3.0 Tesla MRI analysis of the Intra-Uterine Growth Restricted Infant Brain - Comparison of Infants with Normal and Abnormal Antenatal Doppler's.

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3.0 Tesla MRI analysis of the Intra-Uterine Growth Restricted Infant Brain –
Comparison of Infants with Normal and Abnormal Antenatal Doppler’s

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Department of Paediatrics
RCSI

A thesis submitted to the School of Postgraduate Studies, Faculty of
Medicine and Health Sciences, Royal College of Surgeons in Ireland, in
fulfillment of the degree of
Doctor of Philosophy

Supervisors: Dr. Adrienne Foran
Professor Naomi McCallion

2017
Candidate Thesis Declaration

I declare that this thesis, which I submit to RCSI for examination in consideration of the award of a higher 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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Student Number:  12147231

Date: ________________________________________________________________
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<td>A Label Based Encephalic ROI template</td>
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<td>CamBA</td>
<td>Cambridge Brain Analysis software</td>
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<td>Centre for Advanced Medical Imaging</td>
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<td>CRH</td>
<td>Corticotropin Related Hormone</td>
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<td>Insulin-like Growth Factor</td>
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<td>mammalian Target of Rapamycin</td>
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<td>Necrotising Enterocolitis</td>
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Summary

Intra-uterine growth restriction (IUGR) is defined as a failure of a fetus to achieve its expected weight for a given gestational age. These infants have increased mortality and morbidity rates compared to appropriately grown infants. Deficits in cognition, language and social functions may occur. StOOPs (Short-term Outcome Of PORTO study) is a national study incorporating brain imaging to investigate differences in brain development and organisation between two groups of IUGR infants; those with normal and abnormal antenatal Doppler measurements. Antenatal Doppler examination of blood flow in the umbilical artery is used to monitor pregnancies affected with IUGR.

Singleton infants without aneuploidy or congenital anomaly and with an estimated fetal weight < 10th centile were enrolled nationally. Prospective data of delivery and infant characteristics was collected. Non-sedated 3 Tesla MRI of brain was acquired at term corrected gestation. 34 IUGR infants with abnormal umbilical arterial Doppler measurements, 29 IUGR infants with normal measurements and 11 healthy term controls were recruited. Connectivity analyses and voxel based morphometry analysis were performed. Growth and developmental assessments were performed at 1 year of age.

Resting state networks were identified. No group differences were found for the default mode network (DMN). Parts of the dorsal attention network (DAN), sensory networks and thalamic connections all showed systematic disturbance in the Abnormal Doppler group. Volumetric differences were identified between the three groups, which were correlated with one-year outcome measures. Communication scores positively correlate with volume in two cluster areas identified; a brainstem region and a region around the central gyrus. Personal & Social scores are negatively correlated with volume in a region in the right frontal lobe.

Differences between IUGR infant brains based on Doppler status are identifiable at term corrected age. These findings suggest that AbD group may benefit from early intensive interventions.
Acknowledgements

I am grateful for the mentorship provided by both of my supervisors, Dr. Adrienne Foran and Prof. Naomi McCallion, over the past four years, which has amounted both to research supervision and friendship in addition to significant career and personal development.

I would like to express special thanks to Dr. David Watson at the Intelligent Systems Research Centre, Faculty of Computing and Engineering, University of Ulster (Functional MRI processing) and Dr. Roger Tait at the Brain Mapping Unit, Department of Psychiatry, University of Cambridge (Volumetric processing) for their technical assistance, guidance and support throughout the course of this project. I have also enjoyed my time at the Centre for Advanced Medical Imaging at St. James’s Hospital, where the MRI’s were performed and the support that was received from Dr. James Meaney, Dr. Andrew Fagan and Dr. Jason McMorrow as well as Keith McGrath and Aliz Takacs. The support of the consultants and research registrars across the participating units has been invaluable in identifying suitable infants for recruitment. The host institution, the Rotunda Hospital, was especially supportive and provided considerable administrative support, particularly from Donna Aylmer for follow up. I appreciate the continued support and involvement of Dr. Stephanie Ryan and Dr. Ailbhe Tarrant for input on study design and reporting all the scans. The advice and support from Dr. Emma Doyle for the placental histology interpretation from across all sites was greatly valued. I am thankful to the Perinatal Ireland staff – the unit ultrasonographers, Pat Dicker and especially Elizabeth Tully, Fiona Manning and Professor Fergal Malone. I am also very thankful to the Children’s Fund for Health for funding the project and the support I have received from Gayle Kenney and Andrea Neill. The expert talents of Niamh Thorne were invaluable in getting the babies into a deep sleep and keeping the scanning days as smooth as possible.

Finally, I wish to thank the infants and their parents for participating in this study, travelling with their newborns to the imaging centre and remaining enthusiastic about continued involvement in the StOOPS study.
Contributor Statement

During this project, I led in the design of the study, completed the Research and Ethics Committee applications for each participating site of PORTO and St James’s Hospital (8 sites), the Grant application and Scientific Committee application for the Children’s University Hospital and coordinated the study. I remained in weekly contact with the ultrasonographers in the participating sites and the neonatal units. I travelled to all centres and took consent from each participating infant/parent. I collected all the data for each infant at every site. I was present for every scan at the CAMI unit, settling infants to natural sleep and monitoring during the scanning. For the imaging processing, I constructed the study question and learned the steps involved in the image analysis (St. Louis, Derry and Cambridge) which was undertaken in the University of Ulster and University of Cambridge. I dictated the resting state networks to analyse in the fMRI study, which was processed by Dr. Watson (Derry), analysed and interpreted the results and drew the conclusions herein presented. I planned the volumetric analysis in conjunction with Dr. Tait (Cambridge), interpreted the raw results, performed the statistical analysis and drew the conclusions herein presented. I coordinated the 1 year follow up and contact with each family and interpreted the results of the ASQ’s and head circumference measurements and carried out the statistical analysis herein presented.
Dedication

I dedicate this work to my family and friends in both Dublin and Cambridge, who provided endless support and encouragement over the duration of the project, helping me through the long days, frustrations and disappointments and to Katie, for reading through it all.

Most of all I dedicate this to Conall, who never once let me off the hook and pushed me on when I needed it most. I will be forever grateful for the many many deadlines and dinners.
Section 1: Introduction

Chapter 1

Intra-Uterine Growth Restriction
Chapter 1. Introduction – Intra-Uterine Growth Restriction

1. Intra-Uterine Growth Restriction

Intra-uterine growth restriction (IUGR) was first described in 1961 and originally defined as ‘a fetus whose body measurements are below 2 standard deviations of the mean or below the 10th percentile for fetuses of similar sex and gestational age who are born at comparable altitudes above sea level’ (1). The term intra-uterine growth retardation was initially used, but retardation has changed to restriction over time, as it had typically implied abnormal mental function to parents, causing undue distress. Various terms and definitions have been used inconsistently over the years, causing much confusion. Terms such as small for dates, small for gestational age (SGA), chronic fetal distress, fetal growth restriction (FGR) and failure to thrive in utero have been used amongst others, however, they are not all synonymous (2). IUGR is best defined as a failure of a fetus to achieve its expected weight for a given gestational age (3).

SGA is often defined as having a birth weight below the 10th centile for a given population. It does not imply a pathological process and many infants who are SGA will have fulfilled their growth potential and as such are not growth restricted, rather, better described as being constitutionally small. On the other hand, a proportion of growth-restricted infants may have birth weights above the 10th centile for their population, yet have failed to reach their growth potential (4).

Though the terms intra-uterine growth restriction and small for gestational age are closely related they are not interchangeable. IUGR is used to identify those infants who failed to reach their growth potential secondary to a pathological process in utero, usually through an impact on placental development. Several different cutoffs have been used in past definitions, including birth weight below or greater than the 10th centile for gestational age (5, 6). Another approach has been to use a birth weight equal to or lower than 2 standard deviations below the mean in relation to gestational age and gender (7).
This broadly corresponds to the 3rd centile on standard growth charts and it may be clinically relevant as it is known that infants born below this centile represent a significant proportion of those with poor outcomes (8). Kamoji et al assessed extremely growth-restricted infants and suggested the concept of the ‘viability centile’, which falls along the 2nd centile (9). With such a plethora of different centile cutoffs suggested clear guidance on how best to identify IUGR affected infants is not easily determined. The definitions based solely on centile chart cutoffs are based on comparison with population peers, and therefore would exclude those infants with IUGR and birth weight above the 10th centile, and as such fail to assess growth velocity appropriately (10, 11).

Fetal growth velocity assessment through repeated ultrasound measurement has fundamentally changed how intra-uterine growth restriction is defined. It has led to the development of customized charts of birth weight centiles based on the growth potential calculated on an individual or customized basis (12). Evidence of faltering growth, provided by tools such as ultrasound, supplies the strongest indication that the fetus is truly growth restricted. These charts improve differentiation between true cases of growth restriction and those infants who are constitutionally small. Comparison with population-based charts has determined that population-based charts may result in a considerable number of inaccurate assessments of growth, incorrectly labeling some as IUGR and missing others altogether (13-15).

A sub division of intra-uterine growth restriction is that of symmetric (where all anthropometric measurements are proportional) versus asymmetric IUGR (where weight is on a lower centile to head circumferences) (16). Asymmetric growth restriction had been taken to suggest that brain/head growth has been preserved at the expense of body growth. Though a seemingly desirable adaptive mechanism, comparisons of long-term outcomes between symmetric and asymmetric growth restricted infants have not shown a protective brain sparing effect. It has been shown that weight and height gains are similar in both groups postnatally, and that the symmetrically growth restricted infants demonstrate catch up in terms of head circumference (17).
Moreover, Scherjon et al have suggested that those infants with apparent brain sparing have poorer long term cognitive outcome as compared with their symmetrically growth restricted peers (18). Visual function by late school age is not different between the two groups, despite initially accelerated maturation of visual evoked potentials in the first year of life in those preterm infants with antenatal brain sparing (19). Behavioural problems may also be more likely in infants with evidence of fetal circulatory redistribution or brain sparing, suggesting that the brain is not entirely spared. The symmetric versus asymmetric classification evolved as a tool to aid in determining the cause of poor fetal growth. Symmetric IUGR suggested underlying issues as genetic conditions and congenital infections whereas asymmetric IUGR can be said to be related to placental dysfunction, smoking and maternal medical conditions such as pre-eclampsia (PET), hypertension or poorly controlled diabetes.

However, in reality there is a significant degree of overlap between these divisions. As such the classification of symmetric versus asymmetric is not used as routinely as it has been before, especially as it is not particularly helpful in predicting long-term outcomes.

1.1. Aetiology of Intra-Uterine Growth Restriction

IUGR is a common condition encountered in obstetric practice with the incidence reported as varying between 3% and 7% in a population (20). It is a major public health issue due to the associated increase in morbidity in the newborn period as well as the long-term neurodevelopmental and cardiovascular sequelae (21, 22). It also accounts for a substantial proportion of perinatal mortality, alongside fetal malformations and infection (23). Intra-uterine growth restriction can be secondary to fetal, maternal or placental factors and there is often a significant and complex interplay between these issues. As a result, it is difficult to precisely identify the cause in any given case.
1.1.1 Fetal Factors

Genetic factors, infections, multiple gestation and congenital anomalies are implicated in causing growth restriction of fetal origin. Epidemiological studies looking at the reproductive outcome of women born with low birth weight show that growth restriction may be heritable. They have shown that they are twice as likely to deliver a child with intra-uterine growth restriction as compared to women born with a birth weight appropriate for gestational age (24, 25). The risk has also been shown to increase with each pregnancy affected. Genes from both parents may affect birth weight, though maternal genes affect it to a greater degree. A genome-wide association study of birth weight found that mutations in specific alleles at two loci (near CCNL1 and in ADCY5) appear to lower birth weight (26, 27). Congenital imprinting disorders, such as Silver-Russell Syndrome, display how imprinted genes with a parent-of-origin specific expression can affect growth during the pregnancy (28). Silver-Russell syndrome is characterised by growth restriction and poor postnatal growth. Up to 10% of cases have a maternal uniparental disomy of chromosome 7. Abnormalities of karyotype account for the greatest proportion of genetic causes of IUGR, with up to 20% of all cases of growth restriction secondary to such abnormalities (29, 30). These are often apparent early during the course of the pregnancy.

Though most cases will be symmetrically IUGR, a proportion will also be asymmetrically grown (31). Congenital anomalies can impede the ability to maintain appropriate intra-uterine growth, but these account for less than 2% of all cases of IUGR in the absence of a genetic abnormality (32). Twin or higher order multiple pregnancies are associated with intra-uterine growth restriction, the risk for IUGR increasing with the number of fetuses present. The mechanism through which this occurs is thought to be an inability of the environment to provide the required nutrition for all fetuses. Multiple gestations are also at a higher risk for other complications such as twin-twin transfusion syndrome, pre-eclampsia and congenital anomalies.
The interplay between these risks and the stress on nutritional supplies may also play a role in causing growth restriction. Infection during pregnancy, especially at the beginning of the pregnancy, can have a major impact on subsequent growth. However, infection is only responsible for a small proportion of all cases of IUGR. The TORCH infections (TOxoplasmosis, Rubella, Cytomegalovirus and Herpes simplex) are potential causes of IUGR. Viral infections are implicated in IUGR more commonly than other pathogens; cytomegalovirus (CMV) is most frequent of these in the developed world (33). Growth restriction due to CMV infection is secondary to impaired placental development and function (34).

1.1.2 Maternal Factors

Maternal factors causing growth restriction can be broadly separated into those secondary to environmental factors and those due to maternal medical conditions, which affect uteroplacental blood supply. Environmental factors include substance abuse, nutritional status, hypoxaemia, toxins and anatomical issues. Smoking in pregnancy is the single greatest modifiable cause of intrauterine growth restriction (35). As well as causing impaired growth, smoking is associated with a myriad of other in utero complications such as premature birth, placental abruption and stillbirth. Growth restriction secondary to smoking occurs through a number of ways and has an impact through different phases of the pregnancy. Smoking causes vascular damage to the placenta leading to placental insufficiency, which compromises nutritional supply and has its greatest impact in the third trimester when nutritional needs are greatest (36). A reduction in the dimensions of the fetal capillaries present in villi has been shown in the placentas of smokers. This affects blood flow and leads to a reduced area for gaseous exchange and an increased risk for resultant fetal malnutrition (37). Exposure in the first trimester, during periods of significant growth of head and bone growth, affects the biparietal diameter (BPD) and femur length (FL). A direct toxic effect of nicotine, as opposed to a nutrient-restrictive mechanism, has been suggested as a cause for the adverse neurological consequences associated with maternal smoking (38, 39).
Smoking has also been shown to have a genotoxic effect, with a significant increase in chromosomal instability demonstrated in the amniocytes of smokers (40). Environmental tobacco smoke (ETS) exposure, or second hand smoke exposure, is also associated with an increase risk for intrauterine growth restriction (41). Other illicit substances such as heroin and cocaine can cause IUGR. Use of heroin or opiates in pregnancy is associated with IUGR, although this is likely to be multifactorial in nature. Polypharmacy (the concurrent use of several medications) and poor nutrition, combined with episodes of overdose and withdrawal, cause periods of hypoxia affecting fetal growth (42). The use of opiate substitution therapy such as methadone has also been shown to be associated with growth restriction (43). Cocaine can easily cross the placenta and the fetal blood brain barrier. Placental damage is thought to be as a result of the vasoconstriction that is induced by the drug (44). A meta-analysis of studies looking at antenatal cocaine use and postnatal outcomes found that cocaine adversely affects fetal growth (45). Whilst the mechanisms through which all these substances cause poor fetal growth may not be entirely clear it is difficult to establish the magnitude of the direct effect of the drug given the complex environmental and psychosocial factors that are often associated with drug use (46).

Maternal weight and nutritional status have an impact on intrauterine growth restriction. Low pre-pregnancy weight, poor weight gain during pregnancy, obesity and overnutrition impact on fetal growth. Observational data from populations enduring famines or periods of starvation demonstrate significant reductions in average birth weight during the episodes, with return to normal levels on restoration of food supplies (47). Poor nutrition can be as a result of pregnancy complications such as hyperemesis gravidarum or reduced food intake, whether due to reduced availability or conscious effort (48). The fetus is most vulnerable to maternal nutritional status at the earliest stages around implantation and rapid placental development (49). Maternal malnutrition translates to smaller placenta, which affects the adequate transfer of nutrients to the developing fetus resulting in poor growth.
Correction for micronutrient deficiencies has also been shown to improve fetal growth (50). Overweight mothers are more likely to have infants that are macrosomic, the associated complications increasing with the degree of maternal obesity. Pregnancy induced hypertension (PIH) is related to obesity and occurs to a higher degree in those with a body mass index (BMI) \( \geq 30\text{kg/m}^2 \) (51). Maternal hypertensive states, whether chronic or PIH, are well recognised as causing IUGR, though likely through different mechanisms (52-54). As obesity rates are increasing across the developed world the association with IUGR is becoming more evident (55).

Hypoxaemia can also cause IUGR, whether it is secondary to living at altitude or maternal conditions resulting in relative states of hypoxaemia (56). Studies of populations living at altitude have shown that birth weights of infants at altitude can be up to 10% lighter than those born at sea level (57). The degree of growth restriction appears to be directly proportional to increasing altitude above 2000 metres above sea level (58). Infants with IUGR born at altitude have higher risk for neonatal mortality than appropriate for gestational age infants, however it is unclear if it is to the same degree as in infants born at lower altitudes/sea level. A chronic state of hypoxaemia affects maternal circulation and its ability to adjust to the pregnant state. Circulating blood volume may be lower and the rise in cardiac output not as great as compared to sea level. Growth and remodeling of uteroplacental vessels and the uterine artery is curtailed at high altitude compared to low with consequent lowering of uterine artery blood flow at term (59). Evolutionary adaptation is seen in populations living at high altitudes for several generations, such as in Tibet and the Andes, having reduced rates of IUGR and associated complications compared to more recent migrants (60).

A range of medications, produce and therapies has been shown to have a toxic effect on the developing fetus. The toxic effect can be mediated either through a direct effect on the fetus having crossed the placenta, or through impairing placental function. Medications used in the treatment of cancer, epilepsy and hypertension have been implicated in causing IUGR.
Antifolate medications include a spectrum of medicines such as methotrexate (used in the treatment of neoplastic disorders and rheumatoid arthritis), proguanil (malaria treatment) and trimethoprim (a broad spectrum antibiotic). Folic acid is necessary in the synthesis, repair and methylation of DNA. As such is hugely important in supporting rapidly dividing cells and growth as is found in a developing fetus, especially during the first trimester. The mechanism of action is predominantly as a dihydrofolate reductase (DHFR) inhibitor, which is cytotoxic through affecting DNA synthesis. It is through this action that these types of drugs have led to success in treating many malignancies and precisely why they are strongly contraindicated in pregnancy. As well as having a direct effect on the fetus leading to growth restriction it has also been shown that there can be a placentally mediated effect (61). Antiepileptic drugs (AEDs) are well known to have teratogenic potential causing major and minor malformations (62). Selecting the most appropriate AED for use in pregnancy is difficult. However, it is known that the use of multiple AEDs in pregnancy increases the risk significantly. This has informed the treatment plan for epilepsy patients in pregnancy, where they are changed to single agent therapy if possible. Intrauterine growth restriction can result secondary to AED use, but is not their commonest side effect. This growth restriction may also be mediated through their folic acid antagonist properties. Caffeine consumption has previously been thought to cause intrauterine growth restriction, however many of the original studies were poorly designed and did not control for confounding factors such as cigarette smoking (63). The more recent studies in the area suggest that this is not the case and that a caffeine intake of < 300mg/day (< 4 cups of coffee) is unlikely to have a significant effect on fetal growth (64).

Medical therapies that can cause IUGR include those using therapeutic doses of radiation as well as assisted reproduction. Radiation treatment pre pregnancy in the pelvic region can have a lasting effect on the local vascular anatomy, which may lead to growth restriction through effects on future fetoplacental perfusion. Radiation doses of less than 0.05Gy (5 rads) do not pose a risk to the developing fetus and diagnostic imaging procedures typically employ less than this (65).
Although exposure to ionizing radiation during pregnancy ought to be appropriately minimized the risk of a missed diagnosis is often a greater hazard (66). Assisted reproductive techniques are associated with an increased risk of low and very low birth weight infants, and this is more apparent in singleton pregnancies conceived with assisted methods than singleton pregnancies conceived spontaneously compared to multiples. The reason as to why this may be the case is unclear. It is postulated, and supported by observational studies, that subfertility itself is association with poor fetal growth. Women with untreated subfertility issues who conceive spontaneously have similar pregnancy outcomes to those who have assisted reproduction (67).

Intrauterine growth restriction is associated with other maternal environmental factors. Congenital anomalies of the uterus result in growth restriction through effects on placental perfusion and through restrictive biomechanics (68). Extremes of maternal age play a role in IUGR. Older women at the end of their reproductive life are at increased risk for IUGR, which may reflect the advanced age of the biologic material and the additive effects of chronic medical conditions such as hypertension. At the opposite extreme of the spectrum, IUGR is associated with pregnancies in very young mothers. Whereas the advanced age of maternal tissues is thought to lead to poorer function causing IUGR in older women, the cause suggested in younger women is competition for growth factors between the mother, who is still growing, and her developing fetus. A short inter-pregnancy interval is also related to IUGR through a maternal depletion hypothesis, where folate levels have not recovered to normal levels prior to conceiving again (69). Another factor that is associated with IUGR is maternal stress. Chronic stress is associated with elevated levels of corticotropin-related hormone (CRH), which plays a regulatory role in the hypothalamic-pituitary adrenal (HPA) axis and the physiologic response to stress (70). During pregnancy, the placenta is another source of CRH. Over the course of the pregnancy there is an exponential increase in placental CRH levels, which plays a role in parturition. When levels of placental CRH are in excess of normal there is an increased risk for fetal growth restriction and preterm birth (71). Elevated levels of placental CRH are associated with reduction in uteroplacental perfusion (72).
Maternal antiphospholipid syndrome may impact on fetal growth. Antiphospholipid syndrome (APS) is an autoimmune thrombophilic condition. It is characterised by vascular thrombosis and obstetric complications in the presence of antibodies in the blood that attack phospholipid-binding proteins. The antiphospholipid antibodies promote endothelial cell activation as well as monocytes and platelets. This results in an overproduction of tissue factor and thromboxane A2, which is prothrombotic. This, coupled with the hypercoagulable state that is pregnancy, increases the risk for thrombosis. The major obstetric complication of APS is early pregnancy loss; however, it can also result in intrauterine growth restriction, presumably through thrombosis in placental vessels leading to placental insufficiency (73). Women treated with low molecular weight heparin and aspirin over the course of the pregnancy have reduced rates of early pregnancy loss, however, they remain at a higher risk of the pregnancy being affected by intrauterine growth restriction compared to the general population (74, 75).

1.1.3 Placental Factors

Intrauterine growth restriction may also be caused by dysfunction of the placenta or primary placental disease. The healthy status of this organ at the interface between fetus and mother is critical for normal growth and development of the fetus. Animal studies have demonstrated that placental size overestimates functional capacity and that there is a significant potential for redundancy of placental mass (76). However, this does not translate to human placentas to the same degree (77). In humans, abnormalities in the placental vessels and separation of the placenta from the maternal plane have significant impact on function, without necessarily affecting placental size. One of the commonest causes of IUGR is ischaemic placental disease. This is a syndrome that involves preeclampsia, IUGR and placental abruption. Uteroplacental ischaemia and placental insufficiency that begin as early as the point of placental implantation may result in these complications. Inadequate placentation and premature placental separation, though different processes, have many overlapping features that ultimately result in ischaemic placental disease (78).
There are many histological lesions of the placenta that have been identified as related to IUGR. The commonest finding in otherwise idiopathic cases of IUGR is diffuse chronic villitis of unknown aetiology, though changes associated with abnormalities of uteroplacental vasculature, chronic abruption, chronic infectious or inflammatory lesion, infarction, distal villous hypoplasia and vascular thrombosis have all been recognised (79-81).

A discrepancy in the chromosomal makeup between the fetus and the placenta is often seen in cases of IUGR. Confined placental mosaicism (CPM) is where there is a chromosomal mosaicism found in the placenta but not in the fetus (82). Up to 10% of IUGR cases without an obvious cause will have CPM, which is significantly higher than the 1% rate reported in controls undergoing placental examination or chorionic villus sampling (CVS) (83). The chromosomal abnormality identified is often a trisomy, and the influence that CPM has on fetal growth is chromosome specific. The common autosomal trisomies of 21, 18 and 13 are less often detected in CPM than in fetal tissue. The chromosomes most frequently identified in CPM are chromosomes 2, 3, 7, 8 and 16 (84). The placentas affected with CPM tend not to function as well as those without, and have higher rates of placental infarction, increasing the risk of growth restriction and early pregnancy loss. Postnatal catch up in growth is seen in cases of CPM, and developmental outcome appears normal (85).

1.2 Pathophysiology

Maternal, fetal and primary placental diseases along with external factors can disturb transport of nutrients to the fetus and disposal of waste resulting in intrauterine growth restriction. Maternal disorders causing IUGR are often antenatally diagnosed or readily identified through a detailed history once the suspicion of a growth-restricted pregnancy has been raised. How these impinge on fetal growth is often through effects on placental development or function. Fetal disorders may have growth restriction as an isolated primary presentation or IUGR may be one of several features prompting a diagnosis or investigation.
The vast majority of all cases of IUGR in singleton pregnancies are either fetal in origin or secondary to abnormal placental vascular development on the fetal and or maternal side (86-89). As management and early detection can influence outcome in cases caused by placental vascular dysfunction, this is the cohort studied in the work that follows. Understanding placental insufficiency, and how it results in fetal growth restriction, requires an understanding of normal placental development and how interruptions or aberrations to this process could compromise growth.

1.2.1 Normal Placental Development

During each trimester, there are significant placental developmental markers that must be achieved in order to allow for normal functioning. During the first trimester, the cytotrophoblast cells migrate through the syncytiotrophoblast and attach the placental villi directly to the decidua, thus anchoring the villi and establishing placental adherence (Fig.1). The maternal circulation and the intervillous space become linked through angiogenesis. This allows for sufficient support of the growing trophoblast and embryo (90, 91). Once a secure blood supply has been established, the synthetic function of the placental becomes apparent with secretory substances such as human chorionic gonadotrophin and placental lactogen present in maternal circulation. At a local level, paracrine functioning begins and nutrient transport systems differentiate. Villous trophoblasts form providing the interface for nutrient exchange, and once fetal cardiac activity begins, active transference of substances between fetus and the placenta occurs (92).
Providing these processes occur correctly, the placenta maintains vascular autoregulation maximizing efficiency of nutrient transfer between maternal and fetal systems, delivering nutrition to the fetus via the umbilical vein. Over the course of the first trimester this process becomes increasingly more efficient, functioning to maintain enough nutrition to the trophoblast, which is highly active, and delivering the surplus to the fetus (93-95). The transport activities consume the majority of glucose and almost half of the oxygen coming from the maternal circulation, with the remainder used for fetal growth and development (96). Glucose consumption changes in the setting of placental insufficiency. In periods of low glucose supply the placenta will extract up to 80% of available glucose, with minimal uptake by the fetus, with levels of fetal glucose delivery returning to normal during normoglycaemia (97). The main factor determining successful transfer of nutrients from mother to fetus is the placental transport capacity. This is dependent on the expanding surface area of the fetal villous trophoblast and the increased concentration and affinity of transport proteins for glucose, amino acids and fatty acids.
In the second trimester there is a significant increase in the size of the placenta, which is as a result of the increase in synthetic function, transport activity and vascular mass. At the beginning of this trimester the trophoblast invades the maternal spiral arteries (98). There is progressive thinning of the villous trophoblast and a rapid increase in villous surface area occurs up to 26 weeks’ gestation. Fetal cardiac output rises, which significantly increases the villous blood flow, thus increasing the capacity for acquisition of nutrients by the fetus. By the end of the second trimester the vascular compartments on both side of the placenta have low resistance and high capacity systems (99). In the final trimester, the focus is on continued organ development and accumulation of essential body stores to equip the fetus for extra uterine life by ensuring steady amounts of essential nutrients. Uterine perfusion increases from 50ml/min in the first trimester to up to 1300ml/min by the end of the third trimester (100). Fat stores are significantly bolstered to ~20% of fetal body weight to ensure sufficient supplies of fatty acids for myelination and retinal function (101).

