPD with psychopathy</td>
<td>Healthy controls</td>
<td>Cortical thinning</td>
<td>Medial PFC</td>
</tr>
<tr>
<td>(Raine et al., 2000)</td>
<td>MRI</td>
<td>Community ASPD</td>
<td>Alcohol dependents and healthy controls</td>
<td>Reduced volume</td>
<td>Corpus callosum</td>
</tr>
<tr>
<td>(Raine et al., 2003)</td>
<td>MRI</td>
<td>Community ASPD with high psychopathy scores</td>
<td>Alcohol dependents and healthy controls</td>
<td>Increased length</td>
<td>Bilateral occipital lobe, bilateral parietal lobe, left cerelbellum</td>
</tr>
<tr>
<td>(Tiihonen et al., 2008)</td>
<td>MRI</td>
<td>ASPD with alcohol dependence</td>
<td>Healthy controls</td>
<td>Reduced thickness</td>
<td>Right cerebellium</td>
</tr>
<tr>
<td>(Volkow et al., 1995)</td>
<td>PET</td>
<td>Violent offenders</td>
<td>Healthy controls</td>
<td>Increased grey matter volume</td>
<td>FFF, medial temporal cortex</td>
</tr>
<tr>
<td>(Raine et al., 1997)</td>
<td>PET</td>
<td>Murderers</td>
<td>Healthy controls</td>
<td>Reduced glucose metabolism</td>
<td>FFF, corpus callosum, superior parietal gyrus, left angular gyrus</td>
</tr>
<tr>
<td>(Boccardi et al., 2010)</td>
<td>MRI</td>
<td>Psychopathic violent offenders</td>
<td>Healthy controls</td>
<td>Bilateral depression</td>
<td>Hippocampus – longitudinal axis</td>
</tr>
<tr>
<td>(Craig et al., 2009)</td>
<td>DT-MRI</td>
<td>Psychopaths</td>
<td>Healthy controls</td>
<td>Reduced FA</td>
<td>Right UF</td>
</tr>
<tr>
<td>(Glenn et al., 2010)</td>
<td>MRI</td>
<td>Community sample – high psychopathy scorers</td>
<td>Community sample – low psychopathy scorers</td>
<td>Increased volume with greater psychopathy score</td>
<td>Striatum</td>
</tr>
<tr>
<td>(Muller et al., 2006)</td>
<td>MRI</td>
<td>Criminal psychopaths</td>
<td>Healthy controls</td>
<td>Reduced volume</td>
<td>Right superior temporal gyrus</td>
</tr>
<tr>
<td>(Shamay-Tsoory et al., 2010)</td>
<td>CT/MRI</td>
<td>ASPD males with psychopathic traits</td>
<td>Orbitofrontal cortex versus non-frontal lesioned males and healthy controls Successful community psychopaths and healthy controls</td>
<td>Impaired affective empathy performance in both orbitofrontal cortex lesioned and psychopathy group</td>
<td>Orbitofrontal cortex</td>
</tr>
<tr>
<td>(Yang et al., 2005)</td>
<td>MRI</td>
<td>Unsuccessful community psychopaths</td>
<td>Healthy controls</td>
<td>Reduced volume</td>
<td>Prefrontal grey matter</td>
</tr>
<tr>
<td>(Yang et al., 2009a)</td>
<td>MRI</td>
<td>Community psychopaths</td>
<td>Healthy controls</td>
<td>Reduced volume</td>
<td>Bilateral amygdala</td>
</tr>
<tr>
<td>(Yang et al., 2009b)</td>
<td>MRI</td>
<td>Community psychopaths</td>
<td>Healthy controls</td>
<td>Cortical thinning</td>
<td>Amygdala nuclei, basolateral, central, cortical, lateral</td>
</tr>
</tbody>
</table>

**Note:** The table provides a summary of neurological findings in antisocial populations, focusing on brain region abnormalities. The findings include both structural and functional MRI (fMRI) results, highlighting alterations in various brain regions associated with antisocial behaviors. The table lists the author(s), method (MRI, PET, DT-MRI), population studied (ASPD, violent and non-violent schizophrenia, etc.), comparison group (healthy controls, alcohol dependents, etc.), finding (reduced or increased volume, cortical thinning, etc.), and the specific brain regions affected. The findings are integrated into a comprehensive overview of abnormalities in brain regions implicated in antisocial behaviors.
has been relatively understudied although such investigation has been made possible using diffusion tensor magnetic resonance imaging (DT-MRI) (Basser et al., 1994b; Thiebaut de Schotten et al., 2012; Catani et al., 2012) where the ‘connectivity’ of neural systems is assessed using proxy measures of microstructural integrity.

### 1.2. Disconnectivity between frontal and other regions in psychopathy

DT-MRI is particularly used in the assessment of tissue (such as WM networks) where water preferentially diffuses along a particular axis aligned with the tissue’s internal structure and is predicated on the principle that microarchitectural structures, for instance, cell membranes, myelin sheaths, as well as intracellular micro-organelles, act as barriers to the diffusion and free movement of water, and thus limit the spatial motion of these molecules (Malhi and Lagopoulos, 2008). The assessment of the directional dependence of water molecule diffusion in WM is usually quantified through calculation of fractional anisotropy (FA). FA is a measure of the degree of anisotropy or directionality where values range from

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**Table 1 (continued)**

<table>
<thead>
<tr>
<th>Author</th>
<th>Method</th>
<th>Population</th>
<th>Comparison group</th>
<th>Finding</th>
<th>Region</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Deeley et al., 2006)</td>
<td>fMRI</td>
<td>Psychopaths</td>
<td>Healthy controls</td>
<td>Reduced BOLD activation to fearful and happy faces in emotion processing task</td>
<td>Fusiform gyrus, extrastriate cortex</td>
</tr>
<tr>
<td>(Soderstrom et al., 2002)</td>
<td>SPECT</td>
<td>Violent offenders with varying psychopathy scores</td>
<td>Healthy controls</td>
<td>Negative correlation between interpersonal psychopathy factor and perfusion</td>
<td>Frontal and temporal regions, head of caudate, left hippocampus</td>
</tr>
<tr>
<td>(De Brito et al., 2009)</td>
<td>MRI</td>
<td>Community sample of boys – high versus low callous-unemotional trait scorers</td>
<td>Healthy controls</td>
<td>Increased grey matter concentration</td>
<td>Medial orbitofrontal cortex, anterior cingulate cortex</td>
</tr>
<tr>
<td>(Kruesi et al., 2004)</td>
<td>MRI</td>
<td>Conduct disordered adolescents</td>
<td>Healthy controls</td>
<td>Increased grey matter concentration and volume</td>
<td>Bilateral temporal lobe</td>
</tr>
<tr>
<td>(Huebner et al., 2008)</td>
<td>MRI</td>
<td>Conduct disordered adolescent males comorbid with ADHD</td>
<td>Healthy controls</td>
<td>Reduced grey matter volume</td>
<td>Right temporal lobe</td>
</tr>
<tr>
<td>(Sterzer et al., 2007)</td>
<td>MRI</td>
<td>Conduct disordered adolescent males</td>
<td>Healthy controls</td>
<td>Reduced grey matter volume</td>
<td>Bilateral temporal lobe, left hippocampus, left amygdala</td>
</tr>
<tr>
<td>(Berna et al., 2009)</td>
<td>DT-MRI</td>
<td>Healthy adolescents – high versus low on risk taking measure</td>
<td>Healthy controls</td>
<td>High scores positively correlated with FA and negatively with transverse diffusivity</td>
<td>Bilateral anterior insular cortex, left amygdala</td>
</tr>
<tr>
<td>(Marsh et al., 2008)</td>
<td>fMRI</td>
<td>Antisocial children with callous-unemotional traits</td>
<td>Children with ADHD; healthy controls</td>
<td>Reduced BOLD activation to fearful faces on emotional processing task</td>
<td>Amygdala</td>
</tr>
<tr>
<td>(Finger et al., 2008)</td>
<td>fMRI</td>
<td>Antisocial children with callous-unemotional traits</td>
<td>Children with ADHD; healthy controls</td>
<td>Reduced functional connectivity</td>
<td>Between amygdala and vmPFC</td>
</tr>
<tr>
<td>(Jones et al., 2009)</td>
<td>fMRI</td>
<td>Antisocial boys with callous-unemotional traits</td>
<td>Healthy controls</td>
<td>Abnormal BOLD signal to punished errors on reversal learning task</td>
<td>Right amygdala</td>
</tr>
<tr>
<td>(Stadler et al., 2007)</td>
<td>fMRI</td>
<td>Conduct disordered adolescent males</td>
<td>Healthy controls</td>
<td>Reduced BOLD activation to fearful faces on emotional processing task</td>
<td>Right anterior cingulate cortex</td>
</tr>
</tbody>
</table>

et al., 2006). Reduced volume of temporal regions is also seen in ASPD (Barkataki et al., 2006). Taken together, these studies of antisocial adults and conduct disordered children (with and without psychopathic traits) illustrate reduced volume of the temporal lobe and its constituent structures, and deficits in amygdala structure and function.

Apart from these fronto-temporal structures, other regions potentially relevant to psychopathy have been less extensively investigated. For instance, there have been only limited magnetic resonance imaging (MRI) studies assessing the corpus callosum, a major white matter (WM) bundle supporting interhemispheric functional integration, where abnormalities have included increases in volume and length but reduction in thickness (Raine et al., 2003). Also, while reductions in functional connectivity between prefrontal and limbic regions may contribute to antisocial traits (Table 1), their underlying microstructural basis remains unknown. Overall, the wider network of abnormalities in psychopathy has been relatively understudied although such investigation has been made possible using diffusion tensor magnetic
0 (perfectly isotropic diffusion) to 1 (perfectly anisotropic diffusion) (Pierpaoli and Basser, 1996)—so providing a measure of tissue integrity (Horsfield and Jones, 2002; Mori and Zhang, 2006). Mean diffusivity (MD) is another DT-MRI derived parameter used for reporting tissue differences which is calculated by division of the sum of the eigenvalues of the diffusion tensor (which correspond to the magnitude of diffusion in three orthogonal directions) by three. There are however only limited studies examining neural disconnection using DT-MRI in people with psychopathy and/or ASPD.

A recent study by our group using DT-MRI tractography focusing on FA in the uncinate, inferior longitudinal and inferior fronto-occipital fasciculus reported a significant reduction of this measure in only the uncinate fasciculus (UF) of nine psychopaths compared with age- and IQ-matched controls (Craig et al., 2009). Additionally, a significant negative correlation was found between measures of antisocial behaviour (PCL-R Factor 2 scores) and tract volume within this WM pathway, suggesting abnormal connectivity in the amygdala—orbital frontal cortex limbic network. However, as this study was confined to a limited number of WM tracts, it was not possible to assess WM networks on a whole brain level. Consequently, the presence of deficits affecting WM connectivity with other brain regions is yet to be established in either ASPD or psychopathy. Further, our previous study did not examine other indices of WM microstructure, such as MD.

In summary, there is increasing evidence that people with ASPD and psychopathy may have significant differences in the structure and function of frontal, limbic and other brain regions. However, few studies have examined the microstructural integrity or connectivity of their WM networks. Therefore, we undertook the first DT-MRI investigation on a whole brain level of ASPD and psychopathy. We examined WM networks and tested the main hypothesis that people with ASPD and psychopathy have significant differences, based on DT-MRI derived parameters of FA and MD, in microstructural integrity and connectivity as compared to healthy matched controls. Also, we tested an additional hypothesis that within ASPD, severity of psychopathy (as measured by PCL-R) is related to differences in these WM measures.

2. Methods

2.1. Subjects

Study participants were recruited from three specialist forensic inpatient units in south-east London (South London and Maudsley National Health Service Foundation Trust) and south-west London (St George’s Healthcare NHS Trust) as part of our longitudinal work in assessing psychopathy, over a period of eight years. Healthy controls were recruited from the general population through the Institute of Psychiatry, King’s College London by advertisement. Ethical approval was obtained from the Ethics Committee of the South London and Maudsley Trust and Institute of Psychiatry, and St George’s Healthcare Trust. Written informed consent was obtained from participants after full description of the study. Student’s t tests (two-tailed) were used to compare the distribution of continuous data between the two groups.

Participants in both groups were medication free, spoke English as their first language, and were right-handed as assessed by the Annett Handedness Questionnaire. The Wechsler Adult Intelligence Scale—Revised (Wechsler, 1991) was used to measure IQ. All participants (in both groups) were examined by formal psychiatric semi-structured interview using ICD-10 research criteria (World Health Organisation, 1993) in addition to assessment of case notes for a diagnosis of ASPD. Assessment for the presence of comorbid psychiatric illness (e.g., anxiety disorders, substance misuse, schizophrenia, major depression), neurological and extracerebral disorders that may affect brain function, and contraindications to MRI scanning was also performed. Though many individuals with ASPD have a past history of alcohol and/or substance misuse, we attempted to recruit (as far as possible) subjects without comorbidities rather than to control for these post hoc. None of the participants fulfilled criteria for substance misuse or dependence syndrome 6 months prior to recruitment, with the exception of one subject who had harmful use of cocaine.

45 participants were initially recruited into the study (20 ASPD vs 25 controls). However, five with ASPD and 10 controls respectively were unsuitable for further assessment following exclusion for comorbid psychiatric disorder and contraindications to MRI procedures. PCL-R scores were obtained from case notes derived from assessments based at their specialist forensic unit by forensic psychologists fully trained in the administration of the PCL-R, or by a researcher (QD) where the PCL-R had not been administered but where subjects otherwise met inclusion criteria. We therefore included 30 normal intelligence right-handed adult male subjects: 15 with ASPD and a mean PCL-R score of 26 (SD ± 7; range 13–34) aged 39 ± 10 years and with full-scale IQ (FSIQ) 92 ± 13, and 15 healthy controls aged 37 ± 11 years, with FSIQ 99 ± 12. There were no significant differences in age or IQ between participant groups. Those with ASPD had a history of violent offending that encompassed manslaughter, attempted murder and multiple rape with strangulation. In the UK it is accepted practice to define psychopathy as a score of 25 or above on the PCL-R (Coike, 1996; Cooke and Michie, 1999) and 10 of the 15 in the ASPD group scored above this threshold. However, while we were able to obtain total PCL-R scores for the entire patient cohort, it was only possible to acquire Factor 1 and Factor 2 subscores for 12 of the 15 subjects from case notes.

2.2. MRI acquisition protocol

Data were acquired using a 1.5 T GE Signa LX system (General Electric, Milwaukee, WI, USA), with actively shielded magnetic field gradients (maximum amplitude 40 mT m⁻¹). A standard quadrature birdcage head coil was used for both radio-frequency transmission and signal reception. Each DT-MRI volume was acquired using a multi-slice peripherally-gated echo-planar imaging (EPI) sequence, optimised for precise measurement of the diffusion tensor in brain parenchyma, from 60 contiguous 2.5 mm thick slices with field of view (FOV) 240 x 240 mm and matrix size 96 x 96, zero-filled during reconstruction to 128 x 128, giving a final in-plane voxel size of 1.875 x 1.875 mm² (Jones et al., 2002; Kyriakopoulos et al.,
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but is not necessary for our non-parametric statistical weight directed intensity (with no diffusion gradients applied) and image distortions were uniformly distributed in space. Total scan time was approximately 20 min and the relative orientations of the diffusion gradient vectors were based on the electrostatic repulsion algorithm (Jones et al., 1999, 2002).

Following correction of the diffusion-weighted images for image distortions introduced by the diffusion-weighting gradients, in-house software was used to 1) remove non-brain tissue and 2) determine the diffusion tensor in each voxel (Basser et al., 1994a, 1994b). Images of 1) mean T2-weighted intensity (with no diffusion gradients applied) and 2) FA and MD were computed for each subject. Full details are given elsewhere (Jones et al., 2002).

2.3. Pre-processing DT-MRI data

Scans were examined for image corruption or motion artefacts prior to inclusion in the imaging pipeline and none of the acquired scans demonstrated these abnormalities. The subsequent pre-processing steps and analytical methodology have been published previously (Bloemens et al., 2010; Kyriakopoulos et al., 2008, 2009; Sundaram et al., 2010), and are summarised below.

After construction of maps of FA and MD, a voxel-based approach in standard space using SPM2 (Wellcome Department of Imaging Neuroscience, University College London) within MATLAB 6.5.2 (The MathWorks, Natick, MA, USA), aligned, smoothed and segmented the FA and MD images. In a manner analogous to the early voxel-based morphometry (VBM) analysis methods developed for structural T1 and/or T2-weighted MR images, we first performed a two-stage normalisation to standard Montreal Neurological Institute (MNI) space using a study-specific, intermediate template to reduce potential bias due to different degrees of warping that would otherwise be required to match ASPD and control brains to a standard (control subject-based) template.

The mean T2-weighted (nondiffusion-weighted, b = 0) images from each subject were initially aligned to the standard EPI template provided by SPM2. The derived mapping parameters for each subject were then applied to the (inherently co-registered) FA images. The normalised FA images of all subjects were then averaged and smoothed (8 mm full-width at half maximum Gaussian filter) to create a new, study-specific, intermediate template to which each subject’s FA and MD images were then re-registered. Smoothing the data in order to coerce it into the appropriate statistical distribution is a prerequisite for some analytical approaches, but is not necessary for our non-parametric statistical approach where it serves instead to aid between-subject anatomical matching, reduce confounds due to individual variation in WM anatomy and improve the signal-to-noise ratio. We therefore segmented the registered FA images (using SPM’s default a priori tissue probability information) to give maps of the probability of a tissue being either white or grey matter and thresholded the resulting images at a low level (10%) to provide a (deliberately slightly overinclusive) binary mask of WM. We then smoothed the original FA and MD images, before applying these masks to restrict the subsequent statistical testing to WM only. As different smoothing levels can result in varying results (Jones et al., 2005), in the absence of a specific hypothesis about the spatial extent of any abnormalities, we applied a Gaussian smoothing filter of 5 mm full-width at half maximum. Because of our overinclusive mask, the smoothed images will include some mixed grey/WM voxels at the edges of the masked region; however, later analysis steps (see below) further restrict the statistical testing to only core WM regions.

2.4. Image analysis and correlations

2.4.1. DT-MRI group mapping

We examined the statistical significance of between group differences in FA and MD using a non-parametric permutation-based method. Locally developed software, XBAM (version 3.4) (Institute of Psychiatry, http://www.brainmap.co.uk) measures between group differences in standard space at both voxel and cluster levels by fitting an analysis of variance (ANOVA) statistical model. As there were no differences in the mean values in either age or IQ between groups, and the ranges similar, these were not entered as covariates and so the ANOVA model utilised FA or MD as the dependent variable while group classification was the key predictor variable.