1.2.2 Placental Insufficiency

Conditions that result in abnormalities of placental vascular development account for the majority of cases of IUGR. Managing placental insufficiency through maximising transfer of nutrients to the fetus has been given particular attention as a potential therapeutic target for improving IUGR (102). If angiogenesis is disturbed in the first trimester placentation may not be successful, leading to miscarriage. If there is adequate nutritional supply and vascular support to allow for placental adherence, differentiation may proceed. However, maternal adaptation to the pregnancy may also be affected by impaired placental synthetic function and impaired nutrient supply to the fetus. Invasion of the trophoblast can be deficient and confined to the decidual portion of the myometrium. If this occurs the maternal spiral arteries fail to transform in to a low resistance system affecting the autoregulatory function of the placenta. Infarcts in the maternal placental floor may occur along with fetal villous obliteration and fibrosis, which leads to an increase in resistance of placental blood flow.
Such changes in resistance between the two vascular compartments results in a decrease in the effective area for exchange of nutrients and waste materials (103-105). If fetoplacental flow resistance increases throughout the vascular bed as a result of further vascular impairment the placental mass will ultimately reduce in size. Provided compensatory mechanisms sustain fetal development intrauterine growth restriction ensues. The restriction on nutrient transfer to the fetus may only become apparent during the period of rapid fetal growth in the second and third trimesters (88).

Recognised amino acid transport systems (including System A, System L) and molecular regulatory systems such as the mammalian target of rapamycin (mTOR) signaling pathway are altered in placental insufficiency. The mTOR pathway has been shown to act in a nutrient sensor capacity, ‘sensing’ the availability of substrates and acting accordingly (106). Placental insufficiency leads to a reduction in nutrient availability and induces down-regulation in these pathways. These changes augment the nutrient supply in such a way as to protect growth and matching it with availability of substrates (95).

Endocrine function is also impaired in IUGR pregnancies, partly affected by the reduction in the proportion of metabolically active placental mass. Fetal weight at mid-gestation and at term correlates with levels of human placental lactogen (hPL) found in both fetal and maternal systems, as well as with maternal growth hormone levels. In IUGR pregnancies these levels are significantly reduced (107). Glucose and amino acids stimulate the fetal pancreas to release insulin-like growth factor I and II, which are potent stimulators for fetal growth. Inadequate transfer of nutrient substrates affects this endocrine signaling impacting on fetal growth (108). Leptin, which is produced in the placenta and fetal adipose tissue, plays an important role in amino acid and fatty acid transport and stimulates pancreatic growth. Placental produced leptin may also play an important role in placental growth. Fetal and placental leptin levels are reduced in IUGR pregnancies at delivery (109). In order to survive in a suboptimal nutritional environment, the fetus must adapt, and this response sets in motion a chain of events causing growth restriction.
Initially the reduction in nutrient supply prompts a state of catabolism, which if sustained (as in placental insufficiency) causes the fetal basal metabolic rate to decrease, production of trophic hormones, such as insulin-like growth factor 1 (IGF 1), to reduce and decreases tissue sensitivity to these hormones (110). In response to nutrient insufficiency and hypoxia, the brain, heart and adrenal glands are prioritised. This results in adrenal gland hyperplasia and consequent increased glucocorticoid activity, which prompted the initial studies as to whether the resultant increased adrenocorticotrophic hormone (ACTH) accelerated lung maturity. This was postulated to be an adaptive response, detecting a hostile environment and preparing the fetus for survival as earlier delivery is anticipated. The evidence is inconclusive and has raised questions regarding the effects of antenatal glucocorticoids in the setting of IUGR (111, 112). Other organs show evidence of atrophy; the fetal thymus is significantly smaller in IUGR infants (102, 113).

Skeletal muscle development and growth slows; muscle mass is reduced and ultimately total body weight and ponderal index decrease (114). Mesenteric artery blood flow is affected resulting in poorer perfusion to the gastrointestinal tract, which impacts on establishing enteral feeding in postnatal life, gut motility and necrotising enterocolitis (NEC) (115-117). The fetal heart undergoes remodeling in the setting of IUGR / placental insufficiency with changes in both structure and function evident at birth that have significant potential life-long implications (118-120). The response of the fetal brain to placental insufficiency has been well documented, as has the associated ‘brain sparing’ phenomenon (121, 122). Brain sparing describes the situation where blood flow is preferentially redirected to the cerebral circulation at the expense of other organ systems in the setting of placental insufficiency and or hypoxia.

The result of this is to preserve brain growth, and so the infant is born with an increase in head diameter to body weight ratio (123). This effect is induced in both fetal malnutrition and states of hypoxia; however, these often go hand in hand as placental insufficiency leads to a reduction in placental mass and consequent compromise of the umbilical circulation.
In response to hypoxia, neural reflexes are triggered by the carotid body causing vasoconstriction in the peripheral circulation with a concomitant drop in pressure across the cerebral vascular bed and redistribution of blood flow. This is supported by endocrine and local factors, as vasoconstrictive hormones such as vasopressin and angiotensin II levels are found at higher plasma concentrations. In addition, cortisol and ACTH may play a role in sustaining the induced changes (124). Moreover, the carotid body appears to be sensitive to hypoglycaemia, which provides another stimulus for redirection of blood flow to the cerebral circulation in the IUGR fetus (125).

These adaptive mechanisms are not fully functional in earlier gestations and as such the timing of the placental insufficiency insult dictates to what degree the fetus can respond. The ‘brain sparing’ effect is not the panacea for the neurodevelopmental consequences of placental insufficiency. The term suggests that the brain has been protected, however it has been reported that infants with evidence of cerebral redistribution / fetal brain sparing have higher rates of behavioural problems (18, 126). Infants with IUGR may tolerate short periods of hypoxia, however, chronic placental insufficiency and the associated suboptimal nutrition and hypoxia has long-term consequences on the developing brain. If the compensatory mechanisms employed by the fetus to adapt to the hostile environment are inadequate fetal distress ensues. If this is not prevented or acted upon fetal demise results, this has prompted many studies on the best method of antenatal monitoring for IUGR pregnancies.

1.3 In-utero monitoring of Intra-Uterine Growth Restriction

Management of pregnancies affected by intra uterine growth restriction is complex. The processes in place that have caused poor growth are difficult to reverse and improving fetal growth is difficult. Obstetricians are therefore left with empiric approaches such as lifestyle alterations (smoking cessation), bed rest, and optimising the time of delivery. Timing of delivery is critical in improving the outcome of survivors and minimising the risk of fetal death. An abdominal circumference (AC) or estimated fetal weight (EFW) below the 10th centile is usually the first indicator of suboptimal fetal growth.
However, distinguishing between a normal and a pathological growth pattern in this heterogeneous group can be challenging (127). Arbitrarily using the 10th centile cut off to determine IUGR will end up including a majority of fetuses that are growing normally (128). Lower centile cut offs and assessing fetal growth trajectories may better predict adverse outcomes (10, 11). Antenatal detection of IUGR pregnancies is hugely important as perinatal outcomes where it has not been recognised are worse and there is a 5-fold increase in stillbirth rate in pregnancies where IUGR has not been identified (129). Unfortunately, the antenatal detection rate is often quite poor with some studies reporting a detection rate of one third of cases (130, 131). The routine physical assessments of fundal height (FH) measurement and abdominal palpation have poor sensitivities and specificities but are widely used as serial ultrasound to determine EFW is not readily available or feasible. Abdominal palpation and FH measurement is best used as a screening tool to assess growth as opposed to a surrogate for fetal weight measurement and has been shown to improve detection rates, but use alone would miss the majority of cases (132). Sonographic strategies have significantly improved surveillance and outcomes by facilitating timely delivery. These include biometry, cardiotocography (CTG), biophysical profile (BPP) and Doppler surveillance, though the use of one tool in isolation is not advocated.

Biometry is the science and technology of measuring and statistically analyzing biological data. In pregnancy, this refers to the measurement of various parameters with ultrasound to calculate fetal weight. The most common formula used is the Hadlock formula, which is the most widely accepted method. Fetal weight is calculated using a composite measurement of abdominal circumference, fetal head and femur length (133). The accuracy of the estimate is dependent on accurate dating of the pregnancy as well as factors such as equipment, training and expertise of the sonographer. The estimate can be compared to growth charts to determine fetal growth. Gardosi et al proposed customising growth charts that take in to account maternal characteristics including weight, ethnicity, height and parity (12).
These have been shown to be more appropriate at identifying growth failure and the Perinatal Ireland consortium showed that in the PORTO study of IUGR pregnancies that using customised growth charts significantly improved detection rates as compared to standard centiles (134). During the PORTO study a range of obstetric and maternal parameters were collected to develop customised growth charts. Identifying a pathologic growth trajectory in this cohort using these charts is highly predictive of placental insufficiency and poor obstetric outcome as well as predictive of NICU admission (10). The advent of a nationwide electronic maternity and neonatal medical record provides an opportunity to develop customised postnatal growth charts, which can be compared to populational means. Interval scanning fortnightly permits tracking of growth and incorporation of Doppler measurement improves surveillance. Other elements during the ultrasound may be assessed such as amniotic fluid volume, placental morphology and fetal anatomy.

Cardiotocography (CTG) measures fetal heart rate and uterine contractions and provides real time information regarding fetal health. It is widely used and one of the main methods of assessing fetal health. Despite being highly sensitive, it has significant false positive rates when predicting poor outcome, and used in isolation has not demonstrated a reduction in perinatal mortality (135). It is useful in detecting acute hypoxia through fetal distress affecting heart rate, however it is not helpful in cases of chronic hypoxia. Combining CTG with other parameters forms the biophysical profile (BPP). This is a non-invasive measure of fetal well-being composed of 5 elements; amniotic fluid measurement, fetal breathing movements, fetal tone, fetal body movements and CTG (136). A normal score for each parameter is 2 giving a maximum total of 10; however, if the first 4 are normal CTG is often omitted giving a score of 8/8. An abnormal score is predictive of fetal acidaemia and delivery is indicated with scores of ≤ 4/10 (137). Caution is required in assessing at premature gestations and despite being quick to do, if a parameter is absent or abnormal it requires continuous monitoring for 30 minutes to ensure that is the case. As with each method of in utero assessment the ability to detect the fetus at risk of poor perinatal outcome is enhanced when combined with Doppler monitoring.
Doppler sonography in obstetric practice refers to measuring the velocity of blood flow in the umbilical vessels and the fetus. It utilises the Doppler effect, which is the change in frequency of a wave (or other periodic event) for an observer moving relative to its source. In this setting an ultrasound beam encountering circulating blood in a vessel is scattered by millions of red cells, which causes the incident beam to undergo a frequency shift proportional to the speed of red cell movement, which is blood flow velocity (138).

Doppler measurement in obstetric practice was first assessed in the late 1970’s, but it wasn’t until the late 90’s with improvements in technology and technique that it began to have a role in regular practice (139). It has now become widely accepted as the primary investigative tool in assessing IUGR. Doppler interrogation of the umbilical artery is the first line measurement recommended and has been found to be the most useful (127, 140, 141). Other vessels have been studied and may have prognostic value though consensus on which combination has not yet been achieved. Studies assessing ductus venosus, middle cerebral artery, uterine artery and other parameters have been performed. Ductus venosus (DV) measurements were used in the TRUFFLE trial to dictate delivery, after initial enrolment based on abnormal umbilical artery measurements (128). The short-term results reported are favourable, with better outcomes than anticipated compared to previous studies. Despite some authors suggesting DV Doppler may be a better predictor of fetal compromise, these changes are often only seen in a minority of fetuses with IUGR and usually closely followed by CTG changes mandating delivery, which questions its suitability (142, 143).

Controversy also exists as to the pattern of changes seen in Doppler measurements of fetal circulation in terms of the sequence in which various vessels display changes, which has implications for the use of multivessel surveillance in the IUGR setting (144). Doppler measurement of flow in the umbilical artery assesses the resistance to perfusion of the fetoplacental circuit and waveforms can be detected from the beginning of the second trimester onwards. Continuous forward flow throughout systole and diastole results from a relatively low impedance in the umbilical artery (145). A saw tooth pattern is observed with flow in the forward direction (Fig. 2).
As the placenta matures and the number of tertiary stem villi increases over the course of the pregnancy the placenta becomes less resistant to blood flow and the amount of blood flowing in the umbilical artery during diastole increases. Consequently, commonly measured indices such as the resistance index (RI) and pulsatility index (PI) decrease. The resistance index is a measure of pulsatile blood flow that reflects the resistance to blood flow caused by the microvascular bed distal to the site of measurement. The pulsatility index is a measure of the variability of blood velocity in a vessel. A normal RI is < 0.55. The calculations are as follows:

\[
RI = \frac{\text{Peak systolic velocity} - \text{end diastolic velocity}}{\text{peak systolic velocity}}
\]

\[
PI = \frac{\text{Peak systolic velocity} - \text{end diastolic velocity}}{\text{time averaged velocity}}
\]

In placental insufficiency, there is obliteration of small muscular arteries in the placental tertiary villi, increasing resistance to flow. As this continues, a reduction in end-diastolic flow ensues with consequent changes in the umbilical arterial waveform pattern. Initially the RI and PI increase and end-diastolic flow is positive, then there is absent end-diastolic flow (AEDF) and, if it progresses further, reversed end-diastolic flow (REDF) occurs (146). In cases where REDF is present a significant majority of arteries in the placental tertiary villi have been obliterated, and this represents an advanced degree of placental compromise (147, 148). To ensure that the measurements acquired are reliable, the sonographer must be aware of the effect of both physiological and technical factors. The gestational age, fetal heart rate and breathing movements of the fetus affect Doppler measurements. There is a normal progressive decline in impedance to flow within the fetoplacental circuit with increasing gestational age. Whilst fetal heart rate can affect results these are not significant when fetal heart rate is within normal limits. Fetal breathing results in significant changes to intrathoracic pressure and central haemodynamics, which affects variability of the Doppler waveform, so the measurement is usually acquired during fetal apnoea (149). The location along the cord at which the measurements are acquired also impacts on the results. Indices are higher if sampled at the fetal end as compared to the placental end of the cord (150). Another technical issue relating to measurement is the angle of insonation, which is the angle between the ultrasound beam and the direction of flow.
This affects the size of the waveform produced. The higher the angle between the ultrasound beam and the axis of the vessel the smaller the waveform produced, however, the indices are largely angle independent. Regardless, an angle closest to zero is recommended.
Figure 2. Examples of umbilical artery Doppler flow waveforms
A. Normal umbilical artery Doppler flow waveform.
B. Absent umbilical artery Doppler flow waveform.
C. Reversed umbilical artery Doppler flow waveform.

Several studies have demonstrated the correlation between IUGR, deteriorating Doppler parameters, and poor perinatal outcome and mortality (127, 151-155). A deterioration in Doppler indices correlates with increasing pathology in the fetoplacental circuit. When IUGR is caused by placental insufficiency or preeclampsia the fetus is exposed to an adverse nutritive state and hypoxia. Either as a consequence of worsening environment or secondary to duration of exposure to adverse conditions, adaptive survival mechanisms occur – preferential fetal growth at expense of placental growth, change in movement patterns, slowing of fetal growth rate culminating in acidosis and a state of chronic hypoxia. Haemodynamic changes are seen with blood flow redistribution to vital organs such as brain, heart, adrenals and placenta at the expense of other organs. These circulatory changes associated with fetal compromise can be detected by Doppler ultrasound and are detectable prior to other signs of compromise such as loss of fetal heart variability and reactivity and subsequent reduction in fetal breathing and body movements (156, 157). Absent end diastolic flow may improve over time, though this is rarely sustained. A transient improvement in Doppler measurements has been reported post maternal steroid administration in both singleton and multiple gestation pregnancies, though not always identified (433-435). Interestingly the response in severely growth restricted preterm fetuses with abnormal Doppler measurements has demonstrated a divergence in response, with a proportion not showing any improvement in Doppler flow measurements at all (436). The mechanism behind the improvement is postulated to be secondary to a decrease in placental vascular resistance. The transient improvement noted has a mean effect.

The progression to further signs of fetal compromise may be up to several weeks; therefore, AEDF is not an immediate indication to deliver. Reversed end diastolic flow represents a significant degree of compromise, which reflects the limits of the ability of the fetus to survive in utero and if intervention is not prompt death will result. Changes of AEDF and REDF precede signs of fetal distress and can afford clinical teams time to plan delivery, considering gestation, biophysical profile, CTG and logistical factors.
Timing of delivery involves consideration of the above factors in the context of balancing the risk of in utero demise due to delayed delivery with iatrogenic prematurity and the significant morbidity and potential for neonatal death associated with early intervention. Guidelines for delivery in such instances cannot be entirely protocol driven, based on isolated factors such as gestation or Doppler status and as such an individualised approach is employed. Such decisions are harder at earlier gestations, as the main predictors of a successful neonatal outcome are gestational age at delivery and weight at birth. Avoiding in utero demise, improving the chances for postnatal survival, minimising morbidity and allowing time for maturity, frame the decision to deliver with gestational age determining the relative importance of each. Chances for an intact survival are highest when > 29 weeks gestation and birth weight > 800g (158). The guidelines developed to assist in timing of delivery take in to account the various factors reviewed and separate cases of IUGR in to uncomplicated or isolated IUGR and complicated IUGR.

In uncomplicated or isolated cases of IUGR where EFW is < 10th centile and other parameters such as Dopplers are normal delivery is generally delayed until the pregnancy has reached 37 weeks. The DIGITAT study compared induction of labour with expectant management in IUGR pregnancies at term. There was no significant difference in terms of neonatal morbidity or caesarean section rate, however more infants in the expectant group had a birth weight < 3rd centile (159, 160). Given the increase rate of stillbirth in IUGR pregnancies > 37 weeks and an even greater risk when less than the 3rd centile, delaying delivery significantly beyond this is not recommended (161, 162). Complicated IUGR refers to EFW < 10th centile and abnormal Doppler indices. Identification of abnormal Doppler flow is associated with a poorer perinatal outcome regardless of gestational age, and once identified antenatal corticosteroids should be administered when appropriate. Timing of delivery of IUGR pregnancies complicated by abnormal Doppler was reviewed in the GRIT study to assess if delaying delivery versus immediate delivery could afford a survival benefit. Though a trend towards more disability in the immediate group was observed, the results and long-term follow up found no significant difference in primary outcome or long-term outcomes between the groups, suggesting that these infants are being delivered at an appropriate time frame (163, 164).
The current recommendations regarding delivery of complicated IUGR cases suggest increasing surveillance to weekly Doppler examination in cases where the umbilical artery RI is >95th centile with normal flow. More frequent assessments may be indicated depending on the clinical concern and growth trajectory. Such surveillance continues until 37 weeks when delivery should be considered.

These infants should tolerate induction of labour and vaginal delivery. In cases with abnormal Doppler studies earlier delivery is indicated. When AEDF is identified daily CTG should be performed, usually as an inpatient or outpatient if facilities allow and delivery should be no later than 34 weeks. With REDF, admission and close observation instituted with delivery no later than 30 weeks. Consultation between neonatal departments and fetal medicine specialists, ensuring antenatal corticosteroids and magnesium sulphate therapy for neuroprotection complete the guidance. These cases are usually delivered by caesarean section, as these infants are not felt to tolerate induction and vaginal delivery (165).

1.4 Consequences of Intra-Uterine Growth Restriction

An infant born after intra-uterine growth restriction faces a significantly greater number of challenges than an appropriate for gestational age weight (AGA) infant. The nature of these challenges differs depending on gestation and there can be both short-term issues and long-term sequelae. The physical appearance may suggest IUGR in absence of antenatal diagnosis, as there can be typical features. They may have a wizened or older looking face, appear smaller than would be expected for gestation and may have an obviously disproportionately larger head than would expect for their weight. A decrease in muscle mass and redundant skin folds may be seen over buttocks and thighs and reduced amounts of subcutaneous fat. Female genitalia may appear less mature and males with early onset of IUGR secondary to placental insufficiency have higher rates of hypospadias (166, 167). Term babies may show signs of meconium staining and an impression of the skin being more mature, with less vernix and peeling of the hands and feet.
Vernix production may be reduced as a consequence of decreased oestriol synthesis or reduced skin perfusion antenatally. As a result, there is an increased exposure to amniotic fluid, which results in increased desquamation and increased degree of wrinkling (4).

An IUGR infant born at term has a higher likelihood of impaired transitioning from intrauterine to extrauterine life with an increased incidence of persistent pulmonary hypertension of the newborn (168). This is believed to be secondary to chronic fetal hypoxia and oligohydramnios, where hypoxia induces increased synthesis of vasoconstrictors and smooth muscle mitogens such as platelet derived growth factor-β, endothelin-1 and vascular endothelial growth factor (VEGF). Endothelial nitric oxide synthase is also inhibited. In the setting of placental insufficiency infants suffering from chronic hypoxia may poorly tolerate labour, suffer fetal distress, perinatal asphyxia and have higher rates of meconium aspiration (21, 169, 170). The proportion of premature infants affected by IUGR is high and may be higher in developed countries due to improved surveillance and earlier intervention. Premature IUGR infants have increased rates of mortality and morbidity as compared to AGA counterparts (171). Increased rates of death, respiratory distress syndrome (RDS) and necrotizing enterocolitis (NEC) are seen; the use of antenatal steroids reduces the risk for death, severe intraventricular haemorrhage (IVH) and RDS, however, they remain at significantly greater risk as compared to the AGA premature infants (172).

Premature IUGR infants also have increased rates of pulmonary haemorrhage, postnatal corticosteroid use and poorer neurodevelopmental outcome (173, 174). In the post delivery period IUGR infants have difficulty in regulation of temperature control, blood glucose control and blood tests reveal the haematological consequences of chronic fetal hypoxia. They have higher rates of feed intolerance and necrotizing enterocolitis, which has prompted a number of studies regarding how best to introduce feeds to this patient population.

Thermoregulation in the initial period is impaired due to reduced subcutaneous fat stores leading to increased heat loss and impaired thermogenesis, as there is reduced nutrient substrate available and reduced available catecholamine’s, which are required by brown fat for thermogenesis (175).
The hypoglycaemia observed in IUGR infants is early in onset and results from low insulin concentrations in utero, which leads to a decrease in glycogen synthesis and subsequently low available glycogen stores after delivery. The risk of becoming hypoglycaemic increases with increasing degree of growth restriction and IUGR infants are more likely to become symptomatic with hypoglycaemia as compared to AGA infants (176-178).

A number of haematological features are present at birth. Polycythaemia and hyperviscosity result from increased erythropoietin in the setting of fetal hypoxia and the risk increases in relation to the degree of growth restriction (179). Thrombocytopaenia is often noted and is secondary to reduced megakaryopoiesis as a result of fetal hypoxia, the thrombocytopenia in IUGR rarely requires platelet support (180). Immune function may also be impaired with reduced T and B peripheral lymphocytes at birth and neutropaenia is also seen frequently, which may explain the increased rate of nosocomial infections seen in growth-restricted infants (181, 182). IUGR infants have increased rates of feed intolerance and necrotizing enterocolitis (171, 183). The exact mechanism by which NEC occurs is unclear, but a combination of suboptimal gut perfusion in the setting of placental insufficiency and redistribution of blood flow and impaired immune function likely play a role. The practice of delayed initiation of feeds to reduce the burden on an immature gut does not reduce the incidence of NEC, but delays achievement of full enteral feeding and increases the risk of cholestasis (116). IUGR infants, regardless of gestation at birth, have higher mortality rates than AGA infants born at similar gestations. The risk for death increases with the degree of growth restriction and those with a birth weight < 6\textsuperscript{th} centile have the greatest risk (184, 185).

On graduation from the neonatal intensive care unit IUGR infants face a number of challenges. Impaired growth, neurodevelopmental abnormalities and cognitive impairments and the consequences of ‘fetal programming’ – where the hostile in utero environment and the fetal response result in the development of a range of conditions later in adult life.
1.4.1 Impaired Growth

During the first 6 to 12 months of life most infants affected by IUGR will demonstrate an improvement in growth trajectory and catch up with AGA infants, however approximately 10% will remain shorter than their peers (186). The particular cause for intrauterine growth restriction and the degree of growth restriction caused impact on how well an infant can catch up.

Moderately affected infants have a greater potential to achieve catch up growth than those that have been severely affected. These infants are often a normal size by 1 year of age, though long term the accelerated catch up phenomenon may result in earlier onset of puberty, which can result in the final height achieved being slightly less than AGA infants (187, 188). Infants who have been severely growth restricted do not demonstrate the same degree of successful catch up growth and average height is significantly less than AGA infants. One study found that final height in this group was more likely to be less than the 10th centile (odds ratio [OR] 4.13 and 3.32, for boys and girls respectively) (189). Prematurity is an additional factor that further impairs the ability of the IUGR infant to catch up and those with the greatest degree of growth restriction are often born premature (190, 191). The mechanism by which the growth failure occurs in 10% of IUGR remains unclear. They usually do not have a growth hormone (GH) deficiency per se, but the GH secretory patterns may be adversely affected by a prolonged adverse environment, such as is the case with severe placental insufficiency. Growth hormone therapy has been shown to be successful in improving final height in this group (192, 193). Other determinants of successful postnatal growth and final height within normal range are appropriate early nutrition, parental height and normal postnatal head growth.

1.4.2 Fetal Programming

The concept of fetal programming or Barker Hypothesis refers to the process whereby a permanent or long-term change to the physiology, morphology or metabolism of a fetus or neonate occurs in response to a specific insult or stimulus at a critical period in development.
It was first described by Barker in the 1990's and is now widely accepted as an explanation for some causes of a number of chronic diseases arising in adulthood (22, 194). It occurs when the adaptive responses to the adverse intrauterine environment interferes with the normal hyperplasia of tissues, causes inappropriate induction of gene expression or alters the balance of cell types. This may result in changing the epigenetic state of the fetal genome, which results in a change in phenotype without altering the DNA sequence.

These changes may be passed on to future generations (195). Conditions such as ischaemic heart disease, hypertension, chronic kidney disease, type II diabetes, glucose intolerance and obesity have been associated with a low birth weight. Infants who were IUGR may have increased risk for ischaemic heart disease and it has been proposed that it is as a result of fetal malnutrition affecting normal vascular development. A large Swedish cohort study suggested that the association between IUGR and adult ischaemic heart disease is based on fetal growth restriction and that gestational age at birth did not have a role (196). Several studies demonstrate that in the newborn period, infants with IUGR have significantly thicker aortic walls and increased aortic stiffness as compared to AGA infants and this may be involved in the later development of ischaemic heart disease (197-199). The mechanism through which this occurs is not clear and some smaller studies have cast doubt over the strength of the relationship between intrauterine growth restriction and ischaemic heart disease (200, 201). Hypertension may also occur via another mechanism. Animal studies demonstrate that fetal hypoxia induces down regulation of 11β-hydroxysteroid dehydrogenase type 2. Reduced activity of this enzyme in the fetal kidney causes hypertension and hypokalaemia due to sodium retention (202). Chronic kidney disease has also been associated with low birth weight through the impact of reduced nephron number and function (203, 204). Sheep models show that a reduction in nephron numbers by 11% is sufficient to result in adult hypertension (205). The vascular endothelium in IUGR infants and adults has also been shown to have impaired vasodilatory capacity and flow mediated dilation is also decreased (206, 207). IUGR infants as adults are at significant risk for obesity and type 2 diabetes. The ‘thrifty’ phenotype of an IUGR infant described by Barker is programmed to have increased food uptake and fat deposition.
With an abundance of calories available this may lead to obesity. This appears to be mediated through an increase in adipogenesis and impaired appetite regulation. Animal models have demonstrated that there is an increase in expression of PPARγ as well as an upregulation of adipogenic transcription factors regulating it (208). PPARγ regulates fatty acid storage and glucose metabolism and genes activated by it stimulate lipid uptake and adipogenesis by fat cells.