Given that brain changes are likely to extend over a number of contiguous voxels, test statistics incorporating spatial information such as 3D cluster mass (the sum of suprathreshold voxel statistics) are generally more powerful than other possible test statistics, which are informed only by data at a single voxel (Bullmore et al., 1999). The voxelwise statistic images were therefore thresholded at a relatively lenient level of $p < 0.05$, and voxels that were spatially contiguous in three dimensions in the thresholded maps were assigned to the same cluster. The sum of voxel statistics within each cluster was computed for each randomisation to form a distribution of cluster mass under the null hypothesis. As no parametric distribution is known for cluster mass (sum of voxel statistics within each cluster), permutation testing was used to assess statistical significance; the mass of each cluster in the observed data was compared to this randomised distribution, and significant clusters were defined as those that had a greater cluster mass than the randomised distribution at a particular significance level. The number of permutations at the voxel and cluster levels was 1000 which defines the distribution well enough to permit inference about changes between groups (Bullmore et al., 1999). Such a non-parametric approach also overcomes the assumption that parametric methods adopt that the residuals of the model tested will follow a Gaussian distribution (which has been shown to not always be true for DT-MRI data, even after extensive smoothing) (Jones et al., 2005).

At this stage, we considered only those voxels at which all subjects contribute data which, along with the masking procedure above, restricted the analysis to core WM regions, reducing the search volume (and thus the number of
comparisons made) and also avoided testing at the grey/white interfaces, where the high grey/white contrast of FA images exacerbates any edge effects. At the cluster level, rather than set a single a priori p-value below which we regard findings as significant, we calculated, for a range of p-values, the number of clusters which would be expected by chance alone. A stringent cluster significance threshold was then applied to render less than one false positive cluster per analysis. As SPM was used for image registration, XBAM yielded coordinates of significant clusters in MNI space and the identification of significant clusters and WM tracts intersecting them was based on an atlas approach where several WM atlases were used in conjunction to determine the projection and localisation of WM bundles; these atlases were based on both single and multiple subject DT-MRI datasets in MNI space (Catani and Thiebaut de Schotten, 2008; Mori et al., 2005; Thiebaut de Schotten et al., 2011; Wakana et al., 2004). Given that the Talairach coordinate system is most commonly used for reporting findings in the neuroimaging literature, MNI coordinates were subsequently converted to Talairach space via a non-linear transformation (Brett et al., 2002) (details at http://imaging.mrc-cbu.cam.ac.uk/imaging/MniTalairach) and the localisation of WM tracts and their respective anatomical course within cerebral lobes was additionally confirmed using the Talairach atlas (Talairach and Tournoux, 1988). Where significant differences were found on either FA or MD maps, the p-value of each significant cluster is reported in Table 2 alongside the anatomical locations of their respective WM tracts in Talairach space.

In the investigation of cluster level effects, there is an underlying assumption that all regions will equally ‘smooth’, and can therefore be treated equivalently (from a statistical point of view). However, while we restricted our analysis to core WM regions where signal-to-noise ratio is relatively uniform, the effects of physiological noise (e.g., motion artefacts) may still vary across the brain. We therefore concurrently inspected the voxel-level maps (which treat each voxel independently and therefore inherently allow for such local differences in statistics) (Kryiakopoulos et al., 2008).

2.4.2. Post hoc analysis of PCL-R scores
Where significant 3D clusters were found on DT-MRI group mapping analyses, we carried out post hoc tests to determine if significant differences in WM FA or MD were associated with behavioural variation within the entire group with ASPD. As the clusters detected by XBAM may encompass multiple anatomical regions (i.e., were not constrained to lie only within particular WM tracts), we first extracted mean FA in combination with MD for each ASPD subject. To do this, mask images were created from each of the clusters found by the group mapping analysis and applied to each subject’s normalised FA and MD images thereby enabling mean FA and MD values to be calculated over each region for each subject. These were then correlated with PCL-R scores (Factor 1: ‘emotion dysfunction’; Factor 2: ‘antisocial behaviour’ and; total PCL-R) using Pearson product–moment correlation coefficients with Statistical Package for the Social Sciences (SPSS 14.0 for Windows, SPSS Inc., Chicago, IL, USA). Significant correlations are reported where a Bonferroni adjusted alpha of .025 was applied.

3. Results

3.1. Group contrasts of FA using DT-MRI group mapping

3.1.1. ASPD versus controls WM FA (Fig. 1, Table 2)
People with ASPD, relative to controls, had a significant reduction in WM FA; 1) bilaterally in the frontal lobe in the anterior portion of the corpus callosum (genu); 2) in the right hemisphere, only in anterior regions of the brain and in WM tracts that included the genu of corpus callosum, anterior corona radiata and anterior limb and genu of the internal capsule, and frontal course of the uncinate and inferior fronto-occipital fasciculus (IFOF); 3) in the left hemisphere in both anterior and posterior regions of the brain including respectively the genu of corpus callosum and temporo-occipital course of the inferior longitudinal and IFOF, and the retrolenticular part of the internal capsule and posterior thalamic radiation.

3.1.2. ASPD versus controls WM MD (Fig. 2, Table 2)
People with ASPD, relative to controls, had a significant increase in MD only in the right frontal lobe. This was localised to a cluster containing the frontal course of the IFOF and UF, and the genu of corpus callosum and anterior corona radiata. No regions of increased MD were found in the control group.

3.1.3. Within ASPD, an analysis of Factor 1, Factor 2 and total PCL-R scores and differences in WM FA and MD
In the ASPD group, there were significant correlations between PCL-R scores and both WM FA and MD. Mean FA of the cluster in the frontal lobe (Cluster 2) was negatively correlated with Factor 2 (r = −.771, p = .003, n = 12) and total PCL-R (r = −.685, p = .005, n = 15) scores. Additionally in the frontal lobe, there was a significant positive correlation between increased MD (Cluster 3) with Factor 2 scores (r = −.669, p = .017, n = 12). There were no significant correlations between the cluster in the temporo-occipital cortex (Cluster 1) and total or subfactor PCL-R scores.

4. Discussion

We used DT-MRI in conjunction with whole brain voxel-based analyses of MD and FA to compare WM microstructural integrity of 15 adult males with a diagnosis of ASPD and 15 healthy controls matched for age, handedness and IQ. Reductions of FA were present in the frontal lobe of the ASPD group, and these were significantly and inversely correlated with severity of psychopathy (Factor 2, and total PCL-R scores). Also, increased MD was concurrently found in the right frontal lobe of ASPD subjects which showed a significant positive correlation with measures of psychopathy (Factor 2 scores).

The frontal cluster (Cluster 2, Table 2) that showed significant FA reduction and negative correlations with severity of psychopathy in the ASPD group included the genu of corpus callosum. Furthermore, it included right hemisphere structures of the anterior corona radiata and anterior limb and
Table 2 — WM FA and MD differences in ASPD relative to healthy controls (cluster significance threshold for FA maps $p = .0025$; cluster significance threshold for MD maps $p = .005$).

<table>
<thead>
<tr>
<th>Cluster label</th>
<th>Cluster size (number of voxels)</th>
<th>Talairach and Tournoux coordinates</th>
<th>Tract(s) within cluster</th>
<th>Region</th>
<th>Cluster mean FA ASPD (SD)</th>
<th>Cluster mean FA Control (SD)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cluster 1</td>
<td>504</td>
<td>-36 -42 -6</td>
<td>Temporo-occipital course of left ILF and IFOF(^a)</td>
<td>Temporal lobe</td>
<td>.433 (.022)</td>
<td>.466 (.029)</td>
<td>.001378</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-32 -59 4</td>
<td>Left posterior thalamic radiation and retrolenticular part of internal capsule</td>
<td>Occipital lobe</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cluster 2</td>
<td>1027</td>
<td>16 47 -8</td>
<td>Right IFOF and genu of corpus callosum</td>
<td>Frontal lobe</td>
<td>.383 (.020)</td>
<td>.409 (.033)</td>
<td>.000469</td>
</tr>
<tr>
<td></td>
<td></td>
<td>19 30 -8</td>
<td>Right UF</td>
<td>Frontal lobe</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>-14 36 2</td>
<td>Left genu of corpus callosum</td>
<td>Frontal lobe</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>18 2 6</td>
<td>Right genu of internal capsule</td>
<td>Frontal lobe</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td>18 32 7</td>
<td>Right genu of corpus callosum</td>
<td>Frontal lobe</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>23 11 9</td>
<td>Right anterior limb of internal capsule and anterior corona radiata(^a)</td>
<td>Frontal lobe</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Cluster 3     | 325                             | 13 48 -11                         | Right anterior corona radiata, genu of corpus callosum, and IFOF and UF | Frontal lobe | .750 (.026) | .729 (.031) | .004513 |

\(^a\) Location of voxel showing maximum FA difference.
Fig. 1 – Reduced FA in ASPD relative to healthy controls [ascending 2 mm transverse sections].

Fig. 2 – Increased MD in ASPD relative to healthy controls [ascending 2 mm transverse sections].
genus of the internal capsule in addition to the frontal course of the UF and IFOF. Additionally, a right frontal cluster (Cluster 3, Table 2) which encompassed the genus of corpus callosum and anterior corona radiata, as well as the frontal course of the IFOF and UF also showed a significant correlation between increased MD and psychopathy scores. Although FA reduction was also found in posterior regions of the left hemisphere in the tempo-motor-occipital course of the inferior longitudinal fasciculus (ILF) and IFOF, in addition to the retrolenticular section and posterior thalamic radiation of the internal capsule (Cluster 1, Table 2), these did not correlate significantly with measures of psychopathy. Overall, our findings suggest that people with ASPD have WM microstructural abnormalities involving frontal WM networks, and particularly of the right hemisphere.

In a previous report by our group employing DT-MRI tractography examining FA and streamlines (a proxy measure of tract volume) of the UF, ILF and IFOF of nine individuals with psychopathy, FA was found to be reduced only in the right UF; additionally the number of streamlines in the UF bilaterally was correlated negatively with Factor 2 and total PCL-R scores though FA did not show any statistically significant correlations (Craig et al., 2009). The present finding of reduced FA in the right frontal lobe encompassing the UF replicates our original finding, noting that these nine subjects were also included in our larger sample of 15 individuals. The incorporation of more subjects in our current study may have provided additional power to detect further significant correlations with FA.