Epidemiological studies and animal models have shown that these adults are also at risk for glucose intolerance and diabetes. Due to compromised in utero growth there are reduced numbers of pancreatic islets resulting in a decreased ability to secrete insulin. These adults have also been shown to have an impaired insulin response to glucose (209). This is caused by increased rates of hepatic gluconeogenesis, which is relatively resistant to insulin and changes in the intracellular insulin signaling pathways (210). Insulin resistance in IUGR adults is associated with impaired regulation of GLUT4 expression by insulin in muscle and adipose tissue (211). GLUT4 is a glucose transporter through which glucose enters cells when stimulated by insulin. A decreased ability to secrete insulin combined with a potential need for an increased amount of insulin results in glucose intolerance and subsequent diabetes.

1.4.3 Neurodevelopmental Outcome

The neurological and neurodevelopmental consequences of being born IUGR have been well documented (453). Such infants are at increased risk for difficulties with memory, language capabilities, executive functioning problems as well as poorer academic performance and behavioural problems compared to AGA infants. They are also at significantly increased risk for cerebral palsy (CP). Whilst the somatic effects of growth restriction are often quite apparent at birth the neurodevelopmental issues may not be revealed for several years, often on commencing formal education. Several studies have demonstrated a link between IUGR and CP. The exact strength of the association is difficult to assess as the definition of IUGR used in many of the studies included infants that would be classed as small for gestational age as opposed to truly growth restricted (212, 213).
However, when the definition of IUGR is more robust a strong association with cerebral palsy is maintained. Cerebral palsy describes a group of permanent disorders of the development of movement and posture, causing activity limitation, that are attributed to non-progressive disturbances that occurred in the developing fetal or infant brain. The motor disorders of cerebral palsy are often accompanied by disturbances of sensation, perception, cognition, communication and behaviour, by epilepsy and by secondary musculoskeletal problems (214).

The precise mechanism through which CP occurs in IUGR is unclear. It may result as a direct consequence of impaired growth or secondary to chronic hypoxia in the setting of placental insufficiency or through a combination of both processes. Specific changes have been reported in the brains of IUGR infants such as a reduction in grey matter volume, a reduced amount of total DNA in glia cells and neurons, changes in cerebral haemodynamics and reduced myelination. Animal studies have also reported reduced neuron numbers in areas such as the hippocampus and cerebellum (215-218). Chronic hypoxia is also shown to result in changes in neuron number and growth of certain regions of the brain such as cerebellum and forebrain. The injurious effects sustained by the brain during critical periods of growth in the IUGR setting may result in a non-progressive disturbance that leads to cerebral palsy.

The notion of fetal brain sparing was initially thought to be a benign adaptive response and suggested that it afforded a degree of protection from the neurodevelopmental sequelae of IUGR (219). The notion that it was benign or favourable has been disproven with many recent studies showing that fetal brain sparing is in fact related to a worse neurodevelopmental outcome including CP (220, 221). A ‘dose-response’ relationship has also been demonstrated with an increase in risk of CP related to the severity of growth restriction. This has been shown for IUGR infants born at term only; those born prematurely were not seen to have a higher risk compared to AGA controls (212). Term singletons that were severely growth restricted have a 5 to 7-fold risk of developing cerebral palsy compared with term infants who were appropriately grown (222).
The fact that there was no significant difference between premature infants may suggest that the length of time exposed to a hostile in utero environment increases the risk for cerebral palsy, and that cerebral palsy in premature IUGR infants may be as a result of the complications of prematurity that all premature infants are at risk for.

1.4.4 Cognitive Outcome

Cognitive impairments have been well documented in growth-restricted infants. These infants have been shown to score lower in tests on language skills, reading, writing and mathematics compared to AGA infants with consequential poorer academic performance. Memory and executive functioning may also be affected and growth restricted infants are more likely to be reported as having behavioural problems including excessive hyperactivity and attention deficit hyperactivity disorder (ADHD). The long-term neurodevelopmental sequelae also include neurosensory deficits such as visuomotor integration, in which area IUGR infants are 3 times more likely to have deficits in compared to AGA infants (223).

Hack et al have shown that cognitive ability is linked to head size and postnatal growth (224). A head size that remains subnormal at eight months of age is an important predictor of poorer neurocognitive abilities at school age, poor academic achievement and poorer behaviour at 8 years of age (225, 226). The effect of a subnormal head circumference appears to be independent of other factors (227). Birth weight and catch up weight gain is also associated with intelligence quotient (IQ) and neurodevelopmental scores and data from the National Collaborative Perinatal Project (1959-1976) found IQ scores of IUGR infants to be 6 points lower than AGA children (226, 228). Short term memory and recognition memory is affected in IUGR infants, as is the hippocampus, which plays an important role in memory with smaller hippocampal volumes reported (229, 230). However, the memory deficits displayed do not necessarily comply with a typical hippocampal injury, rather characteristic of a deficit in the hippocampal – prefrontal network.
Behavioural problems and hyperactivity disorders are found in greater frequency in children born IUGR compared to controls (231, 232). Several different tools to assess for behavioural issues have been used, ranging from brief screening parental questionnaires to more formal assessments using validated tools such as the Childhood Behavior Checklist (CBCL) and Conners’ Hyperactivity Index. The degree of association differs depending on the tool used, however the association is preserved across studies (233, 234).

Two groups in the Netherlands studied whether fetal brain sparing is associated more with behavioural problems as compared to IUGR infants without evidence of fetal brain sparing. The smaller study of 98 infants did not show a difference, however, the Generation R study of 935 infants concluded that evidence of fetal brain sparing indicates underlying pathology with greater consequences for behavioural problems in later life. The children with higher umbilical/anterior cerebral blood flow ratios demonstrated higher rates of internalising problems, emotional reactivity and difficulties with attention (126, 232). Many IUGR infants are born premature, but it appears that intrauterine growth restriction rather than prematurity increases the risk for symptoms of ADHD in this group of children (234).
Section 1: Introduction

Chapter 2

Imaging Biomarkers of Outcome
Chapter 2. Introduction - Imaging Biomarkers of Outcome

2. Imaging Biomarkers of Outcome

The term biomarker (biological marker) has been used since the 1980’s and is defined as a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacologic responses to a therapeutic intervention (235). In medicine a biomarker can be described as a measurable indicator of the severity or presence of some disease state. More generally, a biomarker is anything that can be used as an indicator of a particular disease state or some other physiological state of an organism. In this regard, an imaging biomarker is a feature of an image that is relevant to a patient’s diagnosis or prognosis. Several different imaging modalities have been employed in neonatology, both to aid in immediate management and also in an effort to predict long-term outcome. Cranial ultrasonography and magnetic resonance imaging are the two most commonly used. Computed tomography (CT) is rarely used in current practice due to the unacceptably high amount of ionizing radiation involved and the consequent long-term neurodevelopmental sequelae. Where there may have been indications for the use of CT in the past, current MRI sequences, availability and access have resulted in a steady decline in the usage of CT in neonatology (236).

2.1 Cranial Ultrasonography

Cranial ultrasonography (CRUSS) has been the main stay of neonatal neuroimaging since the late 1970’s with the advent of real time ultrasonography. Premature infants born in the late second and early third trimester are susceptible to haemorrhagic and ischaemic lesions which are readily identifiable using CRUSS. This is due to a combination of having a highly vascular germinal matrix that has yet to involute and poor cerebral circulatory autoregulation. In the initial phase after delivery there may be periods of physiological instability that challenge the autoregulatory capacity resulting in potential brain injury.
Ultrasound identifies haemorrhages within the ventricular system as well as the parenchyma, but also recognizes non-haemorrhagic lesions such as cystic periventricular leucomalacia, areas of ischaemia and ventriculomegaly. Grading systems have been developed to categorise the findings and to predict outcome. The Papile grading system for intraventricular haemorrhage (IVH) is one of the most widely known and used. In this system a grade 1 IVH is confined to the germinal matrix; a grade 2 involves bleeding in to the ventricle without distension of the ventricle; a grade 3 has blood within and distending the ventricle and grade 4 refers to parenchymal involvement.

These findings have been correlated with long-term outcome, with worsening degree of neurodisability with increasing grade of injury. Grades 3 and 4 and white matter lesions are well associated with adverse neurodevelopmental outcome (237, 238). Regardless of whether the cause of the injury is haemorrhagic or ischaemic, damage in the periventricular area has been associated with neurodevelopmental consequences. Intraventricular haemorrhage associated with white matter injury is much more likely to result in a neurodevelopmental abnormality than an isolated IVH. However, the exact nature of the abnormality can be difficult to predict, ranging from slight cognitive deficits to severe disability. The ability of cranial ultrasound to predict subsequent neurodevelopmental issues is related to gestational age and days of life. A sensitivity of predicting long-term abnormality of 16% at two weeks post delivery, 53% at six weeks of age and 58% at term-corrected has been reported with a high specificity in all age groups (239). Cystic periventricular leucomalacia (PVL) is predictive of cerebral palsy with a positive predictive value of 77% reported in one study (240). Combining the exact location and the size of the cystic lesions identified to determine if a composite of these features would more accurately predict neurodevelopmental outcome is difficult (241).

Ventriculomegaly is another finding that is used as an ultrasound biomarker for longer-term outcome. Ventriculomegaly in the setting of post-haemorrhagic hydrocephalus requiring shunting or intervention is predictive of a poor neurodevelopmental outcome (242). Increasing ventricular size is associated with a worse neurodevelopmental outcome.
Debate exists as to whether cranial ultrasound is as effective as magnetic resonance imaging in predicting neurodevelopmental outcome. Several studies have compared the two modalities with differing results and opinions as to comparability (243-246). A review by Linda de Vries comparing the studies of ultrasound versus MRI brain in the neonatal population provides a useful update on this issue with both the positive and negative predictive values of ultrasound and MRI for a range of injuries that may be seen in a neonate at term, these are presented in table 1 (437). A normal cranial ultrasound scan at term equivalent age is strongly predictive of a normal neuromotor development, however, MRI may provide more detail on subtle structural findings and mild white matter abnormalities (240). As MRI is considerably more expensive, technically challenging and not as readily available, CRUSS can serve as a reliable screening tool, with further imaging in cases with abnormal findings or in particular high-risk groups.

The British Association of Perinatal Medicine (BAPM) recently published a set of recommendations for the use of magnetic resonance imaging in neonatal medicine (438). Specifically, for premature infants at term it quotes that MRI at term equivalent age (38-42 weeks post menstrual age) provides more anatomic detail than cranial ultrasound, which has led to a greater appreciation of the nature and extent of white matter lesions abnormalities; detailed visualisation of the posterior limb of the internal capsule and cerebellum (injury to both of which may carry prognostic significance) and the development of schemes for classifying brain injury. The BAPM recommendations for when magnetic resonance imaging is indicated in the neonate are presented in table 2.
Table 1. Comparison of cranial ultrasound (CUS) and MRI used for the prediction of motor outcome at 18-30 months.

Positive predictive value (PPV), negative predictive value (NPV), intraventricular haemorrhage (IVH), periventricular leukomalacia (PVL), focal infarction (FI), white matter injury (WMI), term equivalent age (TEA).

<table>
<thead>
<tr>
<th>Study</th>
<th>N</th>
<th>Age at Follow up (months)</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>PPV</th>
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<td>Valkama et al. (2000)</td>
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<td>18</td>
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<td></td>
<td></td>
<td>0.65</td>
<td>0.82</td>
<td>0.91</td>
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<tr>
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<td></td>
<td></td>
<td>0.57</td>
<td>0.95</td>
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<tr>
<td>Mod-severe WMI</td>
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<td>0.57</td>
<td>0.98</td>
<td>0.98</td>
<td>0.98</td>
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<tr>
<td>Severe IVH/PVL</td>
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<td>0.92</td>
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<td>Sequential CUS and MRI TEA</td>
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<td>24</td>
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<td>161</td>
<td>24</td>
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<td>Mod-severe WMI</td>
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<td>Leijser et al. (2008)</td>
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<tr>
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<tr>
<td>Neonatal Encephalopathy</td>
<td>Infants with clinical signs of acquired brain injury/neonatal encephalopathy (NE) should undergo neuroimaging. MRI is the <strong>routine recommended</strong> imaging modality of choice for diagnostic imaging in NE</td>
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<tr>
<td>Neonatal Seizure</td>
<td>Newborns with clinical and or electrographic signs of seizures require neuroimaging for diagnostic and prognostic purposes. MRI brain is the <strong>routine recommended</strong> modality of choice.</td>
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<tr>
<td>Congenital Heart Disease</td>
<td>Infants with congenital heart disease have increased risk for neurodevelopmental sequelae, however, in the absence of seizures, abnormal neurological examination and with normal cranial ultrasound <strong>routine MRI</strong> examination is <strong>not recommended</strong>.</td>
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<tr>
<td>ECMO</td>
<td><strong>Routine</strong> MRI brain in infant’s post ECMO is <strong>not recommended</strong> in the absence of abnormal neurological signs unless significant parenchymal abnormality present on cranial ultrasound.</td>
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<tr>
<td>Premature Infants</td>
<td><strong>Routine</strong> MRI brain at term equivalent age is <strong>not recommended</strong> in preterm infants in the absence of abnormal cranial ultrasounds or unexplained neurological signs. MRI of the preterm infant at term equivalent age, with a normal cranial ultrasound scan should not be performed routinely outside the context of research.</td>
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<tr>
<td>Fetal MRI</td>
<td>Fetal MRI should be undertaken as part of a specialist fetal medicine referral. The indications for fetal MRI are: To aid diagnosis, to aid management of a fetus with a known diagnosis and to provide additional information in cases where termination is considered and there is any uncertainty over the diagnosis.</td>
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</table>
2.2 Magnetic Resonance Imaging

There have been many significant improvements since the first neonatal MRI brain scans were performed in the early 1980’s (247). These range from increased strength of the magnets used, resulting in improved image quality, to advanced MRI techniques and complex post image acquisition processing, providing information on function as well as structure. In the past ten years, there have been several studies using MRI to predict long-term neurodisability, with many reporting superiority over CRUSS in this regard. Infants born prematurely, infants with very low birth weights and term infants with hypoxic ischaemic encephalopathy have been studied with MRI, successfully predicting neurodevelopmental outcome. Seminal work by Woodward has demonstrated the ability of term equivalent MRI to predict neurodisability in preterm infants by the age of 2. They found that moderate to severe abnormalities within the white matter was predictive of severe psychomotor delay and cerebral palsy. These findings were also shown to be independent of abnormalities on CRUSS and of other risk factors such as postnatal steroids or bronchopulmonary dysplasia (244). It was also noted that abnormalities in both white matter (moderate to severe) and grey matter abnormalities were predictive of severe psychomotor delay and cerebral palsy, but white matter more so than grey matter. It is also extremely useful in term infants. Infants who suffer from hypoxic ischaemic encephalopathy at birth are treated with therapeutic hypothermia and have an MRI scan performed as part of this treatment package.

The MRI serves as an important predictor of outcome in this high-risk group and provides detailed information that is useful in counseling parents regarding outcome. After a severe asphyxial insult central structures appear to be most at risk such as the deep grey matter and basal ganglia and the perirolandic cortex. These represent areas that are actively myelinating at term and have the highest concentration of N-methyl-D-aspartate (NMDA) receptors. Decreased signal intensity in the posterior limb of the internal capsule (PLIC) may be seen on T1 weighted images, which is a strong predictor of abnormal motor outcome. Evidence of extensive white matter injury is associated with a poor outcome, which is worse if the basal ganglia or thalamus are significantly involved (248-251).
Much work has also been done in the area of IUGR, and using MRI to predict neurodevelopmental outcome. These infants are well documented to be at increased risk for a variety of neurodevelopmental delays, as previously discussed. The MRI changes that have been reported may be partly a function of prematurity, if present, but compounded by poor intrauterine growth. Obvious changes such as white matter abnormalities, defects in myelination and structural lesions have been reported, which can predict long-term problems such as cerebral palsy and visual defects. The other deficits associated with IUGR, such as disabilities of language, recognition memory, abstract reasoning, concentration and attention, may not be as readily identifiable on standard imaging techniques. A reduction in the volumes of the hippocampus and caudate nuclei, as well as overall grey matter volume and total intracranial volume has been reported in IUGR infants when compared to non-IUGR preterm and term infants (229, 252, 253). Advanced MRI techniques such as functional MRI, diffusion tensor imaging, volumetric analysis and tractography could provide information that is not obtainable from cranial ultrasound and this may add considerably to the term equivalent imaging assessment in the setting of IUGR in the future. However, before exploring complex quantitative MRI techniques, a brief review of the basic principles of MRI is required.

2.3 Basic principles of Structural Imaging

The directional magnetic field, or vector, associated with charged particles in motion forms the basis of Magnetic resonance imaging. Nuclei with an odd number of protons have a characteristic motion called precession, which produces a small magnetic moment or vector (Fig. 3 A). The human body is primarily composed of fat and water, both of which contain multiple hydrogen atoms, and so the human body is composed of approximately 63% hydrogen atoms. Hydrogen nuclei have an identifiable nuclear magnetic resonance signal. For these reasons, MRI primarily images the signal from the hydrogen nuclei in the structure being studied. The nuclei precess about the magnetic field direction like gyroscopes, or spinning tops, and this phenomenon is referred to as Larmor precession.
The frequency of this precession is proportional to the strength of the magnetic field applied and is defined by the Larmor frequency; \( \omega = \gamma B \), where \( \gamma \) is the gyromagnetic ratio and \( B \) is the strength of the magnetic field. The gyromagnetic ratio is a nucleus specific constant, and for hydrogen \( \gamma \) is 42.6MHz/Tesla (254).

In order to produce an MR image, the subject is placed in a strong and uniform magnetic field. The field strength of the magnet is measured in Tesla. The majority of commercial systems available currently operate at 1.5T, though systems up to 7T are in use. Once in the scanner the hydrogen nuclei align with the magnetic field and create a net magnetic vector parallel to the magnetic field (Fig. 3 B). A radio frequency (RF) pulse is then applied, perpendicular to the magnetic field of the magnet. The pulse applied is tuned to a specific range of frequencies at which hydrogen protons precess, the Larmor frequency, and causes the net magnetic vector to tilt away from that of the magnetic field of the magnet (Fig. 3 C). On removing the RF signal, the nuclei once again realign so that their net magnetic vector is parallel to the magnetic field of the magnet again, this is referred to as relaxation. During the relaxation period the nuclei lose energy by emitting their own RF signal, which is known as the free-induction decay (FID) response signal. This is measured by the conductive field coil that is placed around the area being imaged (Fig. 3 D). The measured signal is reconstructed to obtain 3D grey-scale MR images.

To form the 3D image, the FID resonance signal must be encoded for each dimension. A 2D Fourier Transform is used to transform the encoded image to the spatial domain. The voxel intensity of the different tissue types, such as white matter and grey matter, depends on the proton density of the tissue; the higher the proton density the stronger the FID response signal. A voxel is a unit of graphic information defining a point in three-dimensional space. A picture element (pixel) defines a point in two-dimensional space.

The MR image contrast is also affected by the tissue specific longitudinal relaxation time, \( T_1 \), and the transverse relaxation time, \( T_2 \). \( T_1 \) measures the time it takes for the magnetic vector of the displaced nuclei to realign with that of the magnet.
T2 represents the time for the FID response signal from a given tissue type to decay. During the scanning process the RF pulse is repeated at a predetermined rate. The period of the RF pulse sequence is known as the repetition time, TR. The FID response signals can be measured at various times during the repetition time period. The time between the RF pulse being applied and the measurement of the response signal is the echo delay time, TE. By adjusting the TR and TE the MR image acquired can be made to contrast different tissue types. Spatial encoding of the MRI signal is accomplished through the use of gradients, which are smaller magnetic fields that perturb the main magnetic field. This causes hydrogen protons in different locations to precess at slightly different rates.
Figure 3 (A). The circles represent hydrogen atoms and the arrows represent the directions of the magnetisation vectors before the protons are exposed to the large uniform magnetic field of the MRI scanner.

Figure 3 (B). When placed in the scanner, the magnetisation vectors of the hydrogen atoms align themselves with the magnetic field. The large arrow represents the direction of the large magnetic field of the MRI scanner.

Figure 3 (C). The radiofrequency pulse is delivered causing a change of direction in the magnetisation vectors of the protons.

Figure 3 (D). As the protons relax they emit a radiofrequency pulse, which is received and an image produced. University of Virginia, 2013. MRI Physics [online]. Available from https://www.med-ed.virginia.edu/courses/rad/cardiacmr/Techniques/Physics.html. [Accessed on 25 May 2015]
The portion of the gradient coils and the associated current that is perpendicular to the main magnetic field causes a force on the coils. These gradients are turned on and off rapidly causing them to vibrate and produces the knocking noise associated with MRI. The structural MR images produced, such as T1- and T2-weighted images, provide high-resolution images of the brain that were not previously possible with other imaging modalities, and do so without utilising ionizing radiation (Fig. 4). Since the first images were produced there have been significant improvements in the quality and the detail provided with these structural images. These allow for assessment of brain development and for identification of the presence of lesions known to be associated with neurodevelopmental sequelae (244, 255). Structural lesions associated with cerebral palsy and myelination defects can be readily appreciated on MR images and are associated with long-term neurodisability. A number of neurostructural imaging studies performed on IUGR infants have shown changes such as in the degree of gyrification and cortical morphometry (256, 257) compared with AGA controls. The length and area of the corpus callosum (CC), a discrete well-defined brain structure, has been related to adverse developmental outcome, and a reduction in its area has been noted in IUGR infants (258-260). The corpus callosum is a thick plate of white matter fibres that serves as the main connection between the cerebral hemispheres, and has been suggested as a surrogate marker of white matter growth. When compared to the gyrification of healthy term infants at birth, IUGR infants do not have the same degree of complexity and a reduction of surface in relation to the sulcation index. These measurements at birth correlate with discrepancies in neurobehavioural assessment at term equivalent age (257).

However, the functional deficits that are recognised in cognitive function, self-regulation, language skills, abstract reasoning, recognition memory, concentration, mood and school performance are not often correlated with focal brains lesions on structural scanning. As these deficits still occur in the absence of an obvious brain injury, quantitative MR imaging techniques have been developed to assess the micro architecture of the brain and how this may be affected during development.
Figure 4. Normal appearances of the brain at term. (a) T1 weighted image. High signal in the posterior limb of the internal capsule (PLIC) (long arrow) and in the ventrolateral nuclei of the thalami (short arrow). (b) T2 weighted image. Myelin in the PLIC has a low signal intensity (long arrow) and in the region of the ventrolateral nuclei of the thalamus (short arrow). Unmyelinated white matter has a low signal intensity on T1 weighted images and high signal intensity on T2 weighted images. Cerebrospinal fluid is hypointense on T1 weighted imaging and hyperintense on T2 weighted imaging.

2.4 Quantitative MRI techniques

The structural images produced by standard MRI techniques provide a wealth of information and high-resolution detail that has resulted in significant advances in both diagnosis and prognosis. It can also play an important role in the counseling of parents regarding long-term developmental expectations and challenges of infants with IUGR, amongst other conditions. In certain infants, the structural anatomic images produced may not show evidence of significant lesions or myelination defects, however, it is well recognised that cognitive and behavioural deficits may occur nonetheless.

This has resulted in the development of quantitative MRI techniques where the MRI scanner becomes more than a means of producing detailed anatomic reconstructions of the brain, but also provides additional useful information that is not readily apparent on initial structural images. These quantitative techniques permit the segmentation of the brain images into various tissues and regions, allowing both volumetric analysis and the measurement of different metabolites from brain tissues in different regions using spectroscopy. Volumetric analysis of premature infants affected by IUGR demonstrated that there was reduced intracranial volume as compared with normally grown premature infants, with cortical grey matter volume reduced by 28% compared to controls. This suggests that the cerebral cortical development is predominantly affected in IUGR (252). Volumetric studies have also suggested that hippocampal and caudate nucleus volumes, along with other structures, are reduced when compared to healthy non-IUGR controls (229, 253, 261). Spectroscopy can be used to assess neuronal development and white matter injury in premature infants by quantifying the N-acetyl aspartate/choline (NAA/Cho) ratio. This normally increases rapidly in the first few months after birth and is in highest concentration in the basal ganglia and deep grey structures. Spectroscopy is also useful in measuring lactate levels in infants with hypoxic ischaemic encephalopathy. Generally, infants with more severe injuries have higher lactate peaks on spectroscopy. The information from spectroscopy may precede anatomical or functional changes and is complementary to standard imaging (262, 263).
There are several other quantitative MRI techniques available including diffusion tensor imaging (DTI), volumetric and functional MRI. As these techniques were used in this study I shall expand on the basic principles of both.

2.4.1 Principles of Functional MRI

Conventional MRI provides images of the fine detail of anatomic structures whereas functional MRI (fMRI) provides useful information on brain function. fMRI was developed in the early 90’s based on work by a number of different groups. The first reports of MR detection of a functional response in the brain came from Belliveau et al, where they used an exogenous contrast agent, gadopenetate, injected into the blood stream. Unfortunately, this measurement was only possible during the brief timeframe when the contrast passed through the areas of interest in the brain (264). Ogawa had identified Blood Oxygen Level Dependent (BOLD) signal as a potential signal for functional imaging of the brain in 1990 and Kwong and Ogawa produced the first images using BOLD as an endogenous contrast agent in 1992 (265, 266). Over the intervening 20 years it has become one of the imaging tools of choice for understanding both how the healthy brain works and how various disease processes or interruptions may affect this. Functional MRI is based on the fact that neuronal activity is associated with cerebral blood flow, and that there are differences in the magnetic properties between oxyhaemoglobin and deoxyhaemoglobin that can be interpreted as an MR signal. There are other forms of contrast that can be used in fMRI, but the BOLD signal is most commonly used and shall be explained herein.

Neural activity requires an increase in blood flow in order to meet demand for oxygen and glucose, as neurons do not possess internal energy reserves. The increase in blood flow is in excess of the demands placed, resulting in a net increase in the balance of oxyhaemoglobin to deoxyhaemoglobin. This results in a drop in the concentration of deoxyhaemoglobin within the tissues. Oxyhaemoglobin is diamagnetic; it has no unpaired electron and therefore no magnetic moment. Conversely deoxyhaemoglobin has an unpaired electron and a magnetic moment.
As deoxyhaemoglobin is significantly paramagnetic, deoxygenated blood differs substantially in its magnetic properties from surrounding tissues (267). It distorts the magnetic field induced by the scanner, causing the nuclei there to lose magnetisation faster via T2* decay. Therefore, when oxygen is not bound to haemoglobin, the difference between the magnetic field applied by the MRI scanner and that experienced close to deoxyhaemoglobin is much greater than when oxygen is bound. During periods of neuronal activity, local blood flow and volume increase with little or no change in oxygen consumption (Fig. 5).

As a consequence of having lower levels of deoxyhaemoglobin in a region of brain tissue, the MRI signal from that region decays less rapidly and so is stronger when it is recorded. Therefore, MR pulse sequences sensitive to T2* show a stronger signal where there is more oxygenated blood and a lesser one where there is not. This small signal increase is the Blood Oxygen Level Dependent (BOLD) signal that is recorded in fMRI (268). This effect increases with the square of the strength of the magnetic field. Consequently, a strong magnetic field and a pulse sequence such as echo planar imaging (EPI), which is sensitive to T2* contrast, are both required (269). As neural activity increases there is a time delay preceding the vasodilation required to increase blood flow and the washout of deoxyhaemoglobin from the region. As a result the BOLD signal that is detected is delayed (270). The signal increases about 2 seconds after neural activity increases then reaches a plateau at 5 to 8 seconds (Fig. 6). After the neuronal activity/stimulation stops the signal falls to baseline in 8 to 11 seconds, after briefly dipping below the baseline, a phenomenon known as the undershoot (271).

Figure 6. Haemodynamic response function
The change in the MRI signal due to neuronal activity is known as the haemodynamic response. As the signal produced is quite small it can be difficult to identify it from the surrounding unwanted signal or ‘noise’ that comes from the scanner and random brain activity. To account for this the stimulus is repeated several times and statistical procedures employed to extract the appropriate signal. Over the course of an fMRI sequence, a series of brain images are acquired as the subject is exposed to a stimulus or performs a task. Changes in the signal measured between the individual images are used to determine the areas of the brain activated during the task/stimulus exposure. The resulting data comprises a sequence of MR images; with each image made up of a number of voxels, which partition the image into equally sized boxes. A voxel is a three-dimensional rectangular cuboid of defined proportions. The intensity of the signal from a voxel corresponds to the spatial distribution of the nuclear spin density in that area. As changes in haemodynamics in the brain occur in reaction to neuronal activity, the MRI signal changes. Consequently, changes in the voxel intensity over time can suggest where and when the neuronal activity is occurring. The response signal from a single voxel over time is called the time series (Fig. 7).

In neonatology, functional MRI is not easily performed. Neonates cannot comply with task based assessments and providing a stimulus to a baby in order to assess a response risks waking a sleeping baby, causing the infant to move and thereby affecting image quality. Resting state functional MRI (rs-fMRI) overcomes these hurdles. Resting state fMRI is a technique that is used to assess regional interactions within the brain when the subject is not performing a task or being exposed to alternating stimuli (272). The brain is constantly active and the energy consumption associated with performing a focused mental task only increases the baseline energy consumption by about 5% (273). As such, spontaneous fluctuations in BOLD signal exist that can be identified on fMRI. This approach has shed light on the functional organisation of the brain and identified a number of networks, which represent specific patterns of reproducible connectivity (Fig. 8). Regions of the brain that are anatomically adjacent are structurally connected, however, this does not imply a functional connectivity.
Functional connectivity refers to an association between different regions of the brain that share functional properties. It is defined as a temporal correlation between spatially remote neurophysiological events, expressed as a deviation from statistical independence across these events in distributed neuronal groups and areas (274).
Figure 7. Time series (depicted in blue and purple) from a single voxel over time representing the change in the BOLD MRI signal detected.

Figure 8. Resting-state networks in a single infant. Each row represents one resting-state network shown at three representative axial sections superimposed on to a T2-weighted infant template.

Resting state functional connectivity refers to connectivity across individual BOLD time points during resting conditions. A number of networks have been described in rs-fMRI studies and these networks have been found to be consistent across studies, differing data acquisition models and different modes of analysis. Several networks have been described including the Default Mode Network (DMN) and the Attentional Network (275, 276). Rs-fMRI studies provide a technique to evaluate how these networks develop and how they may be affected by intra-uterine growth restriction and prematurity. The connectivity present can be analysed in terms of the strength of the correlation signal between regions of the brain, as a whole functional network and the connectivity or relationship between and within networks. The analysis of the data obtained relating to these networks has been important in gaining insight into processes involved in both cognitive and emotional functioning. Resting state fMRI in newborns has provided a tool to investigate these networks at the earliest time frame and discover how they evolve over time, as well as how this development may be affected by injury or adverse conditions. Behavioural assessment in the newborn period does not provide this level of information, further progressing the scope for the use of rs-fMRI in developmental research (277).

The resting state networks (RSN) in the adult brain do not appear to be present, fully formed at birth. Several studies have investigated RSN’s in the neonatal brain and over the first few years of life showing the gradual development and highlighting differences in the neonatal brain. Functional MRI at term identifies prototype networks and differing patterns of connectivity. It appears that networks evolve from a higher short-distance connectivity in infancy to a higher long-range connectivity by adulthood (278). Adult RSN’s also display a stronger lateralisation generally confined to a hemisphere, whereas neonatal RSN’s display a stronger cross hemisphere degree of correlation (279).
2.4.2 Principles of Volumetric Analysis

Morphometry is the measurement of structures or organisms and their component parts. Brain morphometry assesses the brain in terms of shape, mass or volume. It is used in many areas of research including development, aging and disease processes. Subjective assessment of structures or volumes of brain regions does not provide a robust platform for comparing different subjects or assessing for inter-hemispheric differences. As the data obtained from neuroimaging is digital it allows for complex statistical methods to be applied to quantify shape or volume and analysis. Quantitative MRI techniques have been used to generate reproducible numeric measurements that have been used to compare against reference populations and to investigate brain development over time. Given the significant variation in both size and shape of the human head from individual to individual, reference populations are scanned to produce atlases. An atlas is a standardised reference against which scans can be compared and serves as the gold standard for quantitative analysis for a given population. To do this, image registration algorithms ‘warp’ or register the subject image so that the anatomy of a subject aligns with that of the atlas template. Spatial transformation occurs to allow the images to align and the rotation and stretching that occurs during the transformation is determined, which can be analysed to assess the variation between the subject and the reference atlas. Specific regions of interest can also be interrogated through selecting the coordinates of a particular structure and computing the volume. The volumes of structures can then be compared against the atlas reference volume (280).

Volumetric analysis studies in neonatology have shown differences in extrauterine whole brain growth between extremely preterm and moderately preterm infants when compared to term born infants (281, 282). The rate of development of different subcortical regions has also been assessed and the growth patterns for various regions have been shown to not be uniform in nature (283). Volumetric differences have been related to a number of disorders including ADHD and autism in older paediatric populations (284).
The use of volumetric analysis in the neonatal population of at risk groups may identify those at greatest risk, and provide an opportunity to improve outcomes through the institution of interventions during a period of significant plasticity and development of the brain. Initial volumetric analyses required drawing regions of interest on the serial images acquired and calculating the enclosed volume. This was highly labour intensive and not accurate for smaller areas, which led to the development of automated software packages.

2.4.3 Principles of Diffusion Tensor Imaging

Diffusion tensor imaging (DTI) is a magnetic resonance imaging technique that measures the degree of restricted diffusion of water molecules in a tissue and is used to produce neural tract images. The diffusion properties of water in white matter were described in 1990 and the first images using diffusion to map white matter tracts were produced the following year (285, 286). As well as enabling visualisation of white matter tracts it also can assess pre myelination processes during development and can allow quantification of microstructural abnormalities not otherwise detectable with conventional imaging sequences. Due to this ability to identify microstructural details it has developed a role as a biomarker of brain maturation and injury, providing insight in to the effect of prematurity on brain connectivity and functionality (287-289).

Diffusion refers to the random motion of water molecules; this varies across regions of the brain depending on the underlying tissue characteristics. For example, in cerebrospinal fluid (CSF) water molecules can move unhindered in all directions and displacement is equally distributed, this is called isotropic diffusion. In white matter, where the tissue is highly organised, the diffusion of the water molecule is restricted perpendicular to and facilitated along the direction of the fibres and this is known as anisotropic diffusion (Fig. 9). By using these properties, it is possible to assess myelination, fibre density and complexity with DTI. There are a number of quantitative measurements used such as fractional anisotropy (FA) and apparent diffusion coefficient (ADC). Fractional anisotropy is a scalar value between zero and one that describes the degree of anisotropy of a diffusion process.
It increases proportionally with the degree of organisation of the tissue assessed. A value of zero means that a molecule’s diffusion is isotropic (unrestricted in all directions) and a value of one means that diffusion can only occur along one axis and is fully restricted in all other directions. It reflects the fibre density and myelination in white matter and increases during development as a result of decreased water content and the progression of the myelination process.

Figure 9. Isotropic and anisotropic diffusion. (A) When motion is unconstrained, as in the large fluid–filled spaces deep in the brain (i.e., the ventricles, as illustrated in the MR image on the left), diffusion is isotropic, which means that motion occurs equally and randomly in all directions. (B) When motion is constrained, as in white–matter tracts (illustrated on the right), diffusion is anisotropic, meaning that motion is oriented more in one direction than another (e.g., along the y axis rather than along the x axis). Rosenbloom et al. using magnetic resonance imaging and diffusion tensor imaging to assess brain damage in alcoholics. Alcohol Res Health. 2003;27(2):146-152.
As FA in white matter increases over the course of development, the ADC decreases exponentially (290-292). The apparent diffusion coefficient is a measure of the magnitude or degree of impedance of diffusion of water molecules within tissue and is measured by using different b values via changing gradient amplitude. B value measures the degree of diffusion weighting applied, indicating the amplitude, time of applied gradients and duration between the paired gradients. ADC values are calculated and displayed as a parametric map reflecting the degree of diffusion of water molecules through the different tissues. Drawing regions of interest on the ADC map records measurements for a given region and the values are expressed as mm$^2$/s. The ADC pixel values together form the ADC map. On a half logarithmic scale, the signal decay delivers a straight tilted line the slope of which provides the ADC. The faster the signal decay, the steeper the slope and the greater the ADC.

A diffusion image with restricted diffusion appears as hyperintense (bright) whereas on the corresponding ADC map the area of restricted diffusion will appear hypointense (dark). Several studies have shown that white matter injury interrupts the normal developmental increase in FA and associated decrease in ADC, and have correlated these findings with impaired outcome (293-295). DTI measures diffusion in more than six non-collinear directions. Each voxel of an image has one or more pairs of parameters: a preferred direction of diffusion and a rate of diffusion. The properties of each voxel are calculated by vector or tensor math from the six or more directions, each obtained with a different orientation. Therefore, the diffusion tensor calculated describes both the direction of diffusion and the orientation, within three-dimensional space, of the tissue being studied. The spatial organisation of the white matter structures can be represented by a number of methods such as colour maps and fibre tractography. The direction of the fibre is indicated by the tensor's main eigenvector. This is colour coded and creates a map of the tract's position and direction; red for left to right, blue for superior to inferior and green for anterior to posterior. The FA in a given voxel weights the brightness (Fig. 10 A). Fibre tractography enables delineation of specific white matter pathways and is done using fibre tracking algorithms that track a fibre along its whole length by tracking the highest diffusivity from voxel to voxel (Fig. 10 B).
Figure 10 (A). Fractional anisotropy (FA) map. The colours represent the primary direction of diffusion. Red = left-right, green = anterior-posterior and blue = superior-inferior.

As outlined, infants with IUGR are at increased risk for neurodevelopmental sequelae. However, they are not a homogenous group and are frequently managed differently in the initial period dependent on Doppler status, for example those with abnormal Doppler’s antenatally are often commenced on a delayed or slower feeding regime. Nevertheless, by the time of graduation from the neonatal intensive care unit antenatal Doppler status is not factored in to developmental follow up or for stratification of risk for neurodevelopmental sequelae.

The use of resting state functional MRI was chosen as I hypothesised that the hostile environment of placental insufficiency and the potential chronic periods of hypoxia may affect the organisation of the resting state networks and their formation. This could serve as a very early marker of functional impairment. Resting functional MRI has not been investigated specifically in IUGR groups to investigate for differences between groups based on Doppler status. Diffusion weighted imaging has been used extensively in perinatal brain injury and used to aid in timing of ischaemic events. Whilst the injury in intrauterine growth restriction due to placental insufficiency is frequently secondary to periods of hypoxia/ischaemia, at the time of imaging many weeks later significant changes on diffusion imaging would not be anticipated (439). Volumetric analysis was chosen to complement resting state fMRI, where structural differences are not visible nor presented. Volumetric differences in infants affected by IUGR have been demonstrated previously (229). Investigating for differences between the intrauterine growth restricted groups may yield volumetric differences.
2.5 Statement of Hypothesis

I hypothesised that infants with intrauterine growth restriction and evidence of placental insufficiency, as identified by abnormal umbilical artery Doppler measurement, have greater impairment in brain development as compared to infants with intrauterine growth restriction and normal umbilical artery blood flow, and that these differences can be identified using sophisticated MRI brain imaging techniques at term corrected gestation.

2.5.1 Aim of Thesis


2. Investigate for regional brain differences between intrauterine growth restricted infants based on antenatal Doppler status using volumetric analysis.

3. Correlate term corrected MRI findings with 1-year growth and outcome measures using a 12 month Ages & Stages Questionnaire and head circumference growth over first year.

4. Compare the MRI findings and 1 year outcomes of the intrauterine growth restricted groups with a control group to act as a reference group for these analyses and further future longitudinal studies.
Section 2: Materials and Methods

Chapter 3

Materials and Methods
### Chapter 3. Methodology

#### 3.1 Materials

Table 3. Materials used in the StOOPS project.

<table>
<thead>
<tr>
<th>Neonate Assessment</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Item</strong></td>
<td><strong>Specification</strong></td>
</tr>
<tr>
<td>Stethoscope</td>
<td>3M™ Littmann® Classic II Infant Stethoscope, Black Tube, 28 inch, 2114</td>
</tr>
<tr>
<td>Paper measuring tape</td>
<td>Henley disposable tape measure TM30</td>
</tr>
<tr>
<td>Growth Chart</td>
<td>UK Child Growth Foundation ‘4 in 1 Centile Charts’ (296)</td>
</tr>
<tr>
<td>Weighing scales</td>
<td></td>
</tr>
<tr>
<td>Ear plugs</td>
<td>E-A-R EarSoft Yellow Neons UF Foam Ear Plugs (NRR 33)</td>
</tr>
<tr>
<td>Ear muffs</td>
<td>Natus MiniMuffs® Neonatal Noise Attenuators</td>
</tr>
<tr>
<td>Vacuum mattress</td>
<td>NORAS VacuChild Mat It. Nr.: MR11001</td>
</tr>
</tbody>
</table>

| MRI system | |
| **Item** | **Specification** |
| 3 Tesla MRI scanner | Philips Achieva 3.0T TX system |
| Monitor | Medrad® Veris® MR Vital Signs Patient Monitor Model 8600 |
| Pulse oximeter saturations probe | Medrad® Veris® paediatric ‘clip’ probe SN 24368 |

| Equipment | |
| **Item** | **Specification** |
| Laptop | Dell Latitude E6530, 16GB, Quad core processor |
| Software | FMRIB Software Library (FSL) |
| | CONN Toolbox |
| | Montreal Neurological Institute (MNI) atlas |
| | ANTs |
| | ALBERT Neonatal brain atlas |
| | CamBA |
| | PRISM (statistics package) |
| Developmental questionnaire | Ages & Stages Questionnaires® ASQ-3™, 12 month Questionnaire |
3.2 Study Design

Background

StOOPS (Short-term Outcome Of the PORTO Study) is a prospective nested cohort study of infants with intra-uterine growth restriction who were identified through participation in the PORTO study. The StOOPS study compares IUGR infants with abnormal Doppler measurements with IUGR infants with normal Doppler measurements by analysing 3.0 Tesla MRI brain images at term corrected age. As many of the serious long-term effects of IUGR affect neurodevelopment, cognition or neurodisability, early identification of difficulties is of paramount importance in order to access appropriate support services and optimise individual outcome (297-300). Studies using cranial ultrasound in premature infants have shown that major brain destruction by haemorrhagic parenchymal infarction and PVL predicts the later appearance of severe motor impairment. In contrast, however, cognitive outcome cannot be predicted as well by neonatal ultrasound in the absence of impairments in the motor domain (301). The StOOPS study provided an opportunity to investigate if term corrected MRI using sophisticated techniques could identify subtler anatomical correlates of cognitive dysfunction at term, identify which infants are at greatest risk and correlate with longer term outcome.

Since its approval by the US Food and Drug Administration (FDA) in 2000, brain MR imaging at 3.0 Tesla has been increasingly used in clinical practice. It allows for faster and better image acquisition, in a more detailed fashion than the conventional and more commonly used 1.5 Tesla. With the appropriate system protocols and software in place, the use of the stronger magnet is made as safe as with that of 1.5 Tesla. The FDA has analysed the use of MRI in general and found no significant concerns within appropriate specified parameters (last reviewed 2006). MRI scanning is routinely performed with magnetic fields at this 3.0 Tesla level in neonates and infants in both clinical practice and in the field of research (302, 303).
3.0 Tesla MRI offers many technical advantages over conventional 1.5 Tesla MRI that are important for the use of this scanner in the study. An increased magnet strength, such as with 3.0 T, results in an increased contrast to noise ratio when using the BOLD technique with functional MRI studies. This results in an improved sensitivity and specificity and may produce an increase detection in activation by up to 40\% (440). An increased signal to noise ratio (SNR) with 3.0 Tesla scanning can produce images of improved quality or over a decreased scan time as compared to a 1.5 Tesla scanner. Table 4 on the following page summarizes a number of the differences between a 1.5 T and a 3.0 T system and outlining a number of pros and cons for each system. Many of the negative technical issues associated with a 3.0 T system have software developed by the respective companies to overcome these challenges.
### Technical Issues

<table>
<thead>
<tr>
<th>3.0 T MRI PRO</th>
<th>3.0 T MRI CON</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Signal to Noise Ratio (SNR)</strong></td>
<td>Approx. twice that of SNR on 1.5T. Can improve image quality or decrease scan time</td>
</tr>
<tr>
<td><strong>Parallel Imaging (PI)</strong></td>
<td>PI techniques reduce scan time, but are accompanied by loss of signal. Image quality can be comparable to 1.5T due to abundance of signal at 3.0T</td>
</tr>
<tr>
<td><strong>Relaxation Times</strong></td>
<td>Increase T1 relaxation time for solid tissue results in overall improvement in blood vs background tissue contrast in MRA</td>
</tr>
<tr>
<td><strong>Spatial Resolution</strong></td>
<td>Higher spatial resolution results in improved image clarity and diagnostic strength</td>
</tr>
<tr>
<td><strong>Temporal Resolution</strong></td>
<td>Improved temporal resolution occurs with shorter scan time.</td>
</tr>
<tr>
<td><strong>Motion &amp; Breathing Artefacts</strong></td>
<td>Decreased scan time results in reduction in data artefacts</td>
</tr>
<tr>
<td><strong>Functional MRI using BOLD</strong></td>
<td>At 3.0T MRI clinical BOLD functional studies are robust and practical</td>
</tr>
<tr>
<td><strong>Diffusion Weighted Imaging (DWI)</strong></td>
<td>Increased sensitivity for detection of ischaemic lesions in acute stroke</td>
</tr>
<tr>
<td><strong>Spectroscopic Imaging</strong></td>
<td>Improved ability to visualise changes in peaks in metabolites.</td>
</tr>
<tr>
<td><strong>Imaging Coils</strong></td>
<td></td>
</tr>
</tbody>
</table>

### Safety Issues

<table>
<thead>
<tr>
<th>3.0 T MRI PRO</th>
<th>3.0 T MRI CON</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Immediate fringe field surrounding magnet</strong></td>
<td>A ferromagnetic object inadvertently brought near the scanner will experience a sharper increase in attraction toward the magnet versus a 1.5T MRI</td>
</tr>
<tr>
<td><strong>Implanted devices</strong></td>
<td>Not all 1.5T compatible devices have been tested at 3.0T</td>
</tr>
<tr>
<td><strong>Gradient noise</strong></td>
<td>Higher gradient performance at 3.0T MRI results in higher sound pressure levels</td>
</tr>
<tr>
<td><strong>Pulsed radiofrequency (RF) fields</strong></td>
<td>Heating potential is notably higher and more significant at 3.0T</td>
</tr>
</tbody>
</table>
PORTO Study

The PORTO (Prospective Observational Trial to Optimise paediatric health in intra-uterine growth restriction) study was an all-Ireland prospective observational multicentre study evaluating which antenatal sonographic findings were associated with perinatal morbidity and mortality in pregnancies affected by growth restriction, originally defined as estimated fetal weight (EFW) < 10th centile. Perinatal Ireland, a collaborative research consortium on the island of Ireland, conducted the study. Perinatal Ireland is funded through an Imaging Award from the Health Research Board and links 7 maternity hospitals across the island.

During the study period of January 2010 to June 2012 over 1100 consecutive ultrasound-dated singleton pregnancies with an EFW <10th centile were recruited. A number of definitions of IUGR were investigated during the study including EFW or abdominal circumference of <10th, <5th or < 3rd centiles, with or without oligohydramnios and with or without abnormal umbilical arterial Doppler measurement. Abnormal Doppler measurement included pulsatility index >95th centile, absent or reversed end-diastolic flow. Adverse perinatal outcome measures were documented for all cases.

Adverse perinatal outcome was defined as a composite outcome of intraventricular haemorrhage, periventricular leukomalacia, hypoxic ischaemic encephalopathy, necrotising enterocolitis, bronchopulmonary dysplasia, sepsis and or death. The main finding was that abnormal umbilical artery Doppler and EFW < 3rd centile were strongly and most consistently associated with adverse perinatal outcome (127). The study also found that multiple potential patterns of Doppler deterioration were observed as opposed to the previously suggested predictable progressive deterioration (144). A secondary analysis assessing cerebroplacental ratio (CPR) to predict adverse outcome was also performed. The CPR quantifies redistribution of cardiac output by dividing the Doppler indices of the middle cerebral artery (MCA) with that of the umbilical artery (UA). Cerebral redistribution is seen in the brain-sparing phenomenon, which had previously been assumed to be a protective mechanism. The analysis showed that regardless of the calculation method used evidence of brain sparing is significantly associated with an adverse perinatal outcome in IUGR (220).
This work, the StOOPS study, was designed to provide MRI outcome data for a subset of this cohort.

Methods

The StOOPS study is a multicentre prospective nested cohort study. It was conducted across 4 academic perinatal centres in the Republic of Ireland. The definition used in the PORTO study for IUGR was EFW < 10th centile based on sonographic measurements of fetal biparietal diameter, head circumference, abdominal circumference and femur length (Hadlock-4). This diagnosis was made by conventional population based growth standards (133). From July 2011, through to January 2013 120 infants with IUGR were consecutively recruited. Eligible candidates were identified through weekly contact with the Perinatal Ireland PORTO sonographer in each institution. The lead researcher, Dr. Michael Boyle (MB), recruited all candidates. Singleton infants identified as IUGR through the PORTO study with an EFW of < 10th centile and documented antenatal Doppler measurements of the umbilical artery were eligible for inclusion in the study. Two groups were studied 1) IUGR infants with abnormal antenatal umbilical arterial Doppler measurements and 2) IUGR infants with normal antenatal umbilical arterial Doppler measurements. Abnormal Doppler measurements included umbilical artery pulsatility index (UAPI) > 95th centile, absent and reversed end diastolic flow on umbilical artery Doppler measurement.

Infants with complex congenital anomalies, chromosomal disorders and primary brain abnormalities were excluded from the study, as were infants with either subsequent intraventricular haemorrhage Grade 3 or greater or cystic periventricular leucomalacia. Baseline demographic data, antenatal Doppler measurement results, delivery details and neonatal outcome data on all infants were collected. In addition, data from histopathological examination of the placenta was collected, as per current standard local clinical practice for IUGR pregnancies. A control group of full term appropriate for gestational age (AGA) low risk delivery infants was also recruited. The control group was a convenience sample recruited from the Rotunda Hospital in Dublin. Informed consent was obtained at time of recruitment and again on the day of scanning.
Travel expenses and parking on the day of scanning were reimbursed. A 3.0 Tesla MRI brain scan was performed at term corrected gestational age (from 39 to 44 weeks completed postmenstrual age). Term-corrected gestational age has been taken to be many different ranges across the literature and policy documents. Ranges such as 35-42 (NEURO study), 37-42, 38-44 have been quoted (441-443). Imaging at earlier gestations in the quoted ranges poses problems, as the first signs of myelination in the posterior limb of the internal capsule may not be present. The brain continues to actively develop beyond term-corrected, however, significant structural differences (such as degree of myelination or sulcation) would not be readily expected until approx. 3 months’ post term (444). All scans were performed on the same scanner in the Centre for Advanced Medical Imaging (CAMI) at St. James’s Hospital, Dublin. All participants had been discharged, attended the centre from home and had a detailed physical and neurological examination on the day of scanning performed by a clinician. Consultant paediatric radiologists, Dr. Stephanie Ryan and Dr. Ailbhe Tarrant, formally reported all MRI brain scans. Both were blinded to the patient group and postnatal history at the time of reporting. All scans were for research purposes and official reports were filed in patient charts. Clinically significant findings were referred to the consultant neonatologist at the discharging maternity hospital to arrange assessment, follow up and treatment if required. Follow up at 1 year corrected included measurement of an occipito-frontal circumference (OFC) and completion of the 12 month Ages and Stages Questionnaire. Longer-term psychological assessment is ongoing.

The participating centres included in the PORTO study were

- The Rotunda Hospital Dublin
- The National Maternity Hospital Dublin,
- The Coombe Women and Infants University Hospital Dublin,
- Cork University Maternity Hospital,
- University College Hospital, Galway,
- Mid-Western Regional Maternity Hospital Limerick,
- Royal Maternity Hospital Belfast.
The StOOPS study recruited from the units within the Republic of Ireland. Of the units within the Republic of Ireland, patients were ultimately recruited from the three maternity units in Dublin and Cork University Maternity Hospital. Eligible candidates from all the centres were approached, however, those from Limerick and Galway declined participation due to distance required to travel to the CAMI unit in Dublin.

Care of Participants

There was no deviation from standard care for the infants of this study from that of any other patient in the participating centres other than the MRI scan and clinical assessment at term. Infants born with IUGR in these centres were treated along the local protocols in effect in the individual centres and no deviation from these standard practices occurred with the infants enrolled in the StOOPS study. The MRI’s performed were for research purposes and reports were filed in patient’s charts. Any significant abnormality identified was referred to the discharging neonatologist at the maternity hospital for further assessment, follow up and treatment.

Treatment of Data

The antenatal data pertaining to the infants enrolled to StOOPS were obtained from the Fetal Medicine Department in each participating centre. Study data was entered onto a centralised Excel database stored in the Rotunda Hospital on a password-protected computer in a locked office in a swipe accessed non-clinical area. Patient details are anonymised and recorded under an allocated study number. This will be stored for 20 years. Any abnormal MRI scan was referred to MB and acted on immediately. Reports of MRI’s were forwarded to patient charts in phased blocks. Following all clinical and MRI data collection anonymisation was performed and subsequent analysis was conducted blinded to study groups.
Research Group

Principal investigators – Dr. Adrienne Foran and Professor Naomi McCallion
Lead investigator – Dr. Michael Boyle
Research nurse – Niamh Thorne S/N
Co-investigator – Dr. Stephanie Ryan
Co-investigator – Dr. Ailbhe Tarrant

Ethical Approval & Funding

Ethical approval was obtained from the following centres: The Rotunda Hospital, The National Maternity Hospital, The Coombe Women and Infants University Hospital, Cork University Maternity Hospital, University College Hospital Galway, Mid-Western Regional Hospital Limerick and the Children’s University Hospital, Temple Street, Dublin. The scientific committee at the Children’s University Hospital also approved the study. Ethical approval was also sought from St. James’s Hospital, Dublin as the host of the CAMI unit. Funding for the study was obtained from The Children’s Fund for Health with a project grant (PAC 11-53) of €185,790. The Children’s Fund for Health Ltd is the registered charity name of Temple Street Children’s University Hospital fundraising office. Charity Reg No: CHY 13534.

3.3 Patient Recruitment

Eligible infants were identified with the assistance of Perinatal Ireland’s existing personnel and infrastructure in each participating unit. During the course of antenatal monitoring PORTO sonographers informed parents of the StOOPS MRI study and that they may be contacted around the time of delivery. The PORTO sonographers contacted MB on delivery of an infant with an estimated fetal weight < 10th centile. Weekly contact was also made to identify impending deliveries. On admission, or after delivery, women participating in the PORTO study were approached regarding the MRI study. MB travelled to all centres to recruit. Parent information leaflets were distributed, and if interested in being involved in the study parental contact details were obtained. Consent was taken after delivery of the infant.
Contact was maintained with the relevant neonatal intensive care unit (NICU) regarding postnatal course. Contact was made two weeks prior to proposed MRI scan date to arrange a suitable time and confirm continued interest in the study. Control subjects were recruited from the host institution, the Rotunda Hospital. These were term infants with birth weights appropriate for gestational age, born by spontaneous vaginal delivery or elective lower segment caesarean section (LSCS), without complex congenital abnormalities, chromosomal disorders or primary brain abnormalities.
3.4 Clinical Information Gathering

Figure 11. Clinical information gathering algorithm
3.5 Scanning

The MRI scans were performed in the Centre for Advanced Medical Imaging (CAMI) at St. James’s Hospital, Dublin. The unit was established in 2008 following an infrastructural award to develop research magnetic resonance imaging (MRI) in Ireland secured from the Health Research Board. It is a joint venture between Trinity College Dublin and St. James’s Hospital. CAMI provides MRI infrastructure for patient-focused research studies and clinical trials aimed at improving understanding and diagnosis of diseases. Research at CAMI strives to improve patient health through development and execution of state-of-the-art imaging research and aims to deliver direct and rapid translation from bench to bedside. The unit is equipped with a Philips Achieva 3T MRI system, equipped with 32 RF receiver channels and has experienced consultant radiologist, MRI physicists, and radiographer support.