Apart from FA deficits in the UF, the frontal lobe of ASPD subjects in the current study also showed FA deficits in the corpus callosum, internal capsule, anterior corona radiata and IFOF; this is likely to indicate information relay impairments between prefrontal and other brain regions — e.g., inter-hemispherically and with the cingulum, pons, thalamus and tempo-occipital cortices (Catani et al., 2002; Schmahmann and Pandya, 2008). Impaired functioning of the corpus callosum in psychopaths has previously been postulated on the basis of evidence of prolonged interhemispheric transfer time relative to controls (Hatt and Newman, 2007). Lesion studies of the corpus callosum have revealed its role in supporting sensory-motor functional integration, attention, language, interhemispheric transfer of associative learning, and emotional regulation (Bellani et al., 2009; Glickstein and Berlucchi, 2008; Zaidel and Iacoboni, 2003). Consequently, the present findings of reduced FA and increased MD in the corpus callosum of adults with ASPD, which also correlate with measures of psychopathy, may suggest that functions mediated predominantly by the left hemisphere (e.g., approach behaviour, language processing) may be relatively unmodulated by functions mainly mediated by the right hemisphere (including behavioural inhibition and emotion processing) as postulated by Hatt and Newman (2007). This proposed mechanism may also help explain the association of reduced FA in the corpus callosum of dependent cocaine users with increased impulsivity (Moeller et al., 2005) and WM abnormalities in the corpus callosum of adolescents engaged in dangerous behaviour (Berns et al., 2009).

With regard to frontal lobe tracts, abnormalities in the UF are associated with impairments of conditional associative learning (Gaffan and Wilson, 2008; Gutnikov et al., 1997; Parker and Gaffan, 1998). Individuals with ASPD and/or psychopathy demonstrate deficits in reversal learning, a form of conditional associative learning characterised by a failure to 'reverse' a previously rewarded response when it is punished relative to controls (Table 1). Reversal learning deficits may contribute to the perseveration of antisocial behaviour and high levels of recidivism characteristic of the disorder. Further, fibres of the UF connect the orbitofrontal cortex and amygdala, while impaired regulation of amygdala activity by the orbitofrontal cortex may contribute to the behavioural disinhibition encountered both in 'acquired sociopathy' (Brower and Price, 2001) and ASPD and psychopathy (Sarkar et al., 2011). Although abnormalities in the IFOF and anterior corona radiata were also found in the frontal lobe in the current study, functional impairments attributable to these tracts are examined later in the discussion.

Additionally, FA deficits were found in the present study in the anterior limb of the internal capsule which contains thalamo-frontal (anterior thalamic radiation), fronto-thalamic and corticopontine fibres, and interconnects the dorsomedial and anterior thalamic nuclei with both the prefrontal and cingulate cortices. Lesions of the anterior limb of the internal capsule have been associated with 'acquired sociopathy' (Moll et al., 2003). Further, deficits in the anterior limb of the internal capsule have been suggested to lead to impairments in attention, perception and working memory (Buchsbaum et al., 2006; Sepulcre et al., 2008). The anterior limb of the internal capsule together with the anterior corona radiata (which connects the striatum with the anterior cingulate cortex) have been postulated respectively to contribute to attention impairments of alerting and conflict processing (Niogi et al., 2010). Disruption in tracts supporting the functional integration of cortical and subcortical regions involved in memory, attention, volition, learning and visual integration may contribute to the problems of people with ASPD in adaptively responding to altered contingencies in the social and physical environment (including social cues such as facial expressions), expressed in traits such as impulsivity or difficulty inhibiting motivated responses; a low threshold for the discharge of aggression; and failure to learn from aversive experiences.

We also report for the first time in ASPD, WM MD abnormalities of the frontal lobe. MD of the diffusion tensor reflects the magnitude of water molecule movement that is independent of direction and contrasts with FA that assesses the directional preference of such movement (Le Bihan et al., 2001). Increased MD has been reported for instance in vascular and neurodegenerative disorders affecting the brain (Herve et al., 2005; Scula et al., 2010), schizophrenia (Lee et al., 2009; Narr et al., 2009) and autism (Lee et al., 2007) indicating less restricted and thus, increased movement of water molecules. Similarly in our present study, increased diffusion of water molecules in the frontal lobe of those with ASPD suggests a less coherent underlying WM microstructure. Taken together with FA deficits concurrently found in the right frontal lobe, such disorganisation of WM microstructure may have arisen due to abnormal development of glial cells, axons or cell membranes (Dong et al., 2004; Herve et al., 2005). Furthermore, as frontal abnormalities in the current study are lateralised and involve the right hemisphere, there may be
a relationship between lateralisation of abnormalities and emergence of ASPD. Supporting this theory are findings from a previous study that demonstrated impairments in social conduct, social cognition and emotion processing in those with right-sided vmPFC lesions rather than left-sided damage (Tranel et al., 2002). Similarly, right-sided inferior frontal cortex lesions are associated with loss of inhibitory control (Aron et al., 2004) where disinhibition may result from a direct consequence of frontal lobe damage (Brower and Price, 2001) or indirectly through loss of frontal inhibition on temporal lobe structures, particularly, the amygdala (Hoffer et al., 2007).

Given the lateralisation of brain functions noted above in our discussion of the corpus callosum [see also (Doron and Gazzaniga, 2008)], WM microstructural abnormalities localised to the right frontal lobe may further exacerbate the impaired modulation of left hemispheric processing that is potentially associated with abnormalities of the corpus callosum (Hiatt and Newman, 2007); this in turn may additionally contribute to emotion dysfunction (such as emotional shallowness and lack of empathy) and poor impulse control.

Although WM tracts in the posterior brain also showed FA deficits, these did not correlate significantly with psychopathy scores. The ILF and IFOF share projections at the posterior temporal and occipital lobes and are involved in connecting the visual association areas of the occipital lobe, and the auditory and visual association areas and the PFC respectively (Catani et al., 2002, 2003; Kier et al., 2004). Disconnection of the ILF has been associated with impaired communication between the occipital and temporal lobes (including the amygdala) — for instance involving the occipital and fusiform face areas (Catani and Thiebaut de Schotten, 2008; Thiebaut de Schotten et al., 2012) — which may lead to prosopagnosia and deficits in face processing (Fox et al., 2008) as well as visual memory disturbances (Shinoura et al., 2007). Further, the IFOF in humans represents the only direct long range association tract connecting the frontal and occipital lobes (Catani, 2006) and damage to the occipital portion of the IFOF has been associated with visual neglect as a result of impaired modulation by the frontal cortex (Urbanski et al., 2008). FA reduction in the present study was also found in the retrolenticular section and posterior thalamic radiation of the internal capsule which carries fibres of the optic radiation and is thus involved in the visual system. Overall, reduced microstructural integrity of these posterior tracts may contribute to deficits in face processing in antisocial populations, who show significant deficits in recognising fearful, sad, and surprised expressions, with a significantly greater deficit in fear recognition relative to other expressions (Marsh and Blair, 2008) and greater abnormalities of fusiform—extrastriate cortical responses to fearful than happy expressions relative to controls (Deeley et al., 2006).

Deficits in fear processing in antisocial populations have been hypothesised to contribute to impaired moral socialisation, in which the 'at risk' child fails to learn to avoid behaviour that engenders distress in others (Marsh et al., 2008).

There were several limitations to our work. As this was a cross-sectional study, it is unclear whether the frontal deficits encountered are present early in life as a consequence of abnormal brain maturation and thus predispose to the emotional dysfunction and antisocial behaviour of psychopathy and ASPD, or whether they represent cumulative effects of later biopsychosocial factors such as the experience of recurrent involvement in antisocial behaviour or substance misuse. Future studies should therefore aim to assess child cohorts so as to longitudinally characterise WM integrity; and to identify whether WM anomalies predate detrimental lifestyle factors such as substance use, that frequently coexist in antisocial populations. Further, in order to match groups more closely, future studies would benefit from using non-psychopathic/non-ASPD offenders or patients as controls, rather than the healthy community sample used here. This would minimise potential confounds, including the higher incidence of substance misuse disorders, and differing lifestyle and socio-demographic factors. Overall, our current study represents the largest cohort of adults with ASPD analysed by DT-MRI to date, whilst recognising that recruiting suitable participants poses a challenge.

With regard to the PCL-R, while all subjects had total PCL-R scores, a limited number of participants did not have factor scores (see Methods Section 2.1). Additionally, while controls were not assessed on the PCL-R, they were assessed through semi-structured interview using ICD-10 research criteria to assess for the presence of comorbid ASPD. Future studies may wish to implement the PCL-R or the shorter PCL-SV (Hart et al., 1995) to screen for psychopathy in control groups. Another weakness in the current study is that volumetry and neuropsychology measures were not assessed. The inclusion of neuropsychological tests within the present study may have helped to elucidate the type of information processing deficits that mediate the link between neural structural deficits and the behavioural profile of ASPD and psychopathy. Also, as WM FA/MD and structural volume may not be directly related (Lim et al., 1999), where possible, both conventional MRI and DT-MRI should be used in conjunction to investigate potential WM deficits as DT-MRI may be better able to detect pathology in normal appearing WM tissue while conventional MRI is able to ascertain independently occurring WM volumetric changes (Makki et al., 2007; Neil et al., 2002; Rugg-Gunn et al., 2001; Sundram et al., 2010). Moreover, when considering the results of our correlation analyses, it must be remembered that these may not be representative of other brain areas. In particular, as the ROIs over which these correlations were measured were defined by differences between ASPD subjects and controls, there is by definition, some relationship within these ROIs between FA and MD and overall behavioural scores, and our measurements cannot be considered truly independent. Future studies should therefore consider the use of ROIs based on the present study employed in independently acquired samples.

In summary, the current study showed reduced FA and increased MD respectively in areas consistent with a number of WM tracts within the frontal lobe in a group of males with ASPD compared to controls. Furthermore, frontal FA and MD abnormalities in the right hemisphere showed significant correlations with severity of psychopathy. Taken together, these findings suggest that frontal lobe WM microstructural abnormalities in ASPD particularly involve the right hemisphere. Given our pilot investigation, future work is required to longitudinally evaluate frontal lobe abnormalities through methods assessing brain structure, function and connectivity.
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White Matter Integrity in Asperger Syndrome: A Preliminary Diffusion Tensor Magnetic Resonance Imaging Study in Adults

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Introduction

Autism Spectrum disorder (ASD), which includes autism, Asperger syndrome and atypical autism, is an increasingly diagnosed [Baird et al., 2006] and highly genetic [Bailey et al., 1995; Frith, 2001] neurodevelopmental disorder affecting approximately 1:100 children in South London [Baird et al., 2006]. ASD is characterized by significant difficulties in social interaction, communication, and unusual or stereotyped routines and behavior (International Statistical Classification of Diseases and Health Related Problems—10th revision). People with Asperger syndrome typically do not have delay in the acquisition of language but they still show the other characteristic “autistic” impairments. There is consensus that ASD has a biological basis, and it has been proposed that it is a “connectivity” disorder. Diffusion Tensor Magnetic Resonance Imaging (DTI-MRI) allows measurement of the microstructural integrity of white matter (a proxy measure of “connectivity”). However, nobody has investigated the microstructural integrity of whole brain white matter in people with Asperger syndrome. Methods: We measured the fractional anisotropy (FA), mean diffusivity (MD) and radial diffusivity (RD) of white matter, using DT-MRI, in 13 adults with Asperger syndrome and 13 controls. The groups did not differ significantly in overall intelligence and age. FA, MD and RD were assessed using whole brain voxel-based techniques. Results: Adults with Asperger syndrome had a significantly lower FA than controls in 13 clusters. These were largely bilateral and included white matter in the internal capsule, frontal, temporal, parietal and occipital lobes, cingulum and corpus callosum. Conclusions: Adults with Asperger syndrome have widespread significant differences from controls in white matter microstructural integrity.

Keywords: autism; Asperger syndrome; white matter; DTI; connectivity
more severe as the brain matures into adulthood, underlin-
ing the importance of studying brain anatomy of ASD adults as well as children and adolescents.

Diffusion tensor magnetic resonance imaging (DT-MRI) can be used to examine the orientation and integrity of white matter tracts. This is achieved by measuring the magnitude and direction of water diffusion, which can be isotropic (the same amount in every direction) or anisotropic. Diffusion of water molecules in white matter tends to be greater along the direction of white matter tracts and thus predominantly anisotropic, with the degree of anisotropy in a particular tissue often being quantified through its Fractional Anisotropy (FA) value [Basser, Mattiello, & LeBihan, 1994]. The degree of anisotropy depends on a number of factors; for instance, myelination, fibre diameter and density. It is thought that a lower FA may be indicative of altered microstruc-
tural integrity or organization of the fibres [Basser, 1995; Beaulieu, 2002; Pierpaoli & Basser, 1996]. When lower FA is accompanied by higher radial diffusivity (RD) this may be indicative of abnormalities in myelination, although evidence for this comes largely from studies of normal and abnormal development, and animal investigation [Song et al., 2002, 2005].

A prior whole brain DT-MRI study included 7 children with autism and 9 controls [Barnea-Goraly et al., 2004]. They reported that the children with autism had a significant reduction in FA of white matter adjacent to the ventromedial prefrontal cortices, anterior cingulate gyri, temporoparietal junctions, and in the corpus callosum (CC). Subsequently, others investigated mixed samples of children and adults with autism [Alexander et al., 2007; Keller, Kana, & Just, 2007; Lee et al., 2007]. They reported that people with autism have a significant reduction in FA of areas within (or near) the CC [Alexander et al., 2007; Keller et al., 2007], right external capsule [Keller et al., 2007] and the superior temporal gyrus and temporal stem [Lee et al., 2007]. These studies were valuable first steps. However, they included hetero-
geneous clinical populations (in terms of mixed ages) or only studied children; and some did not correct for multiple comparisons. In summary, it has been proposed that ASD is underpinned by cortical "underconnectivity," and with abnormal brain development continuing into adulthood. Further, ASD is a developmental disorder, possibly characterized by grey and white matter over-
growth in very young children and reduced volumes in later childhood [Ben et al., 2007; Courchesne et al., 2001]. Hence, differences in white matter most likely continue to develop as the brain matures into adulthood due to abnormal neuronal loss and synaptic pruning [Muller, 2007]. Also ASD is a heterogenous clinical disorder. However, nobody has yet reported on white matter integrity across the whole brain in adults with ASD, and in a relatively homogeneous population.

Thus, we compared white matter micro integrity (using DT-MRI) in adults with Asperger syndrome and healthy adult controls, using a voxel-based analysis (VBM) to measure FA throughout the whole brain. We hypothe-
sized that people with Asperger syndrome have widespread differences from controls, but that this would mainly affect white matter in brain regions and systems most implicated in the disorder (i.e. frontal, temporal, limbic, basal ganglia and cerebellar regions).

Methods

Subject Recruitment

We recruited 13 male adults fulfilling ICD-10 clinical research criteria for autism—but who did not have a history of language delay. Hence, we defined them as having Asperger syndrome (age≥18, mean age 39 (SD±9.8), mean Full Scale IQ (FSIQ) 110 (SD±15.7)). The age of the subjects with Asperger syndrome ranged from 23 to 54 years and FSIQ ranged from 88 to 133. People with Asperger syndrome were recruited through local support groups, and our clinical research program in autism (supported by the MRC UK A.I.M.S. network). Subjects were diagnosed based on clinical interview, collateral information from family members and review of other information available such as school reports. All assessments were blind to MRI scan data using ICD-10 (International Statistical Classification of Diseases and Health Related Problems—10th revision) clinical research criteria [World Health Organization, 1992]. Diagnosis was made through consensus between three clinicians trained in the Autism Diagnostic Interview (ADI-R, [Lord, Rutter, & Le, 1994]). We were able to confirm our clinical research diagnosis using the ADI-R in six individuals with Asperger syndrome, when their parental informants were willing/available. Another four subjects were assessed on the Autism Diagnostic Observation Schedule (ADOS, [Lord et al., 1989]). Three subjects did not receive additional confirmation of their diagnosis using these methods. Exclusion criteria were a history of major psychiatric disorder (e.g. psychosis), head injury, toxic exposure, diabetes, abnormalities in routine blood tests, drug or alcohol abuse or clinical abnormality on routine MRI.

In addition, 13 male adult controls were recruited locally by advertisement. They did not differ in FSIQ or age (age≥18, mean age 37 (SD±9.6), mean FSIQ 115 (SD±14.4)). The age of the subjects ranged from 25 to 52 years and FSIQ ranged from 89 to 133. All subjects had a structured clinical exam and routine clinical blood tests to exclude biochemical, haematological or chromosomal abnormalities. Exclusion criteria were a history of major psychiatric disorder (e.g. psychosis), head injury, toxic exposure, diabetes, abnormalities in routine blood tests,
drug or alcohol abuse, clinical abnormality on routine MRI, or a medical or genetic disorder associated with autistic symptoms (e.g. fragile X or tuberous sclerosis). Ethics approval was provided by the Joint Institute of Psychiatry and South London and Maudsley NHS Trust Research Ethics Committee. All participants gave informed consent and all experiments were conducted in accordance with the Declaration of Helsinki.

**Neuropsychological Testing**

We measured overall intelligence using the vocabulary, comprehension, similarities, block design and object assembly subtests of the Wechsler Adult Intelligence Scale—Revised [Weschler, 1987].

**MR Acquisition**

Data were acquired using a GE Signa LX system (General Electric, Milwaukee, WI), with actively shielded magnetic field gradients (maximum amplitude 40 mT m⁻¹). A standard quadrature birdcage head coil was used for both RF transmission and signal reception. Each volume was acquired using a multi-slice peripherally gated EPI sequence, optimized for precise measurement of the diffusion tensor in parenchyma, from 60 contiguous near-axial slice locations. Images were acquired with isotropic (2.5 × 2.5 × 2.5 mm) voxels, reconstructed to 1.875 × 1.875 × 2.5 mm. Image acquisition was synchronized to the cardiac cycle using a peripheral gating device placed on the subject's forefinger. Echo time was 107 msec while the effective repetition time was 15 RR intervals. The duration of the diffusion encoding gradient was 17.3 msec giving a maximum diffusion weighting of 1300 sec mm⁻². At each slice location, seven images were acquired with no diffusion gradients applied, together with 64 diffusion-weighted images in which gradient directions were uniformly distributed in space. Full details are given elsewhere [Jones et al., 2002].

**DTI Processing**

Visual inspection was used to ensure all data was of suitable quality for further processing, and three scans were excluded because of data quality issues. The diffusion-weighted images were initially corrected for the effects of eddy-current-induced distortion using in-house software. Group mapping techniques (derived from the VBM analysis methods developed for structural (T1 and/or T2 weighted) images) compare parametric maps of MR parameters (in this case the FA, mean diffusivity (MD) and RD) between subjects, following registration into a standard space. In the current study, calculation of the initial FA, MD and RD images was performed using locally written software. (Full details are given in [Catani, Howard, Pajevic, & Jones, 2002], but briefly: correction for eddy current induced distortions was performed using a mutual information-based registration of all images to the mean non-diffusion-weighted images, using an affine transformation. The diffusion tensor was then calculated for each voxel, using multivariate linear regression after logarithmic transformation of the signal intensities, and maps of FA, MD and RD were generated from the resulting tensor images).