StOOPS was the first paediatric imaging study to be undertaken at the CAMI and as the host institution does not offer paediatric services, ethical approval was required prior to commencing the study. A fully equipped neonatal resuscitation pouch was produced and stored at CAMI for the duration of the study. The MRI scans were performed with a ‘Feed and Wrap’ technique, avoiding the need for sedation. For each study a NRP certified paediatrician (MB) and paediatric intensive care nurse (NT) were present. The Neonatal Resuscitation Program (NRP) is the resuscitation guideline used for newborn life support in the Republic of Ireland. The National Neonatal Transport Programme were consulted prior to commencing the study and agreed to provide transport support should it be required.

Procedure

At the time of enrolment an expected date for MRI and planned future contact were discussed. Two weeks prior to the expected date parents were contacted to confirm continued interest and arrange an exact date and time for the scan. Directions to the unit and instructions regarding feeding were given. The week and day before the scan a text message with date and time confirmation was sent and a contact number offered should the parents need to reschedule or wish to withdraw.
On arrival to the unit, the research team met parents and the study was again discussed and consent confirmed. A clinical assessment was performed prior to imaging using a validated neonatal neurological examination tool, a modified Dubowitz assessment. This assesses tone and posture (10 different tests), tone patterns (5 tests), reflexes (6 reflexes), spontaneous movements (3 types assessed), abnormal signs (3) and behaviours (7 elements) (304). A systemic examination was also performed assessing health, looking for dysmorphism, cutaneous lesions, organomegaly and normality of genitalia. Head circumference and weight were measured and a metal check performed.

The scans were carried out in natural sleep immediately after a feed, when they had been comfortably swaddled. This is a frequently used method for neonatal MR imaging and a standard procedure for performing MRI scans in term corrected infants (302, 305, 306). Timing of feeds was discussed with parents prior to arrival to ensure the infant was hungry and due a feed and parents were instructed to keep the infant awake, where possible, before arrival. Prior to feeding with the usual feed of the infant, E-A-R EarSoft Yellow Neons UF foam earplugs were gently inserted and secured. These have a noise reduction level of 33 decibels. Natus MiniMuffs® Neonatal noise attenuators were then placed around the ear. These have a noise reduction level of 7 decibels. Once the infant was sleeping they were placed on the Noras VacuChild mattress on the scanning table. The suction mattress molded around the body and head of the baby keeping them snug and minimising movement. Foam wedges were placed around the head keeping the protectors in place, supporting the noise attenuating measures employed. Based on studies using 3.0 tesla scanners and similar auditory protective devices, the sound levels experienced by the infants were approximately 70 decibels (307). A Medrad® Veris® paediatric clip pulse oximetry probe was attached to the infant’s foot, which allowed continuous monitoring of infant pulse and oxygen saturations throughout the course of the scan. At this point the scan was commenced. Parents were not permitted to stay within the scanning room during the procedure, but were permitted to watch from the console room.
Figure 12. Infant swaddled and placed in vacuum mattress. Parental consent was obtained for the unrestricted use of these images.
3.6 MRI Sequences

The scanning protocol was as follows.

1. **Survey**
   
The Survey scan is performed to identify head position and for positioning image planes for subsequent sequences.

2. **Ref_scan** (Reference Scan)
   
   A reference scan is acquired prior to clinical data acquisition to measure coil sensitivity profiles.

3. **T1 3D_ NeoNat_new**
   
   A T1 weighted image (also referred to as T1WI or "spin-lattice" relaxation time) one of the basic pulse sequences in MRI and demonstrates differences in the T1 relaxation times of tissues. T1 weighted images in neonates are very useful for demonstrating normal myelination.

4. **DWI_ NeoNat** (Diffusion Weighted Imaging)
   
   A sequence based upon measuring the random Brownian motion of water molecules within a voxel of tissue. In the neonatal brain restricted motion can be detected in both myelinated and unmyelinated white matter tracts and can be used to study changes in global and focal ischaemic injury to the neonatal brain.

5. **T2 VISTA 0.9mm** (Volumetric ISotropic T2 weighted Acquisition)
   
   A T2 weighted 3D fast spin echo sequence that is useful for imaging developing white matter. Used for isotropic volumetric imaging, which supports post image acquisition reformatting.

6. **DTI_ NeoNat** (Diffusion Tensor Imaging)
   
   A sequence that enables the measurement of the restricted diffusion of water in tissue in order to produce neural tract images.

7. **T2*FFE_haem** (Fast Field Echo_haemorrhage)
   
   A gradient echo sequence with T2*-based contrast used to depict haemorrhage, calcification, and iron deposition in various tissues and lesions.

8. **3DI_COW** (Circle of Willis)
   
   A time of flight angiography MRI technique allowing imaging of the circle of Willis without the need of a contrast medium.
9. **SWI_EPI** (Susceptibility Weighted Imaging_Echo Planar Imaging)
SW imaging is an MR imaging technique that exploits the magnetic susceptibility differences of the blood, and iron and calcification in various tissues.

10. **SV_PRESS_35** (Single Voxel_Point Resolved SpectroScopy)
Spectroscopy provides a measure of brain chemistry and uses the same principles as MRI but rather than generating an image, a plot representing chemical composition of a region is generated.

11. **3D_MTR** (Magnetisation Transfer Ratio)
Magnetisation transfer (MT) is a magnetic resonance imaging phenomenon based on the interaction between immobile protons in macromolecules and free water protons of tissues. Although MT is not specific to a particular molecule, there is evidence that molecules associated with myelin dominate the exchange process in the white matter and determine the grey matter (GM)-WM contrast seen at brain MR imaging. MTR measurement taken alone provides a reproducible measurement that is sensitive to myelination in tissue and may provide an index of brain maturation.

12. **fMRI_Resting** (Resting state Functional MRI)
A method of functional brain imaging used to evaluate regional interactions that occur when a subject is not performing an explicit task. Resting brain activity is observed through changes in blood flow in the brain, which creates a blood-oxygen-level dependent (BOLD) signal. Resting-state functional connectivity reveals networks representing specific patterns of synchronous activity.

13. **T2 MV NeoNat** (T2 MultiVane)
MultiVane employs an in-plane motion correction strategy that uses a novel MR signal sampling trajectory in k-space. This sequence was used in cases if there was significant motion artefact.

Not all of the study infants had every sequence performed. The resting state fMRI sequence was placed last as it was the loudest sequence of the study and the motion correction T2 sequence was only used if there had motion artefact on the T2 VISTA sequence.
The mean length of time spent in the scanning room was 55 minutes with a range of 10 to 83 minutes. This time includes periodically checking on infants, resettling if woken and top up feeds in some instances.

3.7 Image Acquisition

A 3D Inversion Recovery prepared Spoiled Gradient Recalled echo (IR-SPGR) sequence was used to obtain high resolution $T_1$-weighted images of the brain, with: Field of view (FOV) = 200 x 161 x 110 mm$^3$, isotropic spatial resolution of 1 mm, TR/TE = 17/4.6 ms, TI = 800 ms, flip angle = 13°, SENSE factor = 2, acquisition time = 3 min 53 s. $T_2$-weighted images were acquired using a 3D VISTA (turbo spin echo) sequence with: FOV = 200 x 161 x 110 mm$^3$, isotropic spatial resolution of 0.9 mm, TR/TE$^{\text{eff}}$ = 2500/452 ms, SPIR fat suppression, SENSE factor = 2, acquisition time = 5 min 28 s. In some subjects where motion artefacts were evident on the $T_2$-weighted images, the acquisition was repeated using a motion-insensitive radial turbo spin echo sequence (2D MultiVane) with: FOV = 160 x 160 x 101 mm$^3$, spatial resolution = 0.4 x 0.4 x 2 mm$^3$, TR/TE$^{\text{eff}}$ = 4000/142 ms, SPIR fat suppression, and acquisition time = 4 min 12 s.

A fast gradient recalled echo $T_2^*$ sequence was acquired with: FOV = 160 x 160 x 98 mm$^3$, spatial resolution = 0.8 x 1.2 x 2 mm$^3$, 38 slices with an interslice gap of 0.6 mm, TR/TE$^{\text{eff}}$ = 1172/16 ms, SENSE factor = 2, NSA = 2, acquisition time = 5 min 28 s.

A high-resolution time-of-flight angiography sequence was acquired covering the Circle of Willis, with the following acquisition parameters: 3D SPGR sequence, FOV = 150 x 108 x 60 mm$^3$, spatial resolution = 0.3 x 0.6 x 0.6 mm$^3$, TR/TE$^{\text{eff}}$ = 25/3.45 ms, Flip angle = 20°, SENSE factor = 2, acquisition time = 2 min 54 s. A separate 3D SPGR sequence was acquired to assess the magnetisation transfer ratio, with: FOV = 160 x 160 x 89 mm$^3$, spatial resolution = 1.4 x 1.5 x 2.4 mm$^3$, TR/TE$^{\text{eff}}$ = 113/4.3 ms, Flip angle = 18°, SENSE factor = 2, with/without an off-resonance radiofrequency excitation pulse, in an acquisition time of 2 min 06 s.
Two SE-EPI diffusion weighted imaging (DWI) acquisitions were performed. The first was a multi-b-value sequence to quantify the apparent diffusion coefficient (ADC) with: FOV = 180 x 180 x 89 mm$^2$, spatial resolution = 1.7 x 1.8 x 2 mm$^3$, 30 slices with 1 mm interslice gap, TR/TE = 4218 / 95 ms, SENSE factor = 2, b-values = 0, 500, 1000 s/mm$^2$, SPIR fat suppression, NSA = 2, acquisition time = 1 min 54 s. The DTI sequence was similar except for: 32-diffusion encoding directions, with b-values = 0 and 1000 s/mm$^2$, TR/TE = 5599 / 67 ms.

Functional connectivity was assessed from a resting state fMRI acquisition with: SE-EPI sequence, FOV = 180 x 180 x 93 mm$^3$, spatial resolution 2.2 x 2.2 x 3 mm$^3$, 28 slices with an interslice gap of 0.35 mm, TR/TE = 2000/28 ms, SPIR fat suppression, SENSE factor = 2, 180 dynamics acquired with dynamic stabilisation ON in an overall acquisition time of 6 min 6 s.

Single voxel MRS was performed with the voxel placed over the basal ganglia region, using the following acquisition parameters: 3D PRESS sequence, voxel size = 18 x 10 x 10 mm$^3$, TR/TE = 2000/36 ms, 2000 Hz spectral bandwidth, NSA = 128, acquisition time = 4 min 52 s.

The imaging sequences were carefully tailored to minimise acoustic noise, for example by reducing the amplitude and switching rate of the magnetic field gradients (particularly beneficial in sequences based on echo planar imaging such as diffusion weighted imaging and resting-state fMRI scans).
3.8 Patient Groupings

The study was composed of three groups: IUGR infants with abnormal Doppler’s IUGR infants with normal Doppler’s and a control group. Infants were recruited from July 2011 to January 2013.

![Flow chart of StOOPS Infants recruitment](image)

Figure 13. Flow chart of StOOPS Infants recruitment
From the 120 prospective study subjects enrolled in to the StOOPS study 48 withdrew prior to the MRI brain scan being performed. Of the 48 who withdrew from the study 37 had changed their minds after discharge from the hospital. The reasons given included the distance required to travel, not wanting to bring their baby for a research scan, feeling that the child had been through enough in hospital and concern regarding the noise from the MRI scanner. A further 10 had an MRI scan appointment arranged, 4 of these failed to attend on the day despite phone call discussions and text message reminders prior to the scan. Subsequent efforts at contact to reschedule were unsuccessful. The remaining 6 had an MRI scan appointment arranged and made contact prior to the scan to reschedule and subsequent efforts at contact to reschedule were unsuccessful.

There were 30 control subjects enrolled over the course of the recruitment period. Ultimately 11 control scans were performed. The 19 prospective controls subjects who withdrew changed their minds on reflection once at home. The main reason given for change of mind in the control group was not wanting to have an MRI scan performed on their child at such a young age and the perceived disruption to the established routine.

Table 5. Study groups – Demographics
Gestational age (GA), Occipitofrontal circumference (OFC).

<table>
<thead>
<tr>
<th></th>
<th>Abnormal Doppler</th>
<th>Normal Doppler</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=34</td>
<td>N=29</td>
<td>N=11</td>
</tr>
<tr>
<td>Infant Characteristics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth Weight (kg)</td>
<td>1.21 ± 0.46</td>
<td>1.91 ± 0.51</td>
<td>3.3 ± 0.29</td>
</tr>
<tr>
<td>Birth Weight Centile</td>
<td>6th</td>
<td>5th</td>
<td>32nd</td>
</tr>
<tr>
<td>GA at Delivery (weeks)</td>
<td>31.4 ± 3.1</td>
<td>36.1 ± 3.2</td>
<td>39.1 ± 1.2</td>
</tr>
<tr>
<td>Sex (Female)</td>
<td>13 (38%)</td>
<td>15 (52%)</td>
<td>5 (45%)</td>
</tr>
<tr>
<td>OFC</td>
<td>27.2 ± 3.1</td>
<td>30.8 ± 2</td>
<td>34.0 ± 1.1</td>
</tr>
<tr>
<td>OFC Centile</td>
<td>13rd</td>
<td>14th</td>
<td>26th</td>
</tr>
</tbody>
</table>
Table 6. Infant and Maternal Demographics and Neonatal Outcome Data. Note: Continuous data presented at mean ± SD or median [inter-quartile range] Spontaneous vaginal delivery (SVD), Caesarean section (CS), Gestational age (GA), Occipitofrontal circumference (OFC), Patent ductus arteriosus (PDA), Cranial ultrasound scan (CRUSS), Retinopathy of prematurity (ROP), Necrotising enterocolitis (NEC), Neonatal intensive care unit (NICU).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>N=74</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (years)</td>
<td>32.1 ± 5.4</td>
</tr>
<tr>
<td>Parity</td>
<td></td>
</tr>
<tr>
<td>Primiparous</td>
<td>16 (22%)</td>
</tr>
<tr>
<td>Multiparous</td>
<td>58 (78%)</td>
</tr>
<tr>
<td>Smoker</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>62 (84%)</td>
</tr>
<tr>
<td>Yes</td>
<td>12 (16%)</td>
</tr>
<tr>
<td>Serology</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>73 (99%)</td>
</tr>
<tr>
<td>Positive</td>
<td>1 (1%)</td>
</tr>
<tr>
<td>Antenatal Steroids</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>24 (32%)</td>
</tr>
<tr>
<td>Yes</td>
<td>50 (68%)</td>
</tr>
<tr>
<td>Delivery</td>
<td></td>
</tr>
<tr>
<td>SVD</td>
<td>22 (30%)</td>
</tr>
<tr>
<td>Instrumental</td>
<td>3 (4%)</td>
</tr>
<tr>
<td>Elective CS</td>
<td>9 (12%)</td>
</tr>
<tr>
<td>Emergency CS</td>
<td>40 (54%)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>33 (45%)</td>
</tr>
<tr>
<td>Male</td>
<td>41 (55%)</td>
</tr>
<tr>
<td>Apgar &lt;7</td>
<td></td>
</tr>
<tr>
<td>1 min</td>
<td>15 (20%)</td>
</tr>
<tr>
<td>5 min</td>
<td>4 (5%)</td>
</tr>
<tr>
<td>Placenta</td>
<td></td>
</tr>
<tr>
<td>Weight (g)</td>
<td>283 ± 119</td>
</tr>
</tbody>
</table>

Neonatal Characteristics at Delivery

| GA at delivery (weeks)       | 34.3 ± 4.1 |
| Weight (kg)                 | 1.79 ± 0.84 |
| OFC (cm)                    | 29.6 ± 3.5 |

Neonatal Outcomes

| Ventilator (1 or more days) | 18 (24%) |
| Infection (culture +)       | 6 (8%)   |
| Antibiotic (1 or more days) | 40 (54%) |
| PDA treatment               | 5 (7%)   |
| CRUSS                       | 46 (62%) |
| ROP                         | 2 (3%)   |
| NEC                         | 7 (9%)   |
| Delayed initiation feeds (> day 1) | 34 (46%) |
| Delayed full feeds (> day 1)  | 47 (64%) |
| NICU stay (days)            | 13 [5 - 44] |

Measurements at MRI

| Weight at MRI (days)         | 3.4 ± 0.6  |
| OFC at MRI (mm)              | 35.7 ± 1.4 |
Table 7. Recruiting Hospital and Social Demographics
National Maternity Hospital, Holles St (NMH), Cork University Hospital (CUH), Not available (N/A)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>N=74</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Centre</strong></td>
<td></td>
</tr>
<tr>
<td>Coombe</td>
<td>6 (8%)</td>
</tr>
<tr>
<td>NMH</td>
<td>12 (16%)</td>
</tr>
<tr>
<td>Rotunda</td>
<td>54 (73%)</td>
</tr>
<tr>
<td>CUH</td>
<td>2 (3%)</td>
</tr>
<tr>
<td><strong>Parental</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Ethnicity</strong></td>
<td></td>
</tr>
<tr>
<td>Irish</td>
<td>44 (59%)</td>
</tr>
<tr>
<td>Non-Irish</td>
<td>23 (31%)</td>
</tr>
<tr>
<td>N/A</td>
<td>7 (9%)</td>
</tr>
<tr>
<td>Combined</td>
<td>27 (36%)</td>
</tr>
<tr>
<td>Parental</td>
<td></td>
</tr>
<tr>
<td>Irish/Non-Irish</td>
<td>3 (4%)</td>
</tr>
<tr>
<td>Both non-Irish</td>
<td>18 (24%)</td>
</tr>
<tr>
<td>N/A</td>
<td>26 (35%)</td>
</tr>
<tr>
<td><strong>Socioeconomic</strong></td>
<td></td>
</tr>
<tr>
<td>Socio-economic class</td>
<td>Maternal</td>
</tr>
<tr>
<td>1</td>
<td>2 (3%)</td>
</tr>
<tr>
<td>2</td>
<td>13 (18%)</td>
</tr>
<tr>
<td>3</td>
<td>10 (14%)</td>
</tr>
<tr>
<td>4</td>
<td>4 (5%)</td>
</tr>
<tr>
<td>5</td>
<td>2 (3%)</td>
</tr>
<tr>
<td>6</td>
<td>4 (5%)</td>
</tr>
<tr>
<td>7</td>
<td>18 (24%)</td>
</tr>
<tr>
<td>10</td>
<td>13 (18%)</td>
</tr>
<tr>
<td>N/A</td>
<td>8 (11%)</td>
</tr>
<tr>
<td><strong>Marital/Family Status</strong></td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>28 (38%)</td>
</tr>
<tr>
<td>Single + partner</td>
<td>12 (16%)</td>
</tr>
<tr>
<td>Single/separated</td>
<td>20 (27%)</td>
</tr>
<tr>
<td>N/A</td>
<td>14 (19%)</td>
</tr>
</tbody>
</table>
Table 8. Demographic and Outcome Data by Group.

Gestational age (GA), Occipitofrontal circumference (OFC), Patent ductus arteriosus (PDA), Cranial ultrasound scan (CRUSS), Retinopathy of prematurity (ROP), Necrotising enterocolitis (NEC).

<table>
<thead>
<tr>
<th></th>
<th>Abnormal Doppler</th>
<th>Normal Doppler</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=34</td>
<td>N =29</td>
<td>N=11</td>
</tr>
<tr>
<td>Maternal and Delivery Characteristics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maternal Age (years)</td>
<td>32 ± 5.5</td>
<td>31.9 ± 5.7</td>
<td>32.6 ± 4.2</td>
</tr>
<tr>
<td>Parity (Primigravida)</td>
<td>7 (21%)</td>
<td>5 (17%)</td>
<td>4 (36%)</td>
</tr>
<tr>
<td>Smoker</td>
<td>2 (6%)</td>
<td>10 (34%)</td>
<td>0</td>
</tr>
<tr>
<td>Serology (Positive)</td>
<td>0</td>
<td>1 (3%)</td>
<td>0</td>
</tr>
<tr>
<td>Antenatal Steroids</td>
<td>34 (100%)</td>
<td>16 (55%)</td>
<td>0</td>
</tr>
<tr>
<td>Delivery</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Emergency Section</td>
<td>29 (85%)</td>
<td>10 (35%)</td>
<td>1 (9%)</td>
</tr>
<tr>
<td>Elective Section</td>
<td>4 (12%)</td>
<td>3 (10%)</td>
<td>2 (18%)</td>
</tr>
<tr>
<td>Assisted</td>
<td>1 (3%)</td>
<td>1 (3%)</td>
<td>1 (9%)</td>
</tr>
<tr>
<td>Spontaneous Vaginal</td>
<td></td>
<td>15 (52%)</td>
<td>7 (64%)</td>
</tr>
<tr>
<td>Apgar &lt; 7 at 5 min</td>
<td>3 (9%)</td>
<td>1 (3%)</td>
<td>0</td>
</tr>
<tr>
<td>Placenta</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight</td>
<td>213.1 ± 68</td>
<td>344.2 ± 104.5</td>
<td></td>
</tr>
<tr>
<td>Neonatal Outcomes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ventilation (&gt; 1 day)</td>
<td>13 (1-24)</td>
<td>4 (1-3)</td>
<td>1 (3)</td>
</tr>
<tr>
<td>Infection (culture +)</td>
<td>6 (18%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Antibiotics (&gt; 1 day)</td>
<td>26 (2 - 42)</td>
<td>12 (2-7)</td>
<td>1 (7)</td>
</tr>
<tr>
<td>Treated PDA</td>
<td>3 (9%)</td>
<td>2 (7%)</td>
<td>0</td>
</tr>
<tr>
<td>CRUSS</td>
<td>31 (91%)</td>
<td>14 (48%)</td>
<td>1(9%)</td>
</tr>
<tr>
<td>ROP</td>
<td>2 (6%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>NEC</td>
<td>6 (18%)</td>
<td>1 (3%)</td>
<td>0</td>
</tr>
<tr>
<td>Delayed Feed &gt; 1 day</td>
<td>28 (82%)</td>
<td>7 (24%)</td>
<td>0</td>
</tr>
<tr>
<td>Time to Full Feed</td>
<td>13.5 ± 6.9</td>
<td>5 ± 5.9</td>
<td>1.6 ± 2.1</td>
</tr>
<tr>
<td>Length of Stay</td>
<td>39 (5 – 122)</td>
<td>8.5 (1 – 69)</td>
<td>3 (2 – 9)</td>
</tr>
</tbody>
</table>

NEC was included if determined by treating physician/unit and patient was treated as such (nil by mouth, IV antibiotics. CRUSS refers to whether an ultrasound was performed. ROP refers to retinopathy stage requiring laser surgery.
3.9 Placental Histology

As part of the PORTO study all the study subjects from StOOPS had placental histology performed. From the Abnormal Doppler group (AbD) 33/34 had a placental histological assessment and 28/29 from the Normal Doppler (ND) group. In each group two subjects had no features to suggest a cause for intra-uterine growth restriction. Of the two placentas from the AbD group without features suggesting a cause for IUGR, one had a minor infarct and the other a microscopic fetal artery occlusion. In the ND group, one had evidence of acute suppurative amnionitis and the other was described as normal. In the AbD group 30/33 (91%) had features of chronic uteroplacental insufficiency (CUPI) whereas 17/28 (61%) of the ND group had evidence of CUPI. A description of CUPI was made on identifying accelerated villous maturation, distal villous hypoplasia and an increased number of syncytiat knots. These features are well described as being associated/causative of intra-uterine growth restriction (308, 309). For the other findings, suggestive of IUGR there was one case of chronic villitis in the AbD group and in the ND group there were four cases with chronic villitis, four with evidence of impaired fetal circulation and one case with a large retroplacental haemorrhage. Impaired fetal circulation was described in the presence of villous oedema, villous sclerosis and/or an increased number of nucleated erythroblasts suggestive of fetal hypoxic exposure.

In the AbD group 31/33 (94%) had histological features supporting a diagnosis of IUGR and 26/28 (93%) of the ND group had histological features supporting a diagnosis of IUGR. There was no difference between the groups in terms of proportions with a placental assessment indicating a diagnosis of IUGR ($\chi^2 = 0.029$, $p = 0.865$), supporting the view that both groups were affected by intra-uterine growth restriction as opposed to the ND group being small for gestational age.
3.10 Statistics

In the absence of data from directly comparable studies, calculation of the sample size required was performed using estimates of the relevant parameters from broadly similar research (252, 279, 294, 310, 311). In addition, techniques and parameter estimates from the paper “Estimating sample size in functional MRI (fMRI) neuroimaging studies: Statistical power analysis” (Desmond and Glover 2002) were employed (312). To achieve 95% power, and with \( \alpha = 0.05 \), varying the other parameters (inter-subject variation \( \sigma_B \), intra-subject variation \( \sigma_W \) and estimated difference between means of the experimental and control condition \( \mu_D \)), between reasonable upper and lower values, yields a sample size of 30 to 40 per group.

Multivariate analyses using the principle of the general linear model were used to adjust for the following co-variates, modelled as continuous variables: age-at-scan, gestational age at birth and birthweight. On this basis, the degree of prematurity was adjusted within the statistical model using the gestational age at birth.

3.11 Analysis

3.11.1 Functional MRI

There are a number of post-processing techniques available for identifying the spatial patterns of coherent BOLD activity found in fMRI data. These are used to assess for functional connectivity between spatially distributed parts of the brain and included seed-based functional connectivity, independent component analysis, clustering, pattern classification and graph theory. In this project seed-based functional connectivity analysis was used. Seed-based functional connectivity analysis correlates between activity in an \textit{a priori} region of interest (ROI) and activity in the rest of the brain, or with the average time series of several distributed ROI’s. It was first used in the mid 90’s when resting-state functional connectivity based on a seed region in the motor cortex demonstrated replicatable patterns with motor task activation (313). There are two core elements to the technique. First the extraction of a time-series from a specified area and then quantifying the similarity between the time series extracted with the time series from other voxels or ROI’s.
Once the time series from the ROI has been extracted several statistical techniques, based on the general linear model (GLM), are available for quantifying the relationship or degree of correlation between the seed or ROI and other voxels or ROI’s. The matrix of correlation coefficients produced can then be interpreted as the functional connectivity of the selected seed region. A high degree of correlation or high correlation coefficient indicates a strong functional connectivity to that area. Several preprocessing steps are required prior to performing the seed-based functional connectivity analysis. The FMRIB Software Library (FSL) tools were used to prepare the raw data (314). First the non-brain tissue, such as skull, is removed using the Brain Extraction Tool (BET) from the structural image (315). Then FAST (FMRIB's Automated Segmentation Tool) is used to segment the 3D image of the brain into different tissue types (Grey Matter, White Matter, CSF, etc.), whilst also correcting for spatial intensity variations (also known as bias field or RF inhomogeneities). The structural and functional images are registered together using FLIRT (FMRIB Linear Image Registration tool). Then from each seed region of interest the mean time series are calculated by averaging across all voxels within the region with a command line tool called fslmeants (FSL mean time-series). Multiple regression analyses are then performed for each participant using FSL FEAT (fMRI Expert Analysis Tool). The CONN functional connectivity toolbox was also used to measure seed to voxel connectivity maps and ROI to ROI connectivity.