Normalization of the FA, MD and RD images was performed using Statistical Parametric Mapping software (SPM2, Wellcome Department of Imaging Neurosciences, University College London, UK). A two-stage registration process was performed, using both the T2-weighted (b = 0) images and the FA images themselves. The mean T2-weighted (b = 0) image for each subject was first registered to the EPI template provided in SPM (although the former is T2 weighted, and the latter T2* weighted, the image contrast is similar, as are the geometrical distortions inherent in any EPI-based acquisition). In both cases, SPM2's default normalization parameters were used (medium regularization; 16 non-linear iterations; trilinear interpolation). The derived warping parameters were then applied to corresponding FA images in order to map the latter into standard space. The normalized FA images of all subjects were then averaged and smoothed (8 mm FWHM Gaussian filter) to create a new, study specific, template to which each subject's FA images were then re-registered. The registered FA images were also segmented (using SPMs default a priori tissue probability information) to give maps of the probability of a tissue being either white or grey matter, and these segmented images were thresholded at a low (10%) probability to provide a binary mask of white matter. Visual inspection of example data sets confirmed that masking at this threshold produced maps, which included all major white matter areas, and did not suffer from any unexpected "holes" (e.g. in low FA regions caused by crossing fibres). An accurate segmentation was not essential, and a relatively liberal threshold was deliberately used, in order to create a slightly "over inclusive" mask.

Scans need to be smoothed in order to reduce confounds due to individual variation in WM anatomy. Smoothing the data in order to constrain it into specific statistical distributions is also a prerequisite for some analysis approaches, but is not necessary for our non-parametric approach (see below). The degree of smoothing to apply is still a subject of much discussion as different smoothing levels result in varying results [Jones, Symms, Cercignani, & Howard, 2005]; in the absence of a specific hypothesis about the spatial extent of any abnormalities, we applied a smoothing filter with an extent similar to the width of many white matter tracts (Gaussian, 5 mm full-width at half maximum) to aid between-subject anatomical matching and improve the signal-to-noise ratio.
Statistics

SPSS (SPSS 12.02 for Windows, SPSS Inc) was used for statistical analysis of non-imaging data. Group differences in age and IQ were examined using independent samples t-tests (two tailed). Level of statistical significance was defined as \( P < 0.05 \) (two tailed).

Differences in white matter FA, MD and RD between the Asperger group and the controls were estimated by fitting an analysis of covariance model at each intracerebral voxel in standard space. Age, performance IQ and verbal IQ were added as covariates. Given that brain changes are likely to extend over a number of contiguous voxels, test statistics incorporating spatial information, such as 3D cluster mass (the sum of suprathreshold voxel statistics), are generally more powerful than other possible test statistics, which are informed only by data at a single voxel [Bullmore et al., 1999]. As no parametric distribution is known for cluster mass, permutation testing was used to assess statistical significance in the analyses described below. A non-parametric approach also overcomes the difficulty that parametric methods assume that the residuals of the model tested will follow a Gaussian distribution (which has been shown not to be true for DTI data, even after large amounts of smoothing [Jones et al., 2005]) and reduces the need for smoothing to constrain the data into meeting this condition.

We first set a relatively lenient \( P \)-value (\( P < 0.05 \)) to detect voxels putatively demonstrating differences between groups. At this stage, we considered only those voxels to which all subjects contributed data; this—along with the masking procedure above—restricts the analysis to core white matter regions, reducing the search volume (and thus the number of comparisons made). This requirement of good “overlap” between the subjects also minimizes testing at the grey/white interfaces, where the high grey/white contrast of FA images exacerbates any edge effects. We then searched for spatial clusters among the voxels highlighted, and tested the “mass” of each cluster (the sum of suprathreshold voxel statistics it comprises) for significance.

Permutation-based testing, implemented in the XBAM package (developed at The Institute of Psychiatry, London, UK http://www.brainmap.co.uk) was used to assess statistical significance at both the voxel and cluster levels [Bullmore et al., 1999]. We performed 1,000 permutations at both voxel and cluster stage of the analysis. For the initial voxel level testing, the distribution is calculated separately at each voxel; this helps minimize potential complications due to the very high dynamic range of FA data, and also makes the analysis less sensitive to differences in (physiological) noise between brain regions. At the cluster level, rather than set a single a priori \( P \)-value below which we regard findings as significant, we calculated, for a range of \( P \)-values, the number of clusters which would be expected by chance alone. We then set the statistical threshold for cluster significance for each analysis such that the expected number of false-positive clusters by chance alone would be less than one, which was \( P = 0.0025 \), and the threshold for cluster size at larger than 10 voxels. It is known, however [Carew, Wahba, & Basser, 2007], that the variance of FA values in the brain depends upon the FA values themselves and, while we restricted our analysis to core white matter regions where this is relatively uniform, the effects of noise may still vary slightly from region to region. We therefore also inspected the voxel level maps (which treat each voxel independently and therefore inherently allow for such local differences in statistics), to ensure that these highlight the same areas as the cluster level results. In this way we retained the additional power of the cluster statistics while controlling for the potential disadvantage of its non-local nature.

Voxels and clusters were localized in Montreal-Neurological-Institute (MNI) space and transformed into Talairach and Tournoux co-ordinates [Brett, Johnsrude, & Owen, 2002; Talairach & Tournoux, 1988]. Resulting voxels and cluster maps were overlaid on the FA template for anatomical assessment. To further localize significant voxel clusters, atlases were consulted [Mori, Wakana, Nagae-Poetscher, & van Zijl, 2005; Talairach & Tournoux, 1988].

Results

Fractional Anisotropy

Adults with Asperger syndrome had a significantly lower FA than controls over large areas of the brain. These changes covered 13, largely bilateral, clusters (Table I and Fig. 1).

Frontal

Two clusters located in the frontal lobe correspond to parts of the inferior fronto-occipital fasciculus (IFO), the forceps minor and anterior corona radiata. On the right the cluster extended into the anterior thalamic radiation (ATR) and the uncinate fasciculus (UNC). On the left a more posterior third cluster extended into this same region, also including parts of the ATR and UNC.

Medial

Two of the frontal clusters described above extend dorsally just into the genu of the CC in both hemispheres. Four more posterior clusters (partly) located in the posterior corona radiata and posterior limb of the internal capsule extend into the CC corresponding to large parts of the splenium and small parts of the dorsal body of the CC, and possibly the corticospinal tracts.
Table I. DTI Group Mapping Analysis of Fractional Anisotropy of Asperger Syndrome vs. Controls

<table>
<thead>
<tr>
<th>Possibly involved tracts</th>
<th>Location</th>
<th>Side</th>
<th>x</th>
<th>y</th>
<th>z</th>
<th>Mean FA (s.d.) Asperger</th>
<th>Controls</th>
<th>P</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Higher FA in Asperger</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC/IFO</td>
<td>Frontal lobe</td>
<td>Left</td>
<td>-14</td>
<td>35</td>
<td>-12</td>
<td>0.387 (0.032)</td>
<td>0.414 (0.024)</td>
<td>0.000374</td>
<td>79</td>
</tr>
<tr>
<td>CC/IFO/ATR/UNC</td>
<td>Medial frontal gyrus</td>
<td>Right</td>
<td>14</td>
<td>32</td>
<td>-7</td>
<td>0.387 (0.039)</td>
<td>0.417 (0.023)</td>
<td>0.000019</td>
<td>296</td>
</tr>
<tr>
<td>IFO</td>
<td>Temporal lobe</td>
<td>Left</td>
<td>-39</td>
<td>-38</td>
<td>0</td>
<td>0.459 (0.063)</td>
<td>0.496 (0.030)</td>
<td>0.000248</td>
<td>83</td>
</tr>
<tr>
<td>IFO/ILF</td>
<td>Temporal lobe</td>
<td>Left</td>
<td>-32</td>
<td>-32</td>
<td>2</td>
<td>0.459 (0.057)</td>
<td>0.497 (0.023)</td>
<td>0.000177</td>
<td>82</td>
</tr>
<tr>
<td>IFO/ILF</td>
<td>Cuneus</td>
<td>Right</td>
<td>25</td>
<td>-70</td>
<td>7</td>
<td>0.375 (0.046)</td>
<td>0.406 (0.028)</td>
<td>0.000231</td>
<td>93</td>
</tr>
<tr>
<td>IFO/ILF</td>
<td>Middle occipital gyrus</td>
<td>Left</td>
<td>-25</td>
<td>-73</td>
<td>7</td>
<td>0.593 (0.046)</td>
<td>0.437 (0.026)</td>
<td>0.000039</td>
<td>178</td>
</tr>
<tr>
<td>CC</td>
<td>Corpus callosum</td>
<td>Left</td>
<td>0</td>
<td>-32</td>
<td>7</td>
<td>0.529 (0.072)</td>
<td>0.594 (0.027)</td>
<td>0.000485</td>
<td>38</td>
</tr>
<tr>
<td>SLF</td>
<td>Frontal lobe</td>
<td>Right</td>
<td>30</td>
<td>24</td>
<td>14</td>
<td>0.393 (0.034)</td>
<td>0.423 (0.023)</td>
<td>0.000139</td>
<td>124</td>
</tr>
<tr>
<td>CST/CC</td>
<td>Parietal lobe</td>
<td>Right</td>
<td>32</td>
<td>-44</td>
<td>22</td>
<td>0.392 (0.049)</td>
<td>0.423 (0.026)</td>
<td>0.000329</td>
<td>61</td>
</tr>
<tr>
<td>CC</td>
<td>Corpus callosum</td>
<td>Right</td>
<td>7</td>
<td>-26</td>
<td>22</td>
<td>0.422 (0.061)</td>
<td>0.468 (0.024)</td>
<td>0.000019</td>
<td>214</td>
</tr>
<tr>
<td>CC/SLF/CST/ATR/UNC</td>
<td>Corpus callosum</td>
<td>Left</td>
<td>-9</td>
<td>-26</td>
<td>23</td>
<td>0.382 (0.037)</td>
<td>0.416 (0.024)</td>
<td>0.000001</td>
<td>1149</td>
</tr>
<tr>
<td>SLF/CG</td>
<td>Cingulate gyrus</td>
<td>Right</td>
<td>16</td>
<td>-13</td>
<td>34</td>
<td>0.405 (0.034)</td>
<td>0.444 (0.021)</td>
<td>0.000009</td>
<td>310</td>
</tr>
<tr>
<td>SLPFG</td>
<td>Cingulate gyrus</td>
<td>Left</td>
<td>-14</td>
<td>0</td>
<td>42</td>
<td>0.454 (0.045)</td>
<td>0.505 (0.031)</td>
<td>0.000289</td>
<td>54</td>
</tr>
</tbody>
</table>

ATR, anterior thalamic radiation; CC, corpus callosum; CG, cingulum; CST, corticospinal tract; IFO, fronto-occipital fasciculus; ILF, inferior longitudinal fasciculus; SLF, superior longitudinal fasciculus; T&T, Talairach & Tournoux coordinates; UNC, uncinate fasciculus.

*Location of most activated voxel.

There were four very small clusters of higher FA in the Asperger group, but all were smaller that 10 voxels.

Mean Diffusivity

Subjects with Asperger syndrome had significantly lower MD in a cluster located in the brain stem (T&T = [7, -21, -23], \( P = 0.0004 \), size = 41 voxels).

Radial Diffusivity

Subjects with Asperger syndrome had higher RD than controls in 16 clusters. They had lower RD than controls in two clusters (Table II and Figs. 2 and 3).

Discussion

We compared white matter microstructural integrity in 13 adult males with Asperger's syndrome to a control group which did not differ significantly in IQ, age or gender. People with Asperger syndrome had a significant decrease in FA of a number of white matter brain regions, and had higher FA in four very small regions.

This is a preliminary study which has a number of limitations, including the relatively small sample size and a lack of ADI-R/ADOS measures in two subjects. Nevertheless we had sufficient power to detect significant group differences in FA. Moreover, the results we report are likely to represent true differences in FA because of the use of a conservative analysis and thresholding method to reduce the risk of type 1 errors (see Methods).

Voxel-based analyses have a risk of false-positive edge effects, and some of our results are indeed located near the ventricles. However, we do not think that edge effects...
Table II. DTI Group Mapping Analysis of Radial Diffusivity of Asperger Syndrome vs. Controls

<table>
<thead>
<tr>
<th>Possibly Involved tracts</th>
<th>Location*</th>
<th>Side</th>
<th>x</th>
<th>y</th>
<th>z</th>
<th>P</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Higher radial diffusivity in Asperger subjects (Fig. 2)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UNC</td>
<td>Inferior frontal gyrus</td>
<td>Right</td>
<td>14</td>
<td>32</td>
<td>-15</td>
<td>0.000940</td>
<td>25</td>
</tr>
<tr>
<td>IFO/ILF</td>
<td>Temporal lobe</td>
<td>Left</td>
<td>-36</td>
<td>-60</td>
<td>-6</td>
<td>0.000204</td>
<td>40</td>
</tr>
<tr>
<td>IFO/ILF</td>
<td>Temporal lobe</td>
<td>Right</td>
<td>38</td>
<td>-63</td>
<td>2</td>
<td>0.000163</td>
<td>54</td>
</tr>
<tr>
<td>CG/IFO/ILF</td>
<td>Cuneus</td>
<td>Left</td>
<td>-16</td>
<td>-75</td>
<td>7</td>
<td>0.000163</td>
<td>28</td>
</tr>
<tr>
<td>CC</td>
<td>Corpus callosum</td>
<td>Right</td>
<td>4</td>
<td>-30</td>
<td>7</td>
<td>0.000061</td>
<td>23</td>
</tr>
<tr>
<td>CPT/CST/STR/PTR</td>
<td>Capsula interna</td>
<td>Right</td>
<td>25</td>
<td>-16</td>
<td>12</td>
<td>0.000797</td>
<td>26</td>
</tr>
<tr>
<td>SLF</td>
<td>Insula</td>
<td>Left</td>
<td>-29</td>
<td>-6</td>
<td>19</td>
<td>0.000797</td>
<td>30</td>
</tr>
<tr>
<td>SLF</td>
<td>Parietal lobe</td>
<td>Left</td>
<td>-29</td>
<td>-51</td>
<td>26</td>
<td>0.000061</td>
<td>23</td>
</tr>
<tr>
<td>CG</td>
<td>Cingulate gyrus</td>
<td>Left</td>
<td>-13</td>
<td>-35</td>
<td>28</td>
<td>0.000307</td>
<td>22</td>
</tr>
<tr>
<td>SLF</td>
<td>Inferior parietal lobule</td>
<td>Right</td>
<td>43</td>
<td>-33</td>
<td>26</td>
<td>0.000960</td>
<td>37</td>
</tr>
<tr>
<td>SLF/CPT/CST/STR</td>
<td>Frontal lobe</td>
<td>Left</td>
<td>-30</td>
<td>-22</td>
<td>27</td>
<td>0.000163</td>
<td>53</td>
</tr>
<tr>
<td>CG</td>
<td>Cingulate gyrus</td>
<td>Right</td>
<td>18</td>
<td>-9</td>
<td>26</td>
<td>0.000797</td>
<td>23</td>
</tr>
<tr>
<td>SLF/CPT/CST/STR</td>
<td>Frontal lobe</td>
<td>Right</td>
<td>25</td>
<td>-14</td>
<td>28</td>
<td>0.000061</td>
<td>122</td>
</tr>
<tr>
<td>CC/CPT/PTR</td>
<td>Precuneus</td>
<td>Left</td>
<td>-16</td>
<td>-45</td>
<td>35</td>
<td>0.000327</td>
<td>26</td>
</tr>
<tr>
<td>CC/CPT/CST/STR</td>
<td>Cingulate gyrus</td>
<td>Left</td>
<td>-13</td>
<td>-14</td>
<td>41</td>
<td>0.000368</td>
<td>30</td>
</tr>
<tr>
<td>CC/CPT/CST/STR</td>
<td>Frontal lobe</td>
<td>Right</td>
<td>20</td>
<td>-30</td>
<td>48</td>
<td>0.000184</td>
<td>21</td>
</tr>
<tr>
<td><strong>Lower radial diffusivity in Asperger subjects (Fig. 3)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCP/ICP/SCP</td>
<td>Cerebellum</td>
<td>Left</td>
<td>-4</td>
<td>-43</td>
<td>-23</td>
<td>0.000471</td>
<td>38</td>
</tr>
<tr>
<td>CC</td>
<td>Corpus callosum</td>
<td>Left</td>
<td>-13</td>
<td>-33</td>
<td>18</td>
<td>0.000264</td>
<td>45</td>
</tr>
</tbody>
</table>

CC, corpus callosum; CG, cingulum; CPT, corticopontine tract; CST, corticospinal tract; ICP, inferior cerebellar peduncle; IFO, fronto-occipital fasciculus; ILF, inferior longitudinal fasciculus; MCP, middle cerebellar peduncle; PTR, posterior thalamic radiation; SCP, superior cerebellar peduncle; SLF, superior longitudinal fasciculus; STR, superior thalamic radiation; T&T, Talairach & Tournoux coordinates; UNC, uncinate fasciculus.

Figure 2. Clusters of higher radial diffusivity in people with Asperger syndrome compared to healthy controls. (Radiological convention; image left = subject's right.) Slices are 2 mm and Z-coordinates range from -24 to +54.

Figure 3. Clusters of lower radial diffusivity in people with Asperger syndrome compared to healthy controls. (Radiological convention; image left = subject's right.) Slices are 2 mm and Z-coordinates range from -40 to +22.

can fully explain our results since most differences we found are not close to the ventricles. Also, even though we specifically excluded children in this study to obtain a more homogeneous adult sample, the age range in our sample is rather large. We therefore covaried in our analyses for age, performance IQ and verbal IQ to limit their influence on our results. We also analyzed the results after excluding the two subjects without ADI-R or ADOS, and this did not substantially change the results.

Despite these limitations, this study is the first comparison of whole brain white matter integrity in adult males with Asperger syndrome and controls. Moreover, our results are in line with prior whole brain studies in children, and in larger populations of (age-mixed) people with autism [Barnea-Goraly et al., 2004; Keller et al., 2007]—although, in our sample of adults, we found more extensive (and bilateral) differences. For
instance, one study in children with autism [Barnea-Goralay et al., 2004] reported reduced FA values in the medial prefrontal areas, anterior cingulate, CC, right (pre) motor area, temporal and occipital regions. Others reported reduced FA in or near the CC and in the retrolenticular portion of the internal capsule in a mixed sample of children and adults with autism [Keller et al., 2007]. Our results also implicate these areas, but also some additional ones. Others also reported lower FA in frontal and temporal regions in autistic children [Cheung et al., 2009; Ke et al., 2009], accompanied by greater FA in the SLF and left occipital pole [Cheung et al., 2009] and greater FA in temporal and frontal lobe [Ke et al., 2009]. We were not able to confirm the FA increases in our sample, and were also not able to confirm altered FA in the cerebellum previously reported in Asperger syndrome [Catani et al., 2008] using tractography-based approaches. Using a different (whole brain) approach to reduce smoothing effects Lee et al. reported lower FA in CC, superior temporal and anterior cingulated gyrus, also in line with our results.