3.11.2 Volumetric Analysis

Voxel based morphometry (VBM) was used for the volumetric analysis performed. This is a neuroimaging technique based on statistical parametric mapping (SPM). The aim of VBM is to identify differences in regional concentrations of grey matter at a local level once global shape differences have been accounted for. The resultant images should be of a high resolution so that partial volume effects do not affect the grey matter extraction, where voxels contain a mixture of differing tissue types. There are a number of steps involved in VBM. The images are normalised to the same stereotactaic space, the grey matter is extracted from the normalised images and the resulting images are smoothed prior to analysing for differences between groups (316).
The spatial normalisation step involves transforming the data acquired from each subject to the same stereotactic space. A stereotactic space is one where the precise position is known within a three-dimensional space. Normalisation is performed by registering each image to the same template image, minimising the residual sum of squared differences between them. Initially, images are matched by estimating the optimum 12-parameter affine transformation using a Bayesian framework based on the knowledge of the normal variability of brain size. Next a nonlinear registration is performed which minimises the residual squared difference between the image and the template as the smoothness of the deformation is maximised. During this process the aim is not for the normalisation to match up the subject with the template perfectly, but rather to correct for global brain shape differences; perfectly exact spatial normalisation would result in the images appearing identical, rendering it impossible to identify significant differences between the subject and the template. Once spatial normalisation has been performed the images are partitioned into the various tissue types such as grey matter, white matter, CSF etc. The next step in the process involves smoothing the grey matter images (Fig.14). Smoothing means that data points are averaged with their neighbours, which results in low pass filtering – high frequencies of the signal are removed whilst lower frequencies are enhanced. Consequently, the sharp edges or boundaries between regions become blurred and improve spatial correlation within the data (317). Convolving with a Gaussian function of a specific width smooths the grey matter images. The Gaussian kernel is a kernel with the shape of a normal distribution curve. The size of the Gaussian kernel defines the width of the curve and as such how much the data is smoothed. The width is expressed as Full Width at Half Maximum (FWHM). Voxel-by-voxel analysis is then comparable to a region of interest approach, as each voxel in the smoothed image contains the average concentration of the grey matter from around the voxel. The smoothing step also compensates for the lower level errors resultant to the inexact nature of the spatial normalisation and improves the signal to noise ratio (SNR) thereby increasing sensitivity. Differences in volume of regions of interest between groups can be assessed by performing a series of t tests at every voxel in the image. Regression analyses are performed across voxels to assess neuroanatomical correlates of cognitive or behavioural deficits if available.

3.12 Follow up

Multiple contact details were obtained for each infant to facilitate longer-term paediatric follow-up. Parents of infants involved in the StOOPS study were told about the 1-year questionnaire and head circumference measurement. Prior to reaching the first birthday parents were contacted about completing the questionnaire on infant development and postal address confirmed. A 12-month Ages and Stages Questionnaire was sent to the infant’s home with a stamped addressed envelope and instructions for completion. A request for measurement of occipito-frontal head circumference (OFC) was included with the questionnaire, and parents were asked to have their GP or public health visitor to perform the measurement at their next visit or vaccination appointment.

The Ages and Stages Questionnaire (ASQ) is a parent-completed questionnaire aimed at estimating the developmental status of infants and young children (318). The questionnaire partly uses parental recall of their child’s abilities and partly instructs parents to test certain tasks at the time of filling in the questionnaire. The ASQ child monitoring system is composed of 19 questionnaires covering the age range of 4 to 60 months.
The ASQ is designed as a screening tool with six questions in each of five domains (communication, gross motor skills, fine motor skills, problem solving and personal social skills). The questions can be answered: ‘yes’ (10 points), ‘sometimes’ (5 points) and ‘not yet’ (0 points). The maximum score is 60 points within each domain. Referral for further evaluation is recommended if the score falls below a given cut-off score (set at – 2 SD from average) in any domain. The items in each questionnaire are chosen to represent a developmental quotient (DQ) of 75-100. The ASQ is composed of questions from the Bayley Scales of Infant Development, Gesell and Developmental Resources: Behavioural Sequences for Assessment and Program Planning (319).

The anthropometric measurements are expressed in both absolute measurements as well as using z scores. Methods using standardised scores such as z-score have the advantage of individualising a growth chart and monitor growth of an individual child with reference to his/her own growth potential and at the same time with reference to population standards. Z-scores also have the advantage of accommodating extreme values of growth. Emerging evidence indicates that z-score is moderately correlated with neurodevelopmental outcome in childhood whereas other measures have failed to achieve significance (445).

As part of the PORTO study all enrolled infants will have a formal psychological assessment beyond 2 years of age, which will be compared with the MRI findings. The 2 year follow up data does not form part of this thesis, but will provide further interesting findings long-term.
Section 3: Results

Chapter 4

Clinical MRI Reports
Chapter 4. Results

Clinical MRI reports

4.1 Introduction

Infants with intrauterine growth restriction have higher rates of later neurodevelopmental issues, especially if combined with prematurity, when compared to appropriate for gestational age weight infants. Structural and functional abnormalities can be demonstrated using magnetic resonance imaging techniques. Hippocampal volume, intracranial volume and cortical grey matter volume reduction have been demonstrated in infants with IUGR (229, 252). Increased rates of cerebral palsy are seen in infants affected by IUGR and may have associated predictive cerebral lesions. These differences may not be apparent with routine clinical imaging. Over the past decade there has been a steady increase in the number of studies of brain development using MRI as this produces high-resolution images without harmful ionising radiation. Image reporting from research studies is highly varied. Funding bodies may stipulate that scans be formally reported; the process by which this occurs varies greatly. Within research studies scans may be cohered together, reported in blocks and often by research fellows with neuroradiology input if suspicious areas are noted (321). All infants in the StOOPS study had formal clinical reports for the MRI scans performed. The StOOPS study investigated for microstructural and functional abnormalities in IUGR infants comparing between IUGR infants with abnormal and normal Doppler’s, however, clinical reports were also reviewed to examine for macrostructural abnormalities and incidental findings.

4.2 Materials and Methods

Two consultant paediatric radiologists (SR and AT) with experience in neonatal MRI neuroimaging formally reported all MRI scans. A reporting framework was devised to standardise reporting and the scans were reported as if requested as clinical scans.
The template included 11 points; Quality of the scan was graded as good, average or poor. Myelination was graded as appropriate or not for term corrected gestation. Midline structures were commented on as normal or not. Ventricles and Parenchyma were described as either normal or abnormal. White matter volume (WMV) was assessed as either being preserved or reduced. Posterior Fossa contents were described and Extra-axial fluid spaces were assessed. The Susceptibility weight imaging (SWI) sequence was assessed for signs of previous haemorrhage or evidence of calcification; abnormalities identified on the diffusion sequences were described and any abnormality noted on the Magnetic Resonance Angiography (MRA) sequence were also described. The radiologists were blinded to the grouping of the study participants and the gestation at birth. The breakdown of the reports is provided in Table 9 and the comments made are expanded on according to group.

4.3 Results

**Control Group**
All studies were reported to be of good quality and all studies were complete except for one where the MRA was not performed. The comments made on the Control scans are as follows.

**Ventricles**
In one study a comment was made noting small spots in the lateral wall of the lateral ventricles, consistent with blood products, but may have been motion artefact. The rest of the study was normal.

**Susceptibility Weighted Imaging**
Two scans had comments regarding the SWI sequence.
1) Commented that it was of poor quality due to motion artefact and not reportable. The rest of the study was normal.
2) The SWI sequence showed 2 small foci of haemorrhage or calcification in the left cerebellar hemisphere and the left occipital lobe. The rest of this study was normal and there were no abnormalities noted on the other structural sequences performed.
Magnetic Resonance Angiography
Two scans had comments regarding the MRA sequence.
1) Commented that it was poor quality due to motion artefact and not reportable.
2) Noted a small posterior cerebral artery (PCA) on the left with distal PCA territory mostly supplied from the posterior communicating artery. The rest of the study was normal.

Table 9. Clinical MRI reports.

<table>
<thead>
<tr>
<th></th>
<th>Control (n=11)</th>
<th>Normal Doppler (n=29)</th>
<th>Abnormal Doppler (n=33)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quality of the Study (Good / Average / Poor)</td>
<td>11/11 Good</td>
<td>25/29 Good</td>
<td>31/33 Good</td>
</tr>
<tr>
<td></td>
<td>4/29 Average</td>
<td></td>
<td>2/33 Average</td>
</tr>
<tr>
<td>Myelination (Appropriate / Delayed)</td>
<td>11/11 Appropriate</td>
<td>29/29 Appropriate</td>
<td>33/33 Appropriate</td>
</tr>
<tr>
<td>Midline Structures (Normal / Comment)</td>
<td>11/11 Normal</td>
<td>29/29 Normal</td>
<td>32/33 Normal</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1/33 Comment</td>
</tr>
<tr>
<td>Ventricles (Normal / Comment)</td>
<td>10/11 Normal</td>
<td>26/29 Normal</td>
<td>29/33 Normal</td>
</tr>
<tr>
<td></td>
<td>1/11 Comment</td>
<td>3/29 Comment</td>
<td>4/33 Comment</td>
</tr>
<tr>
<td>Parenchyma (Normal / Comment)</td>
<td>11/11 Normal</td>
<td>22/29 Normal</td>
<td>29/33 Normal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7/29 Comment</td>
<td>4/33 Comment</td>
</tr>
<tr>
<td>White Matter Volume (Preserved / Reduced)</td>
<td>11/11 Preserved</td>
<td>29/29 Preserved</td>
<td>33/33 Preserved</td>
</tr>
<tr>
<td>Posterior Fossa Contents (Normal / Comment)</td>
<td>11/11 Normal</td>
<td>28/29 Normal</td>
<td>33/33 Normal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1/29 Comment</td>
<td></td>
</tr>
<tr>
<td>Extra-Axial Fluid Spaces (Normal / Comment)</td>
<td>11/11 Normal</td>
<td>29/29 Normal</td>
<td>33/33 Normal</td>
</tr>
<tr>
<td>Susceptibility Weight Imaging (Normal / Comment)</td>
<td>9/11 Normal</td>
<td>23/28 Normal</td>
<td>28/32 Normal</td>
</tr>
<tr>
<td></td>
<td>2/11 Comment</td>
<td>5/28 Comment</td>
<td>4/32 Comment</td>
</tr>
<tr>
<td>Diffusion Sequence (Normal / Comment)</td>
<td>11/11 Comment</td>
<td>28/29 Normal</td>
<td>33/33 Normal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1/29 Comment</td>
<td></td>
</tr>
<tr>
<td>Magnetic Resonance Angiography (Normal / Comment)</td>
<td>8/10 Normal</td>
<td>22/26 Normal</td>
<td>25/33 Normal</td>
</tr>
<tr>
<td></td>
<td>2/10 Comment</td>
<td>4/26 Comment</td>
<td>8/33 Comment</td>
</tr>
</tbody>
</table>
Normal Doppler (ND) Group
25 of the 29 scans were reported to be of good quality and 4 were reported to be average. In one study, there was no SWI or MRA performed and in 2 others there was no MRA sequence, all other scans were complete. The comments made on the ND scans are as follows. Comments followed by a superscript symbol signify more than one comment in that particular study. Each type of superscript symbol is specific to a single study and subject for ease of cross-reference.

Ventricles
Three scans had comments on the ventricles.

1) A small amount of subependymal haemorrhage was noted in the lateral wall of the right lateral ventricle, no other ventricular abnormality identified. The rest of the study was normal.
2) A tiny focus of calcification in the right caudate thalamic groove seen on the T2* sequence. Otherwise there is no ventricular abnormality identified. §§
3) A small amount of blood (vs vessel) noted in the lateral wall of the right lateral ventricle. The rest of the study was normal.

Parenchyma
Seven scans had comments made regarding the parenchyma.

1) Two separate tiny foci of increased T1, decreased T2 signal in the right peririgonal region and the right posterior centrum semiovale were noted. They did not show up on the T2* sequence; however, they likely represent small foci of haemorrhage or calcification. The rest of the study was normal.
2) A focus of calcification on the surface of the medial aspect of the left cerebellar hemisphere. A second focus was noted more superiorly on the surface of the right cerebellar hemisphere, probably due to some haemorrhage into the extra axial spaces at birth. Otherwise posterior fossa contents are normal. §§
3) A small focus of increased T2, decreased T1 signal in the right caudate head, which may represent a dilated Virchow Robin space. There is also a decrease in the T2 signal of the right putamen. This appeared more artefactual than real as in the same slices the left cerebral parenchyma was increased in signal. The increase in signal in the right putamen was not appreciated on either the DWI or T1 sequence. The rest of the study was normal.

4) The T1 sequences showed a few punctuate foci of increased signal in the periventricular signal bilaterally, right>left. These foci were not seen on the T2* sequence and were not very noticeable on the T2 sequences and probably represent foci of petechial haemorrhage in the periventricular white matter. The rest of the study was normal.

5) The T2 sequence showed decreased signal in the right frontal horn which on correlation with the T2* is due to calcification/old haemorrhage. ^^

6) A focus of T1 hyperintensity in the left posterior periventricular white matter was noted. Linear, extending towards the lateral ventricle, low signal on T2, was not seen on the T2* and may represent a vessel. Two other foci of T1 hyperintensity were noted in the right posterior periventricular white matter which were low signal on T2 and not seen on the T2*. These most likely represent tiny focal haemorrhages. ##

7) Three punctate bright T1 lesions noted lateral to the left lateral ventricle. The rest of the study was normal.

**Posterior Fossa**
In one study three spots of blood were noted in cerebellum. **

**Susceptibility Weighted Imaging**
Five scans had comments regarding the SWI sequence.

1) Two foci of previous haemorrhage or calcification in the midline posterior to the cerebellum were noted. The rest of the study was normal.

2) The susceptibility sequence showed one tiny focus of calcification in the left cerebellar hemisphere. The rest of the study was normal.
3) The susceptibility sequence showed 2 subtle foci of calcification/ old haemorrhage in the cerebellum. One focus on the surface of the right hemisphere and was not visible on the conventional sequences. The other focus was in the left cerebellum and visible on the T1 and T2 sequences (dark on both). Otherwise posterior fossa contents were normal as was the rest of the study.

4) The susceptibility sequence showed right frontal periventricular/subependymal blooming, most likely due to previous haemorrhage. ^^

5) The susceptibility sequence showed one focus of calcification in the left cerebellar hemisphere. ##

**Diffusion**
In one study the diffusion noted restricted diffusion in the two foci of calcification in the posterior fossa. There was no other diffusion abnormality. §§

**Magnetic Resonance Angiography**
Four scans had comments regarding the MRA sequence.
1) The right anterior cerebral artery and the left posterior communicating artery were not visible on the MRA. The rest of the study was normal.
2) Poor quality sequence due to motion artefact, unable to report on MRA. The rest of the study was normal apart from an incidental finding of a scalp cystic lesion over the fontanelle and in contact with superior sagittal sinus and its draining veins.
3) Poorly developed right posterior cerebral artery on MRA. ++
4) Absent beginning of anterior cerebral artery (ACA) so both ACA from right. The rest of the study was normal.
Abnormal Doppler (AbD) Group
31 of the 33 scans were reported to be of good quality and 2 were reported to be average. In one study there was no SWI performed, all other scans were complete. The comments made on the AbD scans are as follows. Comments followed by a superscript symbol signify more than one comment in that particular study. Each type of superscript symbol is specific to a single study and subject for ease of cross-reference.

Midline Structures
In one study the posterior pituitary was not seen. The remainder of the midline structures including the chiasm and craniocervical junction was normal. The rest of the study was normal.

Ventricles
Four scans had comments regarding the ventricles.

1) The presence of a developmental venous anomaly was queried. The rest of the study was normal.
2) Three tiny foci of cortical heterotopia were noted in the wall of the right lateral ventricle (one anterior, one peritrigonal and one posterior). The rest of the study was normal.
3) Three distinct foci of subependymal cortical heterotopia were noted in the lateral wall of the right lateral ventricle. ^^^
4) Foci of abnormal signal consistent with blood products were seen in the walls of both lateral ventricles. ###

Parenchyma
Four scans had comments regarding the parenchyma.

1) Right frontal cortical dysplasia where one of the medial sulci in the right frontal lobe extends to the frontal horn of the right lateral ventricle was noted. ^^^
2) A focus of abnormal signal in the right posterior parietal area consistent with a prior focus of haemorrhage in the deep white matter was noted. Abnormal signal in the thalami would be consistent with prior ischaemia. 

3) Subtle increased T2 signal in a periventricular distribution on the T2 sequences. T1 images of this region were normal. 

4) A few small foci of increased T2 signal were noted in the body of the right caudate nucleus. These were not visible on the T1, T2 * or DWI images. They did not appear to involve the PLIC. The largest measures 5mm in diameter. They were of indeterminate significance. The rest of the study was normal.

**Susceptibility Weighted Imaging**

Four scans had comments regarding the SWI sequence.

1) The susceptibility sequence showed two small foci of previous haemorrhage or calcification in the left cerebellar hemisphere. The rest of the study was normal.

2) The susceptibility sequence showed one tiny focus of old blood in the left caudothalamic groove suggesting a previous left grade 1 IVH. No other evidence of previous haemorrhage or calcifications. The rest of the study was normal.

3) The susceptibility sequence showed 2 abnormal foci, one in either occipital pole, the left focus might represent a vessel. The right focus was a little larger and although it may also represent a vessel, it may also represent a small parenchymal focus of calcification/haemorrhage. This area was included on the MRA and there was no abnormality seen. 

4) The susceptibility sequence showed one tiny focus of calcification in the left cerebellar hemisphere. The rest of the study was normal.

**Magnetic Resonance Angiography**

Four scans had comments regarding the MRA sequence.

1) Absent beginning of anterior cerebral artery (ACA) so both ACA from right. The rest of the study was normal.

2) Absent beginning of ACA so both ACA from right. The rest of the study was normal.
3) Poor quality sequence, unable to report. The rest of the study was normal.

4) Absent proximal left posterior cerebral artery (PCA). The rest of the study was normal.

The reports and comments were reviewed and interpreted as to whether the study was normal given comments made. In the control group 10/11 (91%) were considered essentially normal, 15/29 (52%) in the ND group and 20/33 (61%) in the AbD group (Fig. 15). There was no statistical significance in the proportion of normal scans between the groups; \( \chi^2 \) is 5.2064, \( p = 0.074036 \). The commonest finding across the groups was of small cerebellar haemorrhages (CH). These were not clinically significant and the subjects did not have known IVH’s during the course of the NICU admission.

![Figure 15. Proportion of normal scans in each study group.](image)
The cerebellar haemorrhages noted were small and the significance unknown. There were 6/29 in the ND group and 2/33 in the AbD group. There was no significant difference between these groups for cerebellar haemorrhage ($\chi^2 = 2.9314$, $p = 0.086$). Of these 8 infants 2 were extremely low birthweight (ELBW), both in the AbD group, and no very low birthweight (VLBW) infants. The mean gestational age of the ND group with CH was 37 weeks and 29 weeks in the AbD group. One of the study subjects from the AbD group had evidence of ischaemia in the thalami, which is an injury associated with subsequent development of cerebral palsy (322).

Incidental Findings
Two infants in the AbD group had evidence of periventricular cortical heterotopia. The first child had no other abnormality noted on the MRI scan and had a normal developmental assessment using the Ages and Stages Questionnaire at 1 year and no parental concern. The second child also had evidence of frontal cortical dysplasia in the right hemisphere where one of the medial sulci in the right frontal lobe extended to the frontal horn of the right lateral ventricle. She was referred to paediatric neurology for repeat imaging and review. At 6 months she had normal development, after which the family relocated to Poland and were lost to follow up. There was a case where there was an incidental finding of a scalp cystic lesion over the fontanelle in a subject from the ND group. This was removed prior to 1 year of age and was a lymphatic structure that was not found to be in contact with superior sagittal sinus and its draining veins as suggested on the original MRI scan. That family did not engage in 1-year follow up.
4.4 Conclusion

The clinical MRI reports provided valuable macrostructural information. The number of infants with small cerebellar haemorrhages was unexpected. Neonatal cerebellar haemorrhages were thought to be relatively rare and seen on post mortem studies. Improved cranial ultrasound and detailed MRI have shown that small cerebellar haemorrhages are present more frequently than previously believed (323). Cerebellar haemorrhages are associated with low birth weight and low gestation (<26/40) as well as with vaginal instrumental delivery. An increased incidence in VLBW and ELBW infants is recognised, however a specific association with IUGR has not previously been made (324, 325). The StOOPS infants with cerebellar haemorrhage were not extremely preterm or of a very low birthweight implying that IUGR itself may be a potential risk factor for CH. Rates of cerebral palsy are higher in IUGR infants, however, only one of the StOOPS infants had a lesion readily predictive of CP, suggesting the causative lesions may not be obvious at a macrostructural level. Periventricular heterotopia is not typically associated with IUGR, but was found in two of the AbD infants. This finding is generally associated with normal intelligence and development, but with an increased risk for epilepsy in later life. However, if symptomatic in childhood, it is associated with a poorer developmental prognosis (326). The incidental findings identified highlight the importance of formal reporting for images acquired for research studies.

Whilst validated scoring systems for neonatal MRI brain scoring exist, during the time of reporting there was none based on MR using 3 Tesla (244, 446). Given constraints of time and funding both reporting radiologists preferred to use a system they were familiar with to facilitate the reporting process.
Section 3: Results

Chapter 5

Resting-state functional MRI of the Intra-Uterine Growth Restricted Infant
Chapter 5. Results

Resting-state functional MRI of the Intra-Uterine Growth Restricted Infant

5.1 Introduction

As it matures to the adult form the human brain changes substantially in both structure and function (327). The first year of life represents a period of significant increase in total brain volume, and may be a vulnerable stage during which disruption of normal developmental processes may have long-lasting or permanent effects on brain structure and function (328). The process by which these changes occur is nonlinear and understanding normal brain maturation is essential for understanding neurodevelopmental disorders (329). Studying this process of functional change over time is challenging, especially in the neonatal period. The neurological sequelae of intra-uterine growth restriction are known to include both cognitive and motor problems - abnormal perceptual abilities, motor slowing, cognitive delay, poor attention and memory with subsequent poor school performance (330). As discussed elsewhere, a sonographic estimated fetal weight of < 10th centile is the most commonly accepted definition of a fetus not achieving its target weight, however, a substantial proportion of these fetuses will be physiologically normal infants who are small for gestational age (SGA). True growth restriction is commonly the result of placental insufficiency, when dysfunction of the fetal-placental perfusion leads to a reduced delivery of oxygen and nutrition from the placenta (331), resulting in hypoxia and acidosis in the fetal circulation (332). Altered placental function can be detected by Doppler ultrasound of fetal vessels and used to help discriminate between IUGR and SGA infants (333). Abnormal Doppler waveform patterns in the umbilical artery such as absent (AEDF) or reversed (REDF) end-diastolic flow, are well recognised as markers of IUGR.

Reduced umbilical flow leads to chronic hypoxia resulting in fetal blood flow redistribution, i.e., brain sparing, where brain growth is preserved at the expense of other organs.
Whether abnormal Doppler measurements are associated with neurodevelopmental sequelae has been the subject of several studies, with differing conclusions. Some suggest AEDF is not associated with adverse neurodevelopmental outcome on antenatal assessment, whilst REDF has been found to be associated with a wide range of problems at school age (334, 335).

However, both AEDF and REDF have also been reported as independent predictors of neonatal death or cerebral palsy in IUGR infants, which suggests that neurological consequences of AEDF cannot be completely discounted (336, 337). The Doppler status of an IUGR infant is also relevant on admission to a neonatal intensive care unit, as infants with abnormal Doppler’s start a slower feeding regime from those with normal antenatal measurements to minimise morbidity such as necrotising enterocolitis. However, by discharge, Doppler status is not routinely factored in to discharge planning, particularly when determining risk for poor neurodevelopmental outcome or the degree of follow-up required.

The cerebral cortex is organised in a modular manner, consisting of many parallel-distributed networks (338, 339). The impact of prenatal and perinatal insults on the developing functional architecture of the brain around birth is uncertain. During the second half of the gestation and the immediate perinatal period, important cortico-cortical and corticothalamic connections are known to develop (340, 341). Disturbances of nutrition and oxygenation in this period are therefore likely to impact on the development of the normal functional architecture. Resting-state fMRI (rs-fMRI) permits the study of the functional brain circuits around birth, and so provides a better understanding of the large-scale organization of the developing brain, and of how this organisation may be affected by adverse prenatal and perinatal conditions (342). As rs-fMRI is independent of task performance, it has allowed for examination of these functional brain circuits in neonatal populations using relatively short sequences (343). Functional connectivity measures derived from rs-fMRI have been shown to be extremely valuable in studying age-related changes in the structure of neural networks (344).
Findings from rs-fMRI studies are shown to be consistent and reproducible across and within study groups (345, 346). Resting state functional MRI (rs-fMRI) thus has become a prominent and reliable tool in the investigation of the connectivity properties of large-scale brain networks (313, 346). It has provided significant insights into brain development, origin of individual differences and brain disorders (342, 347, 348). Brain connectivity has been demonstrated to be affected by fetal growth restriction as has been evidenced by neuronal migration deficits, a reduction in dendritic processes and reduced efficiency in cortical networks as seen with decreased long-range connections (447). A functional MRI study on children who had been born prematurely suggested that those affected by IUGR use different brain regions and a more diffuse network to perform tasks, which may interfere with goal-directed activity and may reflect inefficient attentional control, when compared to those without IUGR (448). It is known that IUGR impacts on functional resting state networks with more hyper connected, but sub-optimally organised functional networks. This has also been correlated with neurobehavioural scores underlining the capacity for its use as a biomarker of altered development (449). The specific aim of this study was to determine whether infants, antenatally identified as having IUGR, had changes in rs-fMRI functional brain connectivity depending on their umbilical arterial Doppler status i.e. a comparison between those with Abnormal versus Normal Doppler antenatal studies.

5.2 Materials and Methods

Subjects
Infants were recruited through participation in the StOOPS study (Short-term Outcome Of infants in the PORTO Study). The PORTO study identified and followed growth-restricted pregnancies recruiting from the largest obstetric units on the island of Ireland. Infants in the cohort were antenatally identified as growth restricted if they had an estimated fetal weight < 10\textsuperscript{th} centile and detailed serial Doppler ultrasound measurement studies of the umbilical artery were performed as per study protocol (127). Infants were eligible for inclusion if their estimated fetal weight was less than the 10th centile, they were a singleton pregnancy and they did not have any identified complex congenital problems, chromosomal disorders or primary brain abnormalities.
All StOOPS infants were scanned at corrected full term gestational age. Scanning was performed on non-sedated infants after feeding to facilitate natural sleep. They were then placed in a vacuum immobilisation mattress (Noras MRI products GmbH, Germany) designed to mold to their shape, supporting them in a comfortable position within the head coil and greatly minimising the potential for head motion. Acoustic noise was minimised through the use of earplugs in conjunction with neonatal earmuffs (MiniMuffs, Natus Medical Inc., USA). From the StOOPS cohort 38 infants had an fMRI sequence for analysis. 8 infants were subsequently excluded due to excessive head movement during the sequence.

Anatomical MR images were analysed by 2 experienced paediatric radiologists. Mean gestational age at delivery in the normal Doppler (ND) group was 35 weeks and 5 days versus 31 weeks and 2 days in the abnormal Doppler (AbD) group. Relevant neonatal demographic details provided in Table 10.

Table 10. Demographic information. Normal Doppler’s (ND), Abnormal Doppler’s (AbD), Occipitofrontal circumference (OFC)

<table>
<thead>
<tr>
<th>Infants (Male)</th>
<th>ND</th>
<th>AbD</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infants (Male)</td>
<td>15 (7)</td>
<td>15 (7)</td>
<td>ns</td>
</tr>
<tr>
<td>Birth Weight (grams)</td>
<td>1901 (820-2870)</td>
<td>1180 (510-1920)</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Gestation at birth (weeks)</td>
<td>35 + 5 (28+1 - 40+3)</td>
<td>31 + 2 (26+1 - 36+0)</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Age at scan (weeks)</td>
<td>42 + 6 (40+1 - 45+5)</td>
<td>42 + 3 (40+3 – 45+4)</td>
<td>ns</td>
</tr>
<tr>
<td>OFC (cms)</td>
<td>35.8</td>
<td>34.9</td>
<td>ns</td>
</tr>
</tbody>
</table>

**MRI Image acquisition**

The infants were scanned in natural sleep on Philips Achieva 3 Tesla scanner using an 8-channel receive-only head coil (Philips Medical Systems, Best, The Netherlands). A 3D Inversion Recovery prepared Spoiled Gradient Recalled echo (IR-SPGR) sequence was used to obtain high resolution T1-weighted images of the brain, with the parameters as follows:
Field of view (FOV) = 200 x 161 x 110 mm$^3$, isotropic spatial resolution of 1 mm, time repetition/time echo/flip TR/TE = 17/4.6 ms, TI = 800 ms, flip angle = 13°, SENSE factor = 2, acquisition time = 3min 53 s. T$_2$-weighted images were acquired using a 3D VISTA (turbo spin echo) sequence with: FOV = 200 x 161 x 110 mm$^3$, isotropic spatial resolution of 0.9 mm, TR/TE$_{eff}$ = 2500/452 ms, SPIR fat suppression, SENSE factor = 2, acquisition time = 5min 28 s.