Voxel-based analytic DTI methods do not give tract specific information. Therefore, although we are able to name regions which include possibly affected tracts, the actual involvement of specific tracts requires the use of alternative methods such as tractography [Catani et al., 2008]. Nevertheless, our findings suggest altered organization of the CC. This adds to evidence from other structural [Barnea-Goralay et al., 2004; Chung, Dalton, Alexander, & Davidson, 2004; Egaas, Courchesne, & Saitho, 1995; Hardan, Minshew, & Keshavan, 2000; Keller et al., 2007; Lee et al., 2007; Manes et al., 1999; Piven, Bailey, Ranson, & Arndt, 1997; Vidal et al., 2006; Wailer et al., 2005] and functional studies [Just et al., 2007; Mason, Williams, Kana, Minshew, & Just, 2008] that the CC is abnormal in ASD, and may partially explain some clinical features of the disorder. For example, recent fMRI studies reported that people with ASD have significantly lower interhemispheric connectivity during various language, working memory and visuospatial imagery tasks [Just et al., 2004; Kana, Keller, Cherkassky, Minshew, & Just, 2006; Koshino et al., 2005].

We found that the Asperger group had significantly reduced FA in a number of frontal regions, that contain the uncinate fasciculi, inferior fronto-occipital fasciculi and the ATR. Differences in frontal lobe development, anatomy and function are frequently reported in ASD [Carper & Courchesne, 2000; Carper, Moses, Tigue, & Courchesne, 2002; Herbert et al., 2004; Levitt et al., 2003; Luna et al., 2002; Sundaram et al., 2008] and frontal lobe deficits have been linked to abnormalities in Theory of Mind [Baron-Cohen et al., 1999; Castelli, Frith, Happ, & Frith, 2002; Frith & Frith, 1999; Happe et al., 1996], executive functioning [Luna et al., 2002] joint attention [Mundy, 2003] and language [Just et al., 2004; Muller et al., 1998] in ASD. Further, others have reported that people with ASD have significant differences in the activation of cortical motor areas [Muller, Pierce, Ambrose, Allen, & Courchesne, 2001] and motor abnormalities are one of the earliest observable behavioral features in ASD. Hence, our results add support to the work of others [Just et al., 2004], suggesting that people with ASD have significant differences in frontal connectivity.

In addition we found that people with Asperger syndrome have significant differences in FA of various parietal lobe regions. These results are in line with previous studies in ASD that reported abnormalities in parietal lobe anatomy [Carper et al., 2002; Courchesne, Press, & Yeung-Courchesne, 1993; Haznedar et al., 2000; Piven, Arndt, Bailey, & Andreasen, 1996; Thakkar et al., 2008] and add to evidence of underconnectivity in the temporo-parietal area [Barnea-Goralay et al., 2004; Cherkassky, Kana, Keller, & Just, 2006; Just et al., 2007]. The areas with reduced FA have been linked to, for instance, emotion processing [Hollman & Gilmore, 1998], ToM [Castelli et al., 2002], and determining the relevance of stimuli to the self [Northoff & Bermpohl, 2004] and deficits in these domains are core clinical features of ASD. Further, Thakkar et al., reported lower FA and abnormal BOLD activation during executive function tasks bilaterally in the anterior cingulate gyrus [Thakkar et al., 2008].

In our investigation people with Asperger syndrome had a significant reduction of FA bilaterally in the temporal lobe and perisylvian area. Areas with significantly reduced FA bilaterally contained the ILF, SLF and the IFO. This supports suggestions from prior DTI [Barnea-Goralay et al., 2004; Conturo et al., 2008; Keller et al., 2007; Lee et al., 2007] and structural and functional studies [Boddeart et al., 2004; Gendry Messe et al., 2005] that these regions are abnormal in people with ASD. The temporal and perisylvian areas are vital for language and social cognition, and abnormalities in these areas may explain some of the difficulties ASD patients have in these domains. For example, the superior temporal area is important in auditory processing [Just et al., 2004; Meyer et al., 2005], social perception of movement [Allison, Puce, & McCarthy, 2000], imitation [Jacoboni, 2005] and ToM [Castelli et al., 2002]. Additionally, damage to the temporal region is thought to be related to visually specific semantic, emotional, memory and language deficits [Catani, Jones, Donato, & fytche, 2003; Mandonnet, Notou, Gatifoglou, Capelle, & Dufau, 2007; Vigneau et al., 2006]. Thus, the significant reduction in FA we found in the temporal and perisylvian areas in people with ASD may be associated with abnormal connectivity between brain regions important in the understanding of salient social information and language.

We also found reduced FA values in the occipital lobes in people with Asperger syndrome. White matter areas which differed from controls included those adjacent to
the the analysis of data. All authors contributed to revising the analysis of data. All authors contributed to revising the article for important intellectual content and gave final approval of the version to be published. No other authors have contributed substantially to this manuscript.

References


theory and permutation, for a difference between two groups of structural MR images of the brain. IEEE Transactions on Medical Imaging, 18, 32–42.


White Matter Integrity in Asperger Syndrome: A Preliminary Diffusion Tensor Magnetic Resonance Imaging Study in Adults

Oswald J.N. Bloemen, Quinton Deeley, Fred Sundram, Eileen M. Daly, Gareth J. Barker, Derek K. Jones, Therese A.M.J. van Amelsvoort, Nicole Schmitz, Dene Robertson, Kieran C. Murphy, and Declan G.M. Murphy


The authors noticed two errors in their article:

1. In the following reference, the author’s first and middle initial are reversed:

This reference should instead read as follows:

2. Also, in Table I there is an error in the first column header. “Higher FA in Asperger” should instead be “Lower FA in Asperger”. Below is the corrected table.

Table I. DTI Group Mapping Analysis of Fractional Anisotropy of Asperger Syndrome vs. Controls

<table>
<thead>
<tr>
<th>Possibly Involved Tracts</th>
<th>Location*</th>
<th>Side</th>
<th>x</th>
<th>y</th>
<th>z</th>
<th>Mean FA (SD) Asperger</th>
<th>Controls</th>
<th>P</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lower FA in Asperger</td>
<td>CC/IFO</td>
<td>Frontal lobe</td>
<td>Left</td>
<td>-14</td>
<td>35</td>
<td>-12</td>
<td>0.387 (0.032)</td>
<td>0.414 (0.024)</td>
<td>0.000374</td>
</tr>
<tr>
<td>CC/IFO/ATR/UNC</td>
<td>Medial frontal gyrus</td>
<td>Right</td>
<td>14</td>
<td>32</td>
<td>-7</td>
<td>0.387 (0.039)</td>
<td>0.417 (0.023)</td>
<td>0.000019</td>
<td>296</td>
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<td>IFO</td>
<td>Temporal lobe</td>
<td>Left</td>
<td>-39</td>
<td>-38</td>
<td>0</td>
<td>0.459 (0.063)</td>
<td>0.496 (0.030)</td>
<td>0.000248</td>
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<td>Temporal lobe</td>
<td>Left</td>
<td>-32</td>
<td>-32</td>
<td>2</td>
<td>0.459 (0.057)</td>
<td>0.497 (0.023)</td>
<td>0.000177</td>
<td>82</td>
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<tr>
<td>IFO/ILF</td>
<td>Cuneus</td>
<td>Right</td>
<td>25</td>
<td>-70</td>
<td>7</td>
<td>0.375 (0.046)</td>
<td>0.406 (0.028)</td>
<td>0.000231</td>
<td>93</td>
</tr>
<tr>
<td>IFO/ILF</td>
<td>Middle occipital gyrus</td>
<td>Left</td>
<td>-25</td>
<td>-73</td>
<td>7</td>
<td>0.393 (0.046)</td>
<td>0.437 (0.028)</td>
<td>0.000039</td>
<td>178</td>
</tr>
<tr>
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<td>Corpus callosum</td>
<td>0</td>
<td>-32</td>
<td>7</td>
<td>0.507 (0.072)</td>
<td>0.594 (0.027)</td>
<td>0.000485</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>SLF</td>
<td>Frontal lobe</td>
<td>Right</td>
<td>30</td>
<td>24</td>
<td>14</td>
<td>0.393 (0.034)</td>
<td>0.423 (0.023)</td>
<td>0.000139</td>
<td>124</td>
</tr>
<tr>
<td>SLF/CC</td>
<td>Parietal lobe</td>
<td>Right</td>
<td>32</td>
<td>-44</td>
<td>22</td>
<td>0.392 (0.044)</td>
<td>0.432 (0.026)</td>
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<tr>
<td>CC</td>
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<td>7</td>
<td>-26</td>
<td>22</td>
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<td>0.468 (0.024)</td>
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<td></td>
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<tr>
<td>CC/SLF/CSF/ATR/UNC</td>
<td>Corpus callosum</td>
<td>Left</td>
<td>-9</td>
<td>-26</td>
<td>23</td>
<td>0.382 (0.037)</td>
<td>0.416 (0.024)</td>
<td>0.000001</td>
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<tr>
<td>SLF/CG</td>
<td>Cingulate gyrus</td>
<td>Right</td>
<td>16</td>
<td>-13</td>
<td>34</td>
<td>0.405 (0.034)</td>
<td>0.444 (0.021)</td>
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<tr>
<td>SLF/CG</td>
<td>Cingulate gyrus</td>
<td>Left</td>
<td>-14</td>
<td>0</td>
<td>42</td>
<td>0.454 (0.045)</td>
<td>0.505 (0.031)</td>
<td>0.000289</td>
<td>54</td>
</tr>
</tbody>
</table>

ATR, anterior thalamic radiation; CC, corpus callosum; CSF, cingulum; CST, corticospinal tract; IFO, fronto-occipital fasciculus; ILF, inferior longitudinal fasciculus; SLF, superior longitudinal fasciculus; T&T, Talairach & Tournoux coordinates; UNC, uncinate fasciculus.

*Location of most activated voxel.

The authors regret the errors.