Functional connectivity was assessed from a resting state fMRI scan using a spin echo - echo planar imaging (SE-EPI) sequence sensitised to blood oxygen level-dependent (BOLD) contrast, with: FOV = 180 x 180 x 93 mm$^3$, spatial resolution 2.2 x 2.2 x 3 mm$^3$, 28 slices with an interslice gap of 0.35 mm, TR/TE = 2000/28 ms, SPIR fat suppression, SENSE factor = 2, 180 dynamics acquired with dynamic stabilisation in an overall acquisition time of 6min 6 s.

**fMRI Data Preprocessing and Statistical Analysis**

Image preprocessing was performed using FSL (FMRIB, Oxford University, U.K.) in conjunction with Conn Toolbox and SPM8 (Wellcome Department of Imaging Neuroscience, London, UK) (349). The first five volumes of the rs-fMRI data from each subject were discarded to allow for equilibrium effects and the remaining 175 volumes per subject were motion and slice timing corrected and low pass filtered (cutoff 100 sec.). Within-run grand mean intensity scaling was also performed. Atlas transformation of the functional data to Montreal Neurological Institute (MNI) space was achieved in a two-stage process. FLIRT was used to register the fMRI image to the infants T2 brain stripped structural scan. The T2 structural image was then registered to the MNI152 reference image with the resulting registration matrix used to convert the T2 registered fMRI image to MNI152 space and resliced to 2x2x2 mm voxels. As removing the global signal has become controversial, non-neuronal sources of noise were estimated and removed using a component based reduction method (350-352). The anatomical image of each infant was segmented into white matter (WM), grey matter and cerebral spinal fluid (CSF) masks. WM and CSF masks were then eroded to reduce partial volume issues. The first three principal components of the WM and CSF signals were removed from the fMRI images using regression. The 4D residuals were then standardized for each original voxel time series by subtracting their mean value, dividing by the standard deviation and adding 100.
The standardized 4D images were then smoothed using a 5mm full width half maximum (FWHM) kernel. All seed-based resting-state correlation analyses were performed on these processed 4D images (Fig. 16, 17). Seed region ROIs were created as 5mm radius spheres centred at coordinates from the Montreal Neurological Institute (MNI) atlas (Table 11). Time series were averaged across all voxels in each seed ROI. These time series were then normalised for input into the first level (individual subject) correlation analyses.
Figure 16. Pre-analysis processing algorithm.
Resting state functional MRI (rs-fMRI), Brain Extraction Tool (BET), FMRIBs Automated Segmentation Tool (FAST), FMRIB Software Library (FSL), time series (ts), FMRI Expert Analysis Tool (FEAT), General Linear Modeling (GLM)

Resting state functional MRI (rs-fMRI), Brain Extraction Tool (BET), FMRIBs Automated Segmentation Tool (FAST), FMRIB Software Library (FSL), time series (ts), FMRI Expert Analysis Tool (FEAT), General Linear Modeling (GLM)
Extract hub ts
Default Network
Attention Network
Sensory Networks

Seed masks in
resting state
connectivity hubs

Res4D
Norm

GLM
Whole brain
correlation
analysis for
each seed for
each subject

FEAT

Group
Differences

GLM
Group Analysis

Correlation
Maps

FEAT Group
analysis, corrected at
the cluster level
z >2.3, p<0.01

Figure 17. Seed based analysis algorithm.
time series (ts), FMRI Expert Analysis Tool (FEAT), General Linear Modeling (GLM).
Table 11. Seed region of interest (ROI) co-ordinates Montreal Neurological Institute (MNI) atlas

<table>
<thead>
<tr>
<th>Network</th>
<th>Network Hub</th>
<th>Hemisphere</th>
<th>MNI Co-ordinate</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Default Mode Network</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Posterior Cingulate</td>
<td></td>
<td></td>
<td>0 -53 26</td>
</tr>
<tr>
<td>Lateral Parietal Cortex</td>
<td>Left</td>
<td></td>
<td>-48 -62 36</td>
</tr>
<tr>
<td></td>
<td>Right</td>
<td></td>
<td>46 -62 32</td>
</tr>
<tr>
<td>Medial Prefrontal Cortex</td>
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<td></td>
<td>0 52 -6</td>
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<tr>
<td>Hippocampal Formation</td>
<td>Left</td>
<td></td>
<td>-24 -22 -20</td>
</tr>
<tr>
<td></td>
<td>Right</td>
<td></td>
<td>24 -20 -22</td>
</tr>
<tr>
<td><strong>Dorsal Attention Network</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frontal Eye Field</td>
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<td></td>
<td>-38 -4 48</td>
</tr>
<tr>
<td></td>
<td>Right</td>
<td></td>
<td>40 -4 48</td>
</tr>
<tr>
<td>Intra-Parietal Sulcus</td>
<td>Left</td>
<td></td>
<td>-24 -58 52</td>
</tr>
<tr>
<td></td>
<td>Right</td>
<td></td>
<td>22 -58 54</td>
</tr>
<tr>
<td>Middle Temporal Complex (MT+)</td>
<td>Left</td>
<td></td>
<td>-56 -60 -2</td>
</tr>
<tr>
<td></td>
<td>Right</td>
<td></td>
<td>54 -58 -4</td>
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<tr>
<td><strong>Sensory Networks</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Auditory</td>
<td>Left</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>Right</td>
<td></td>
<td>43 -26 12</td>
</tr>
<tr>
<td>Motor</td>
<td>Left</td>
<td></td>
<td>-36 -25 57</td>
</tr>
<tr>
<td></td>
<td>Right</td>
<td></td>
<td>36 -25 57</td>
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<td></td>
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</tr>
<tr>
<td></td>
<td>Right</td>
<td></td>
<td>30 -88 0</td>
</tr>
<tr>
<td><strong>Thalamus</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thalamus</td>
<td>Left</td>
<td></td>
<td>-12 -19 4</td>
</tr>
<tr>
<td></td>
<td>Right</td>
<td></td>
<td>14 -19 4</td>
</tr>
</tbody>
</table>
Networks Analysed

Two signal networks, three sensory networks and the thalami were selected for analysis. The two signal networks chosen were the default mode network and the dorsal attention system (353-358). These networks have multiple, bilateral regions that show strong correlations with one another and also important negative correlations for regions between networks (358, 359). The sensory networks consisted of the Visual, Auditory and Motor systems. The thalamus is a vital subcortical gray matter region, which integrates neural activity from widespread neocortical inputs and outputs so it was also included in the investigation. Atlas coordinates of the centres of the signal and sensory networks were based upon the data presented by Van Dijk (343).

Hub-to-Hub Analysis

In order to identify patterns of intra-network correlation and any group differences, the time series for each seed ROI in each network were cross-correlated. For group analysis correlations were converted to a normal distribution by Fisher’s z transformation, generating \( z(r) \) correlation scores. In addition, the thalamus time series were tested against all other network hubs. One sample t-tests were performed to identify intra-network connectivity’s which were greater than zero, significance set at \( p<0.05 \) – one-tail. For group differences two-sample t-tests were performed, significance set at \( p<0.05 \) – two tail.

Hub-to-Voxel Analysis

Multiple regression analysis using the FSL FEAT tool was performed on the extracted seed ROI time series. This analysis produced individual infant-level correlation maps of all voxels that were positively or negatively correlated with each of the network ROIs. Higher level (group) analysis was subsequently carried out using FMRIB’s Local Analysis of Mixed Effects. The general linear model (GLM) was applied to test for group averages and differences between the two groups. Thresholds were generated for Z statistic images using clusters determined by \( Z > 2.3 \) and a family-wise error–corrected cluster significance threshold of \( p<0.05 \).
**Higher Level (Group) Analysis**

Individual 3D correlation maps for all seeds were then converted to a normal distribution by Fisher’s $z$ transformation, generating $z(r)$ correlation maps. Analysis was then performed on this transformed data to identify brain voxels which displayed a significant correlation with a given seed region, independent of group (F-statistic). The resulting cluster maps were converted to binary masks and used as pre-threshold masks when testing for group differences. This process ensured that group differences were only identified in voxels with confirmed connectivity to the seed ROI in question.

**5.3 Results**

*Hub to hub connectivity*

Default Mode Network (DMN) - Table 13(A) contains the inter-hub connectivity’s found between DMN hubs. In these infants, the DMN was somewhat fragmented as the hippocampal formation hubs, although connected to one another, did not display any connectivity with the rest of the network. The connectivity in the posterior part of the system appeared more developed than the anterior portion. The medial prefrontal cortex hub displayed connectivity with the posterior cingulate but had very weak connectivity to the right lateral parietal region and exhibited no connectivity with the left. No connectivity differences between groups were found. The connectivity pattern is illustrated in Figure 18.

Dorsal Attention Network (DAN) - Table 13(B) contains the inter-hub connectivity’s found between the DAN hubs. Most of the neural correlations detected involved contralateral connections and all analogous hub pairs displayed their expected interconnection. The interconnections shown represent the best correlation values obtained. The left hemisphere hubs displayed sparser connectivity with other parts of the network than the right (Fig. 18). No group differences were noted.
Sensory Networks - Table 13(C) contains the inter-hub neural correlations found for the sensory hubs. Consistent interconnections were found between analogous sensory hubs in each hemisphere. Visual seeds displayed only one significant interconnection with the other sensory systems (VisCtx_R to MCtx_R). In contrast the motor and auditory seeds displayed both ipsilateral and contralateral connectivity (Fig. 18). No group differences were found.

Thalamic connectivity - Table 12 contains information about the neural correlation between the thalamic seeds and the other networks. Only significant correlations are shown. Both thalami showed connectivity with the right and left hippocampal formation seeds in the DMN. With regard to the DAN both thalami displayed connectivity with the right frontal eye field (FEF_R) seed. For the sensory networks, right and left thalami showed ipsilateral and contralateral connectivity with the auditory and motor seeds. No connections were confirmed with the visual seeds. No group differences were found.
Table 12. Pearson correlation coefficients (mean and s.e) for thalamo-thalamic and thalamus connections cross-correlated with the time series for each seed ROI in each network. One sample t-tests were performed to identify intra-network connectivity's which were greater than zero, significance set at p<0.05 – one-tail. Only significant correlations are shown. All p-values <0.001.

<table>
<thead>
<tr>
<th>Thalamic Connectivity</th>
<th>DMN</th>
<th>DAN</th>
<th>Sensory</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thal_L</td>
<td>0.62</td>
<td>(0.04)</td>
<td></td>
</tr>
<tr>
<td>Thal_R</td>
<td>0.19</td>
<td>(0.03)</td>
<td></td>
</tr>
<tr>
<td>HF_L</td>
<td>0.19</td>
<td>(0.03)</td>
<td></td>
</tr>
<tr>
<td>HF_R</td>
<td>0.14</td>
<td>(0.03)</td>
<td></td>
</tr>
<tr>
<td>FEF_R</td>
<td>0.14</td>
<td>(0.03)</td>
<td></td>
</tr>
<tr>
<td>AudCxs_L</td>
<td>0.17</td>
<td>(0.03)</td>
<td></td>
</tr>
<tr>
<td>AudCxs_R</td>
<td>0.26</td>
<td>(0.03)</td>
<td></td>
</tr>
<tr>
<td>MCx_L</td>
<td>0.11</td>
<td>(0.03)</td>
<td></td>
</tr>
<tr>
<td>MCx_R</td>
<td>0.19</td>
<td>(0.04)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Thalamus Left, Right (Thal_L, Thal_R), Default Mode Network (DMN), Dorsal Attention Network (DAN), Hippocampal Formation Left, Right</th>
<th>FEF_L, Thal_L, Thal_R, Thal_R, Frontal Eye Field Right (FEF_R), Auditory Cortex Left, Right</th>
</tr>
</thead>
</table>
Table 13. A) Default Network, B) Attention Network and C) Sensory Networks. Inter-hub Pearson correlation coefficients (mean and s.e.) connections cross-correlated with the time series for each seed ROI in each network. One sample t-tests were performed to identify intra-network connectivity’s which were greater than zero, significance set at p<0.05 – one-tail. *** p<0.001, ** p<0.01, * p<0.05; ‘nc’ designates no statistical evidence of connection.

<table>
<thead>
<tr>
<th>Network</th>
<th>pC</th>
<th>LatPar_L</th>
<th>LatPar_R</th>
<th>mPFC</th>
<th>HF_L</th>
<th>HF_R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Default</td>
<td>0.21 (0.04)**</td>
<td>0.15 (0.04)**</td>
<td>0.13 (0.04)**</td>
<td></td>
<td>0.08 (0.03)**</td>
<td>0.4 (0.04)**</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Posterior Cingulate (pC), Lateral Parietal Cortex Left, Right (LatPar_L, LatPar_R), Medial Prefrontal Cortex (mPFC), Hippocampal Formation Left, Right (HF_L, HF_R)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Network</th>
<th>FEF_L</th>
<th>FEF_R</th>
<th>IPS_L</th>
<th>IPS_R</th>
<th>MT+_L</th>
<th>MT+_R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Attention</td>
<td>0.28 (0.04)**</td>
<td></td>
<td>0.09 (0.03)**</td>
<td>0.07 (0.04)**</td>
<td>0.13 (0.04)**</td>
<td>0.14 (0.03)**</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frontal Eye Field Left, Right (FEF_L, FEF-R), Intra-Parietal Sulcus (IPS_L, IPS_R), Middle Temporal Complex Left, Right (MT+_L, MT+_R)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Network</th>
<th>AudCtx_L</th>
<th>AudCtx_R</th>
<th>MCtx_L</th>
<th>MCtx_R</th>
<th>VisCtx_L</th>
<th>VisCtx_R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensory</td>
<td>0.37 (0.04)**</td>
<td>0.16 (0.04)**</td>
<td>0.20 (0.03)**</td>
<td>0.16 (0.03)**</td>
<td>0.35 (0.04)**</td>
<td>0.09 (0.03)**</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>0.46 (0.05)**</td>
</tr>
<tr>
<td>Auditory Cortex Left, Right (AudCtx_L, AudCtx_R), Motor Cortex Left, Right (MCtx_L, MCtx_R), Visual Cortex Left, Right (VisCtx_L, VisCtx_R)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 18. Hub to hub connectivity within the different networks. Top – Default Network, Middle – Attention Network, Bottom – Sensory Network. All connections shown were significant at $p = 0.05$ or better.
Whole brain (seed to voxel) connectivity analysis

In the whole brain connectivity analysis, the resting state fMRI BOLD signals collected from the AbD and ND infants displayed multiple networks demonstrating correlated neural activity. Networks were identified for seed regions in the DMN, DAN, sensory cortices and thalami and these network properties were similar for the two groups but with some important exceptions. Summary descriptions of the connectivity maps for the various networks and their component hubs are given below. The following pages contain z(r) group correlation maps showing the statistically significant connectivity patterns for the distributed hubs of the DMN, DAN and Sensory networks for all the infants in the study. Right hemisphere representations only are shown in the figure for homotopic seed pairs, as correlation patterns were essentially similar for both right and left seeds.

Within the DMN (Fig. 19-21) there were indications of a consistent network structure across the infants. For example, the posterior cingulate/precuneus showed evidence of its expected connectivity with the medial prefrontal cortex and lateral parietal cortex. The lateral parietal seeds demonstrated connectivity with both the medial prefrontal (extending to middle and superior frontal zones) and posterior cingulate brain regions. However, network structure appeared incomplete or underdeveloped, as no evidence of connectivity between the hippocampal formation and the other DMN hubs was found.
Figure 19. Group analysis z(r) correlation maps for the Posterior Cingulate seed region within the Default Mode Network. For bilateral seeds the results for the right hemisphere are used for illustration. The Z statistic images were thresholded using clusters determined by Z > 2.3 and a family-wise error-corrected cluster significance threshold of p<0.05.
Figure 20. Group analysis z(r) correlation maps for the Lateral Parietal Cortex seed region within the Default Mode Network. For bilateral seeds the results for the right hemisphere are used for illustration. The Z statistic images were thresholded using clusters determined by Z > 2.3 and a family-wise error-corrected cluster significance threshold of p<0.05.
Figure 21. Group analysis z(r) correlation maps for the Hippocampal Formation seed region within the Default Mode Network. For bilateral seeds the results for the right hemisphere are used for illustration. The Z statistic images were thresholded using clusters determined by Z > 2.3 and a family-wise error–corrected cluster significance threshold of p<0.05.
For the DAN network (Fig. 22-24) homotopic seed regions showed the expected neural interhemispheric connectivity with each other, however, ipsilateral connections were stronger and more widespread than contralateral projections. Inter-connections within the network were also noted to be less well developed. The frontal eye field (FEF) seeds showed widespread correlated neural activity to bilateral regions of the precentral gyrus and the middle temporal gyrus, including locations proximal to middle temporal complex (MT+). Other connections include areas of the striate and extrastriate visual cortices. Ipsilateral connections were also noted with subcortical areas particularly the putamen. The intra-parietal sulcus (IPS) network seed displayed bilateral connectivity with precentral gyrus areas consistent with FEF locations and areas of the striate and extrastriate visual cortices and regions in proximity to the ipsilateral MT+. Connectivity was also noted with superior frontal gyri and the cerebellum. MT+ seeds showed connectivity with striate and extrastriate visual cortices and evidence of consistent connectivity with the contralateral precentral and postcentral areas encompassing FEF and ipsilateral areas in proximity to IPS.
Figure 22. Group analysis z(r) correlation maps for the Frontal Eye Field seed region within the Dorsal Attention Network. For bilateral seeds the results for the right hemisphere are used for illustration. The Z statistic images were thresholded using clusters determined by Z > 2.3 and a family-wise error–corrected cluster significance threshold of p<0.05.
Figure 23. Group analysis $z(r)$ correlation maps for the Intra-Parietal seed region within the Dorsal Attention Network. For bilateral seeds the results for the right hemisphere are used for illustration. The Z statistic images were thresholded using clusters determined by $Z > 2.3$ and a family-wise error–corrected cluster significance threshold of $p<0.05$. 
Figure 24. Group analysis z(r) correlation maps for the Middle Temporal Complex seed region within the Dorsal Attention Network. For bilateral seeds the results for the right hemisphere are used for illustration. The Z statistic images were thresholded using clusters determined by $Z > 2.3$ and a family-wise error–corrected cluster significance threshold of $p<0.05$. 
For the sensory networks, homotopic seed pairs showed the expected neural interhemispheric connectivity, although connectivity maps did display broader and better regional connectivity in the same hemisphere as the seed (Fig. 25-27). For auditory cortices (AudCtx) connectivity involved auditory areas in both hemispheres but also extended posteriorly into visual areas including precuneus, intracalcarine cortex and lingual gyrus. There was evidence of correlated activity in the amygdala and anterior hippocampus. In the case of motor cortex (MCtx), bilateral connectivity was seen in the precentral and postcentral gyri, extending into visual areas including precuneus, intracalcarine cortex and lingual gyrus and parts of the auditory cortex. Subcortical connectivity was noted bilaterally to parts of the basal ganglia; mainly putamen. In the visual cortex (VisCtx) widespread correlated neural activation was found in the visual cortices. Activations were seen in motor areas including the postcentral gyrus as well as areas of the superior frontal gyrus. For both right and left (VisCtx) seeds these latter activations were in the right hemisphere only.
Figure 25. Group analysis z(r) correlation maps for the Auditory Cortex seed region within the Sensory networks. For bilateral seeds the results for the right hemisphere are used for illustration. The Z statistic images were thresholded using clusters determined by Z > 2.3 and a family-wise error–corrected cluster significance threshold of p<0.05.
Figure 26. Group analysis z(r) correlation maps for the Motor Cortex seed region within the Sensory networks. For bilateral seeds the results for the right hemisphere are used for illustration. The Z statistic images were thresholded using clusters determined by $Z > 2.3$ and a family-wise error–corrected cluster significance threshold of $p<0.05$. 

Sensory Network
Figure 27. Group analysis z(r) correlation maps for the Visual Cortex seed region within the Sensory networks. For bilateral seeds the results for the right hemisphere are used for illustration. The Z statistic images were thresholded using clusters determined by $Z > 2.3$ and a family-wise error–corrected cluster significance threshold of $p<0.05$. 

Sensory Network
The thalamic seeds displayed widespread bilateral connectivity to postcentral motor areas. In addition, correlated activity was found bilaterally in parts of the hippocampus and in the putamen. Finally, both thalami had significant connectivity with regions of the cerebellum including the vermis and lobules V and VI bilaterally.

*Group Differences in whole brain connectivity*

None of the DMN seeds showed whole brain connectivity differences between the abnormal (AbD) and normal Doppler (ND) infants. In the DAN analysis, the AbD infants displayed reductions in connectivity between the left frontal eye field (FEF_L) and regions in the left precentral gyrus. In addition, the right intra-parietal sulcus (IPS_R) showed connectivity reductions to striate and extrastriate cortex. In the sensory network analysis, the left auditory cortex (AudCtx_L) showed increased connectivity in the AbD infants to regions including the left superior parietal lobule and the left striatum, including putamen and nucleus accumbens, and parts of the left anterior hippocampus and amygdala. The right visual cortex (VisCtx_R) showed reduced connectivity in the AbD infants with right postcentral and intra-parietal areas. The right thalamus (Thal_R) displayed reduced connectivity to various lobes of the right and left cerebellum in the AbD group. These included lobules I-IV and lobule V. Connectivity differences were more marked for the left cerebellum. See Figures 28-30 and Table 14.
Figure 28. Brain regions identified with significantly altered connectivity in the AbD infants compared to the ND infants with various hubs in the Dorsal Attention Network. These Z statistic images were thresholded using clusters determined by $Z > 2.3$ and a family-wise error–corrected cluster significance threshold of $p<0.05$. 

Dorsal Attention Network
Sensory Networks

Figure 29. Brain regions identified with significantly altered connectivity in the AbD infants compared to the ND infants with various hubs in the Sensory Networks. These Z statistic images were thresholded using clusters determined by $Z > 2.3$ and a family-wise error–corrected cluster significance threshold of $p<0.05$. 
Figure 30. Brain regions identified with significantly altered connectivity in the AbD infants compared to the ND infants with various hubs in the Thalamic Network. These Z statistic images were thresholded using clusters determined by Z > 2.3 and a family-wise error–corrected cluster significance threshold of p<0.05.
Table 14. Network seed regions showing significantly altered brain connectivity with various brain regions in the AbD compared to ND infants. The size, maximum Z score and MNI coordinate for each cluster is shown as well as main anatomical regions involved, corrected for multiple comparisons. Networks: DMN – Default Mode Network; DAN – Dorsal Attention network. Network Seeds: FEF_L – Left Frontal Eye Field; IPS_R – Right Intra-Parietal Sulcus; AudCtx_R – Right Auditory Cortex; VisCtx_R – Right Visual Cortex; Thal_R – Right Thalamus.

<table>
<thead>
<tr>
<th>Network</th>
<th>Hub</th>
<th>Cluster</th>
<th>Voxels</th>
<th>$Z_{\text{max}}$</th>
<th>x</th>
<th>y</th>
<th>z</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMN</td>
<td>None</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DAN</td>
<td>FEF_L</td>
<td>1</td>
<td>531</td>
<td>3.83</td>
<td>-14</td>
<td>-16</td>
<td>64</td>
<td>Left precentral gyrus and some left superior frontal gyrus</td>
</tr>
<tr>
<td>IPs_R</td>
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<td>1376</td>
<td>4.1</td>
<td>-6</td>
<td>-76</td>
<td>-10</td>
<td></td>
<td>Right occipital pole and cuneus, left cuneus</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1253</td>
<td>4.2</td>
<td>-40</td>
<td>-60</td>
<td>14</td>
<td></td>
<td>Left angular gyrus and left lateral occipital gyrus</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1110</td>
<td>3.97</td>
<td>10</td>
<td>-90</td>
<td>8</td>
<td></td>
<td>Left and right lingual gyrus and right lateral occipital gyrus</td>
</tr>
<tr>
<td>Sensory</td>
<td>AudCtx_R</td>
<td>1</td>
<td>548</td>
<td>4.57</td>
<td>20</td>
<td>4</td>
<td>-10</td>
<td>Right putamen, right nucleus accumbens, right anterior hippocampus, right amygdala</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>587</td>
<td>3.71</td>
<td>-28</td>
<td>-58</td>
<td>42</td>
<td></td>
<td>Left superior parietal lobule, left precuneus, left lateral occipital gyrus, left angular gyrus</td>
</tr>
<tr>
<td>Thalamus</td>
<td>Thal_R</td>
<td>1</td>
<td>449</td>
<td>3.52</td>
<td>-4</td>
<td>-50</td>
<td>-14</td>
<td>Cerebellum left lobes I_V, right lobe V</td>
</tr>
</tbody>
</table>
In order to better visualize these connectivity changes detected between the two groups, average correlations at the Zmax coordinates were compiled for all the network components showing group differences. To check consistency of connectivity patterns within each cluster, connectivity patterns at other major local maxima were investigated. The connectivity changes seen at Zmax were found to be typical of those at other local maxima. Group correlation comparisons are contained in Figure 31. In general, AbD infants showed reduced positive correlations between seed and other brain regions with the exception of the connectivity with the AudCtx_R.

Figure 31. Comparison of the seed to cluster correlation co-efficients (at $Z_{\text{max}}$ coordinates) in the high-level group GLM analyses in the two infant groups; IUGR infants with normal Doppler’s (ND) and abnormal Doppler’s (AbD).

Network seed: FEF_L – Left Frontal Eye Field; IPS_R – Right Intra-Parietal Sulcus; AudCtx_R – Right Auditory Cortex; VisCtx_R – Right Visual Cortex; Thal_R – Right Thalamus.
5.4 Conclusion

Using resting state fMRI data with seed correlation techniques, this study investigated for possible changes in neural connectivity in infants antenatally identified as IUGR and categorised according to their Doppler status (abnormal Doppler (AbD) versus normal Doppler (ND) measurements). Intra-network and network-to-brain connectivity in several key network systems were studied. In this study the DMN network appears separated into two compartments, one involving the hippocampal formations and the other encompassing the remaining hubs. The apparent fragmentation of the DMN seen in this study may reflect the immature condition of the network at this early stage. This would also be consistent with the finding that medial temporal regions (hippocampus, parahippocampal gyrus) become increasingly correlated to the DMN with age (360). Although intra-network seed-to-seed analysis confirmed general similarities in connectivity patterns between the two populations, differences were noted for the seed-to-voxel whole brain analysis. No group differences were found for the DMN, which suggests that this network development has not been significantly perturbed by adverse prenatal factors. In contrast, parts of the DAN, sensory networks and thalamic connections all showed systematic disturbance in the AbD group. Within the DAN system the left frontal eye field (FEF) and the right intra-parietal sulcus (IPS) were identified as areas with reduced connectivity to other brain regions in the AbD infants; regions adjacent to the precentral gyrus and parts of the striate and extrastriate visual cortices respectively. FEF and IPS areas are important in many aspects of visually guided behaviour including involvement in control of visual attention and eye movements and are known to collaborate in directing sustained attention to external stimuli (361, 362).

Within the sensory networks connectivity changes are also shown. Decreased connectivity is noted between the auditory cortex and superior parietal lobule (SPL) in the AbD infants. This might reflect a loss of top-down attentional control of processing that could have ramifications for the development of various cognitive functions including language development, which is known to be impaired in IUGR infants (363).
In the AbD infants the left auditory cortex also displayed increased connectivity to the anterior hippocampus, amygdala and striatum including the putamen and nucleus accumbens. The striatum is one of the major long-range targets of the auditory cortex and is known to be important in the processing of auditory perceptual space and permitting the convergence of multiple sensory inputs (364-367). The nucleus accumbens mediates motivational effects of emotionally significant stimuli and is important in integration of information from the hippocampus and amygdala (368, 369). The putamen is proposed to be involved in the encoding of acoustical signal properties thus reducing processing demands of auditory analysis in the temporal cortex and negative correlations have been established between the striatum and the auditory cortex. Connectivity between the visual cortex and other brain regions is also changed in the AbD infants, with reduced connectivity between the right visual cortex and regions in the right postcentral gyrus and superior parietal lobule. Posterior parietal areas such as the superior parietal lobule and postcentral gyrus are known to serve visuomotor integration processes which demand a high level of accuracy of spatial representation and attentional resources. They are important for the integration of signals from diverse sensory areas and across modalities (370). Visuo-spatial attention functions may be primarily lateralised to right parietal regions, which would fit with the lateralisation of the findings in this study (371, 372). Disturbances in visuomotor circuitry would be consistent with visuomotor deficits noted previously in IUGR infants (230).

Finally, with regard to neural connectivity to the thalamus, the AbD infants displayed reduced connectivity to parts of the right and left cerebellum from the right thalamus. Regions identified were lobules I-IV and V. The thalamus is a vital subcortical gray matter region, which integrates neural activity from widespread neocortical inputs and outputs, and is believed to modulate and facilitate communication in all areas of the cerebral cortex (373, 374). All links between the cerebellum and the cerebral cortex relay through the thalamus, which projects cerebellar inputs to motor and non-motor cortices (375).
The anterior cerebellum (lobules I-V) is particularly implicated in sensorimotor function while lobules I-IV have demonstrated strong co activations with the thalamus which is an important node in the cerebro-ponto-cerebellar-thalamic-cortical loop; central for movement, cognitive and emotional processes (375-378). Strong resting state functional connectivity has also been found between lobules I-IV and visual systems (377). Functional deficits in a wide range of domains are evident in IUGR populations in later life (363). The reduction in connectivity in the AbD infants suggests there may be compromised communication within neural loops that include thalamo-cerebellar components. This may compromise diverse functions in the AbD infants including motor, cognitive and emotional areas and subsequently developmental trajectory. Functional deficits in a wide range of domains are evident in IUGR populations in later life (363).

At the time of study design in 2010 there were no suitable freely available neonatal 3T MRI brain atlases (451). An atlas used by Kazemi et al from 2007 was based on a subject number of 7 and was not felt to be robust enough for the intended use in this study (450). Subsequent to the finalisation of study design and the granting of ethical approval an atlas by Kuklisova-Murgasova was published (452). A decision to proceed with the Montreal Neurological Institute template was made as a further delay would have meant loss of many PORTO infants for the study. The use of an appropriate 3T MRI brain atlas would be superior and a repeat of the study using such an atlas would be interesting to compare the results. However, both groups were treated the same using the same template and as such I feel that the results generated are less likely to be spurious.

This study demonstrates that compared to IUGR infants with normal Doppler’s, those with abnormal Doppler’s appear to be at particular risk as they demonstrate alterations of connectivity within attentional network systems and between these systems and sensory, motor and thalamic component processes. Such deficits could influence their ability to control and direct attentional resources and so affect how they interact with their surroundings and how they engage within the learning environment.
This work will be correlated with the ongoing prospective psychological follow up of this cohort where we speculate that those growth-restricted infants with abnormal Doppler’s may display signs of learning/concentration deficits as suggest by these findings.
Section 3: Results

Chapter 6

Volumetric analysis of the Intra-Uterine Growth Restricted Infant Brain
Chapter 6. Results

Volumetric analysis of the Intra-Uterine Growth Restricted Infant brain

6.1 Introduction

Accurately assessing volumes of various regions of the brain is popular in neurodevelopmental and neuropsychiatric research and has resulted in several valuable insights into the adult brain through anatomical segmentation. Translating adult approaches into the neonatal period is difficult due to the several differences in both brain structure and the distinctiveness of the images produced in the neonatal period (379). Due to the relative lack of myelin and the rapid brain growth during this period, adult-based atlases may be less accurate for segmentation of the newborn brain. Over the past decade significant advances have been made, improving the accuracy and reliability of these findings in neonates. Age-appropriate atlases and improved volumetric techniques are freely available to investigate the effects of both preterm birth and various causes of brain injury in this population (380). Consequently, many studies assessing brain volumes in different capacities have been undertaken. The earliest studies suggested impaired brain growth in preterm infants; however, more recent studies suggest that the cortical surface area as opposed to the total brain volume may be affected (381, 382). Initial studies were only able to investigate for a small number of regions of interest, as accurate detailed whole brain segmentation was not readily available. Another potentially limiting factor is that the boundaries between structures and regions are indistinct due to the state of myelination of the subject and intersubject variability. Rapid changes, the relatively small size of anatomical structures and the significant variability between subjects also impacts on volumetric analysis in the neonate (383).

Volumetric analysis of premature infants affected by IUGR has revealed that they demonstrate reduced intracranial volume as compared with normally grown premature infants, with cortical grey matter volume in particular reduced by 28% compared to controls. This suggests that the cerebral cortical development is predominantly affected in IUGR (252).
Volumetric studies have also suggested that hippocampal and caudate nucleus volumes, along with other structures, are reduced when compared to healthy non-IUGR controls (229, 253, 261). Recently young adults born small for gestational age at term have also been shown to have a global reduction in brain volume as well as regional reduction in cortical surface area. These reductions were more pronounced in those who were growth restricted (454).

The specific aim of this study was to determine whether infants, antenatally identified as having IUGR, had global or regional volumetric differences depending on their umbilical arterial Doppler status i.e. a comparison between those with Abnormal versus Normal Doppler antenatal studies.

6.2 Materials and Methods

Subjects
Infants were recruited through participation in the StOOPS study (Short-term Outcome Of infants in the PORTO Study). Infants in the cohort were antenatally identified as growth restricted with an estimated fetal weight < 10th centile and detailed serial Doppler ultrasound measurement studies of the umbilical artery performed as per study protocol (127). Eligibility and exclusion criteria have previously been described. Infants were eligible for inclusion if estimated fetal weight was less than the 10th centile, they were a singleton pregnancy and they did not have any identified complex congenital problems, chromosomal disorders or primary brain abnormalities. All StOOPS infants were scanned at corrected full term gestational age. Scanning was performed on non-sedated infants after feeding to facilitate natural sleep. They were then placed in a vacuum immobilisation mattress (Noras MRI products GmbH, Germany) designed to mold to their shape, supporting them in a comfortable position within the head coil and greatly minimising the potential for head motion. Acoustic noise was minimised through the use of earplugs in conjunction with neonatal earmuffs (MiniMuffs, Natus Medical Inc., USA).
The entire StOOPS cohort of 73 infants had volumetric sequences for analysis. 14 infants were subsequently excluded due to excessive head movement during the sequence, affecting the transformation. Anatomical MR images were analysed by 2 experienced paediatric radiologists. Relevant neonatal demographic details provided in Table 15.

Image Acquisition

A 3D Inversion Recovery prepared Spoiled Gradient Recalled echo (IR-SPGR) sequence was used to obtain high resolution T₁-weighted images of the brain, with: Field of view (FOV) = 200 x 161 x 110 mm³, isotropic spatial resolution of 1 mm, TR/TE = 17/4.6 ms, TI = 800 ms, flip angle = 13°, SENSE factor = 2, acquisition time = 3 min 53 s. T₂-weighted images were acquired using a 3D VISTA (turbo spin echo) sequence with: FOV = 200 x 161 x 110 mm³, isotropic spatial resolution of 0.9 mm, TR/TE_{eff} = 2500/452 ms, SPIR fat suppression, SENSE factor = 2, acquisition time = 5 min 28 s.
Table 15. Demographic information. Normal Doppler (ND), Abnormal Doppler (AbD), Occipitofrontal circumference (OFC), Neonatal intensive care unit (NICU), Patent ductus arteriosus (PDA), Ultrasound (US), Retinopathy of prematurity (ROP), Necrotising enterocolitis (NEC). ** Not significant between control and ND groups, * Not significant between ND and AbD groups. Comparison of proportional differences between groups was performed using Chi squared analysis and Comparison between continuous variables was made using the analysis of variance (ANOVA), followed by Post Hoc Tukey’s analysis, where appropriate.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>ND</th>
<th>AbD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infants (Male)</td>
<td>9 (5)</td>
<td>22 (11)</td>
<td>28 (18)</td>
</tr>
<tr>
<td>Antenatal Steroids</td>
<td>0</td>
<td>12 (55%)</td>
<td>28 (100%)</td>
</tr>
<tr>
<td>Delivery – Instrumental/Emergency</td>
<td>0</td>
<td>7 (32%)</td>
<td>24 (86%)</td>
</tr>
<tr>
<td>Birth Weight (grams)</td>
<td>3330 (2980 – 3820)</td>
<td>2050 (1050 – 2870)</td>
<td>1170 (580 – 2230)</td>
</tr>
<tr>
<td>Birth Weight (centile)</td>
<td>38 (10 – 75)</td>
<td>1.45 (0.1 – 23)</td>
<td>4 (0.3 – 24)</td>
</tr>
<tr>
<td>Gestation at birth (weeks)</td>
<td>39+1 (37+0 – 41+0)</td>
<td>37+0 (28+3 – 40+3)</td>
<td>31+2 (27+1 – 36+1)</td>
</tr>
<tr>
<td>OFC at birth (cms)</td>
<td>34.1 (32.5 – 36)</td>
<td>31 (27.5 – 34.5)</td>
<td>27.5 (21.3 – 33)</td>
</tr>
<tr>
<td>NICU Course</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ventilated</td>
<td>0</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>(days)</td>
<td>(1 – 3)</td>
<td>(2 – 7)</td>
<td>(1.9 (1 – 24)</td>
</tr>
<tr>
<td>Culture positive Infection</td>
<td>0</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Antibiotic course</td>
<td>1</td>
<td>9</td>
<td>21</td>
</tr>
<tr>
<td>(days)</td>
<td>(2)</td>
<td>(2 – 7)</td>
<td>(4 (2 – 42)</td>
</tr>
<tr>
<td>Treated PDA</td>
<td>0</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>(Surgical)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abnormal Cranial US</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>(Medical)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ROP</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>NEC</td>
<td>0</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Start feeds (days)</td>
<td>1</td>
<td>1.7 (1 – 10)</td>
<td>3.3 (1 – 12)</td>
</tr>
<tr>
<td>Days to full feeds</td>
<td>1</td>
<td>4.2 (1 – 27)</td>
<td>12.8 (1 – 31)</td>
</tr>
<tr>
<td>Length of stay</td>
<td>3 (2 – 4)</td>
<td>6 (2 – 69)</td>
<td>33 (5 – 89)</td>
</tr>
<tr>
<td>Age at MRI (weeks)</td>
<td>42+5 (39+1 – 45+2)</td>
<td>43+2 (41+6 – 47+5)</td>
<td>42+2 (40+1 – 47+1)</td>
</tr>
<tr>
<td>Weight at MRI (grams)</td>
<td>3900 (3000 – 4800)</td>
<td>3300 (2600 – 5000)</td>
<td>3250 (2300 – 4500)</td>
</tr>
<tr>
<td>OFC at MRI (cms)</td>
<td>36.5 (33.5 – 37.5)</td>
<td>36 (33 – 39)</td>
<td>35.5 (33 – 37.5)</td>
</tr>
</tbody>
</table>
Volumetric analyses were conducted on the sample using two different methodologies.

**Analysis I**
In the first analysis, a study specific template was generated using ANTs (Advanced Normalisation Tools), an open source software for multidimensional image registration, segmentation and statistical analysis. This is used to generate a large deformation atlas, which can be used to compare topologically dissimilar groups and comparing anatomical growth and development. Large deformation image registration frameworks are based on structural comparisons of the geodesic distances between members of the group as opposed to linear averaging to estimate mean atlas shapes (384). Geodesic refers to the geometry of curved surfaces, the geodesic distance being the shortest possible line between two points on a sphere or other curved surface. The normalisation step maps individuals to common anatomy and the average shape and appearance image is then iteratively estimated (Fig. 32). The degree of transformation that occurs from a subject to the atlas template is captured by the *Jacobian*. The Jacobian accounts for the changes in the infinitesimal lengths, areas and volumes that occur when changing the basis of a coordinate system. The study specific template was generated from the T1 structural images and based on the Montreal Neurological Institute (birth - 4.5 years) standard space template (306, 385). Voxel based morphometry was then performed with differences in volume of regions of interest between groups assessed by performing a series of *t* tests at every voxel in the image.
Analysis II

The second VBM analysis used the ALBERT (A Label Based Encephalic Roi Template) neonatal brain atlas (380). The ALBERT template is a neonatal brain atlas, based on 20 neonatal brain datasets (15 ex preterm and 5 term infants) and segmented into 50 regions of interest. The absolute size of total and regional brain volumes were calculated for each infant from the voxel size and the number of voxels in the 50 identified regions of interest (Fig. 33). The proportional volume of each ROI was obtained as a fraction of the total brain volume. Comparison between groups was made using the analysis of variance (ANOVA), followed by Post hoc Tukey’s analysis where appropriate. Statistical significance was accepted for alpha < 0.05. Between groups analysis was performed using the CamBA (Cambridge Brain Activation) suite of programs. CamBA is a software repository based at the Brain Mapping Unit in Cambridge and contains software pipelines for a number of different analyses including second- level linear modeling of within- and between-group effects (386, 387). Regression analyses were performed across voxels to assess neuroanatomical correlates with the 1-year outcome results from the ASQ-3.
Table 16. ALBERT labels.

<table>
<thead>
<tr>
<th>Temporal Lobe</th>
<th>Posterior Fossa</th>
</tr>
</thead>
<tbody>
<tr>
<td>1;2 Hippocampus</td>
<td>17;18 Cerebellum</td>
</tr>
<tr>
<td>3;4 Amygdala</td>
<td>19 Brainstem</td>
</tr>
<tr>
<td>5;6 Anterior temporal lobe, medial part</td>
<td><strong>Insula and Cingulate Gyri</strong></td>
</tr>
<tr>
<td>7;8 Anterior temporal lobe, lateral part</td>
<td>21;20 Insula</td>
</tr>
<tr>
<td>9;10 Parahippocampal and ambient gyri, ant. part</td>
<td>33;32 Cingulate gyrus, ant. part</td>
</tr>
<tr>
<td>25;24 Parahippocampal and ambient gyri post. part</td>
<td>35;34 Cingulate gyrus, post. part</td>
</tr>
<tr>
<td>11;12 Superior temporal gyrus, middle part</td>
<td><strong>Frontal Lobe</strong></td>
</tr>
<tr>
<td>31;30 Superior temporal gyrus, post. part</td>
<td>37;36 Frontal lobe</td>
</tr>
<tr>
<td>13;14 Middle and inferior temporal gyrus, ant. part</td>
<td><strong>Occipital Lobe</strong></td>
</tr>
<tr>
<td>29;28 Middle and inferior temporal gyrus, post. part</td>
<td>23;22 Occipital lobe</td>
</tr>
<tr>
<td>15;16 Fusiform gyrus, ant. part</td>
<td><strong>Parietal Lobe</strong></td>
</tr>
<tr>
<td>27;26 Fusiform gyrus, post. part</td>
<td>39;38 Parietal lobe</td>
</tr>
<tr>
<td><strong>Basal Ganglia</strong></td>
<td><strong>Corpus Callosum</strong></td>
</tr>
<tr>
<td>41;40 Caudate nucleus</td>
<td>48 Corpus callosum</td>
</tr>
<tr>
<td>43;42 Thalamus</td>
<td><strong>Ventricles</strong></td>
</tr>
<tr>
<td>45;44 Sub-thalamic nucleus</td>
<td>49;50 Lateral ventricles</td>
</tr>
<tr>
<td>47;46 Lentiform nucleus</td>
<td></td>
</tr>
</tbody>
</table>
Figure 33. A series of slices from the orthogonal planes of the atlas data set of a randomly chosen male preterm infant, which has manually been labelled using the ALBERT protocols. Numbers correspond to the anatomical regions as listed in Table 16.
6.3 Results

Analysis I –
The first analysis performed produced a study specific template formed from the 9 Control infants, 22 Normal Doppler IUGR infants (ND) and the 28 Abnormal Doppler IUGR infants (AbD). Voxel based morphometry was then performed with differences in volume of regions of interest between groups assessed by performing a series of \( t \) tests at every voxel in the image. Differences were identified based on study groups and sex.

Male infant brains demonstrated an increase in volume in the left frontal lobe in the regions of the superior, middle and medial frontal gyri, as compared to female infants, \( p < 0.001 \) (Fig. 34). Female infant brains had greater volumes in two distinct areas, the occipital lobe in the region of the lingual gyrus and the cuneus bilaterally, \( p < 0.0001 \); and the central grey nuclei (including the thalamus and posterior limb of the internal capsule), \( p < 0.001 \) (Fig. 35). Figure 36 shows the study specific template with sex differences.

Differences were also identified between the groups based on their Doppler status. The areas where volume differences were seen were in the lower diencephalon and frontal lobes, specifically the inferior frontal, orbital and paraterminal gyri (Region 1); right temporal lobe region, specifically including the medial temporal and postcentral gyri, and left temporal lobe region, specifically including the medial temporal and postcentral gyri. The greatest volumes in these regions were found in the Control group, followed by ND and then AbD groups. Differences between groups was also seen in the cerebellum, particularly in the region of the cerebellar nuclei, and in these areas, the greatest volume was again seen in the Control group, followed by AbD then ND groups, Table 17 (Fig. 37, 38)
Table 17. Data expressed as means and standard deviation. * Significant between all three groups, + not significant between Ctrl and ND, ** not significant between ND and AbD groups.

<table>
<thead>
<tr>
<th>Region</th>
<th>Control (mm$^3$)</th>
<th>ND (mm$^3$)</th>
<th>AbD (mm$^3$)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Region 1</td>
<td>3811 ± 228</td>
<td>3496 ± 269</td>
<td>3167 ± 294</td>
<td>p &lt; 0.0001*</td>
</tr>
<tr>
<td>Temporal Lobe Right</td>
<td>3474 ± 288</td>
<td>3295 ± 433</td>
<td>2852 ± 392</td>
<td>p &lt; 0.0001†</td>
</tr>
<tr>
<td>Temporal Lobe Left</td>
<td>4160 ± 453</td>
<td>4063 ± 485</td>
<td>3497 ± 394</td>
<td>p &lt; 0.0001†</td>
</tr>
<tr>
<td>Cerebellum Right</td>
<td>1416 ± 208</td>
<td>1163 ± 166</td>
<td>1195 ± 141</td>
<td>p = 0.0007**</td>
</tr>
<tr>
<td>Cerebellum Left</td>
<td>2095 ± 382</td>
<td>1698 ± 229</td>
<td>1756 ± 291</td>
<td>p = 0.0031**</td>
</tr>
</tbody>
</table>
Figure 34. Volumetric differences between the sexes, region of increased volume in male brain.
Figure 35. Volumetric differences between the sexes, regions of increased volume in female brain. Yellow arrow pointing to region in occipital lobe.
Figure 36. Study specific template with volumetric differences by sex. Red and peach colours indicate increased volume in female infant brains. Blue colour indicates increased volume in male infant brains.
Figure 36 contd. Study specific template with volumetric differences by sex. Red and peach colours indicate increased volume in female infant brains. Blue colour indicates increased volume in male infant brains.
Figure 36 contd. Study specific template with volumetric differences by sex. Red and peach colours indicate increased volume in female infant brains. Blue colour indicates increased volume in male infant brains.
Figure 37. Study specific template with significant volumetric differences based on study groupings.
Figure 38. Study specific template with significant volumetric differences based on study groupings. Yellow arrow pointing to region of significant difference in right temporal lobe.
Figure 39. Study specific template with significant volumetric differences based on study group highlighted in coloured areas. Greatest volume in the Control group. See Table 17.
**Analysis II – Volumetric analysis**

The mean ± SD brain volume, estimated by adding all 50 ROI’s, was 339.3 ± 11.9 cm$^3$ for the control group, 349.1 ± 19.5 cm$^3$ for the ND group and 348.5 ± 19.5 cm$^3$ for the AbD group. This difference was not significant. Hemispheres also did not differ between the right and the left side for the ND and AbD groups, there was a significant difference between hemispheres in the control group, p < 0.05; there was no difference in hemispheres between groups (control, right hemisphere 162.6 ± 11.7 cm$^3$, left hemisphere 168.2 ± 11.9 cm$^3$; ND, right hemisphere 168.2 ± 12.2 cm$^3$, left hemisphere 172.5 ± 12.3 cm$^3$; AbD, right hemisphere 168.4 ± 12.4 cm$^3$, left hemisphere 171.8 ± 12.4 cm$^3$).

In Tables 18-20 the absolute volumes of the ROI’s for each of the groups are presented. For absolute volumes, hypothesis testing for multiple groups was performed using the analysis of variance (ANOVA); p < 0.05. Individual intergroup comparisons were made using Tukey’s Post hoc test, given that group sizes differed. Both analyses adjusted for type 1 error and consequent false discovery rate. In IUGR infants with abnormal Doppler’s (AbD) the volumes of the right medial and inferior temporal gyri (anterior part) were smaller than IUGR infants with normal Doppler’s (ND) whilst the volume of left parahippocampal and ambient gyri (posterior part) and right frontal lobe were smaller in the control group compared to both AbD and ND groups. The volume of the left lateral ventricle was larger in the AbD group as compared to the control group, but not significantly larger than the ND group and no significant difference between ND and control groups. Left hippocampal volumes were greater than right for each group, the greatest difference was noted in the control group though not significantly so (Ctrl 172mm$^3$, ND 163 mm$^3$, AbD 153 mm$^3$).
In Tables 21-23 the results of normalisation of each structure volume for the whole brain volume for each of the groups are presented. Bilaterally, fractional regional volumes in the medial and inferior temporal gyri (anterior part) were larger in the control group. On the right the Ctrl and ND groups were significantly greater than the AbD group, whereas on the left significance was seen in Ctrl vs AbD only. The fractional regional volume of the brainstem was also greater in the control group, significantly greater than the ND group. The lateral ventricles bilaterally had fractional regional volumes smaller in the control group, significantly in Ctrl vs AbD. Unilaterally, the fractional regional volume of the right frontal lobe was smaller in the Control group compared to the AbD group.

The left medial and inferior temporal gyri (posterior part) fractional volume was larger in the ND group compared to the AbD group and the left parahippocampal and ambient gyri (posterior part) fractional volume was significantly larger in both the ND and AbD groups compared to the Control group.
Table 18. Absolute volumes in mm$^3$ for the regions of interest (ROI) in the temporal lobe ALBERT labels. Normal Doppler (ND), Abnormal Doppler (AbD), anterior (ant.), posterior (post.) * denotes significance for Ctrl vs ND and Ctrl vs AbD, but not ND vs AbD, ** denotes significance for ND vs AbD only. ANOVA with Tukey’s post hoc analysis; p < 0.05.

<table>
<thead>
<tr>
<th>ROI</th>
<th>Control</th>
<th>ND</th>
<th>AbD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Right</td>
<td>Left</td>
<td>Right</td>
</tr>
<tr>
<td><strong>Temporal Lobe</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hippocampus</td>
<td>649</td>
<td>821</td>
<td>715</td>
</tr>
<tr>
<td>Amygdala</td>
<td>488</td>
<td>579</td>
<td>516</td>
</tr>
<tr>
<td>Anterior Temporal lobe,</td>
<td>1034</td>
<td>1151</td>
<td>1188</td>
</tr>
<tr>
<td>medial part</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anterior Temporal lobe,</td>
<td>1182</td>
<td>1330</td>
<td>1352</td>
</tr>
<tr>
<td>lateral part</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parahippocampal &amp; Ambient</td>
<td>1161</td>
<td>1270</td>
<td>1202</td>
</tr>
<tr>
<td>gyri ant.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parahippocampal &amp; Ambient</td>
<td>989</td>
<td>1168</td>
<td>1056</td>
</tr>
<tr>
<td>gyri post.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Superior Temporal gyrus,</td>
<td>4892</td>
<td>5448</td>
<td>5053</td>
</tr>
<tr>
<td>middle part</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Superior Temporal gyrus,</td>
<td>1693</td>
<td>2509</td>
<td>1760</td>
</tr>
<tr>
<td>post.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Middle and Inferior</td>
<td>4553</td>
<td>4795</td>
<td>4301</td>
</tr>
<tr>
<td>Temporal gyrus ant.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Middle and Inferior</td>
<td>6264</td>
<td>6286</td>
<td>6482</td>
</tr>
<tr>
<td>Temporal gyrus post.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fusiform gyrus ant.</td>
<td>940</td>
<td>1123</td>
<td>899</td>
</tr>
<tr>
<td>Fusiform gyrus post.</td>
<td>1179</td>
<td>1408</td>
<td>1185</td>
</tr>
</tbody>
</table>
Table 19. Absolute volumes in mm$^3$ for the regions of interest (ROI) as per the ALBERT labels. Normal Doppler (ND), Abnormal Doppler (AbD). * denotes significance for Ctrl vs ND and Ctrl vs AbD, but not ND vs AbD.

<table>
<thead>
<tr>
<th>ROI</th>
<th>Control</th>
<th>ND</th>
<th>AbD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Right</td>
<td>Left</td>
<td>Right</td>
</tr>
<tr>
<td><strong>Posterior Fossa</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cerebellum</td>
<td>14654</td>
<td>14382</td>
<td>13975</td>
</tr>
<tr>
<td>Brainstem</td>
<td>6126</td>
<td>5846</td>
<td>5871</td>
</tr>
<tr>
<td><strong>Insula &amp; Cingulate Gyri</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insula</td>
<td>4148</td>
<td>4359</td>
<td>4305</td>
</tr>
<tr>
<td>Cingulate gyrus anterior part</td>
<td>3309</td>
<td>3172</td>
<td>3594</td>
</tr>
<tr>
<td>Cingulate gyrus posterior part</td>
<td>2897</td>
<td>2923</td>
<td>3098</td>
</tr>
<tr>
<td><strong>Frontal Lobe</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frontal lobe</td>
<td>48184</td>
<td>49953</td>
<td>51440</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Occipital Lobe</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Occipital lobe</td>
<td>19975</td>
<td>19011</td>
<td>20483</td>
</tr>
<tr>
<td><strong>Parietal Lobe</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Parietal lobe</td>
<td>33536</td>
<td>34842</td>
<td>34070</td>
</tr>
</tbody>
</table>

Table 20. Absolute volumes in mm$^3$ for the regions of interest (ROI) as per the ALBERT labels. Normal Doppler (ND), Abnormal Doppler (AbD). * denotes significance for Ctrl vs AbD only.

<table>
<thead>
<tr>
<th>ROI</th>
<th>Control</th>
<th>ND</th>
<th>AbD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Right</td>
<td>Left</td>
<td>Right</td>
</tr>
<tr>
<td><strong>Basal Ganglia</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caudate nucleus</td>
<td>1623</td>
<td>1684</td>
<td>1659</td>
</tr>
<tr>
<td>Thalamus</td>
<td>3382</td>
<td>3601</td>
<td>3541</td>
</tr>
<tr>
<td>Sub-thalamic nucleus</td>
<td>361</td>
<td>346</td>
<td>359</td>
</tr>
<tr>
<td>Lentiform nucleus</td>
<td>3973</td>
<td>4264</td>
<td>4026</td>
</tr>
<tr>
<td><strong>Corpus Callosum</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corpus callosum</td>
<td>2232</td>
<td>2379</td>
<td>2335</td>
</tr>
<tr>
<td><strong>Ventricles</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lateral ventricles</td>
<td>1541</td>
<td>1732</td>
<td>1614</td>
</tr>
</tbody>
</table>
Table 21. Fractional regional volumes for the regions of interest (ROI) in the temporal lobe ALBERT labels. Normal Doppler (ND), Abnormal Doppler (AbD), anterior (ant.), posterior (post.).

<table>
<thead>
<tr>
<th>ROI</th>
<th>Control</th>
<th>ND</th>
<th>AbD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Right</td>
<td>Left</td>
<td>Right</td>
</tr>
<tr>
<td><strong>Temporal Lobe</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hippocampus</td>
<td>0.19</td>
<td>0.24</td>
<td>0.20</td>
</tr>
<tr>
<td>Amygdala</td>
<td>0.14</td>
<td>0.17</td>
<td>0.15</td>
</tr>
<tr>
<td>Anterior Temporal lobe, medial part</td>
<td>0.30</td>
<td>0.34</td>
<td>0.31</td>
</tr>
<tr>
<td>Anterior Temporal lobe, lateral part</td>
<td>0.35</td>
<td>0.39</td>
<td>0.31</td>
</tr>
<tr>
<td>Parahippocampal &amp; Ambient gyri ant.</td>
<td>0.34</td>
<td>0.37</td>
<td>0.35</td>
</tr>
<tr>
<td>Parahippocampal &amp; Ambient gyri post.</td>
<td>0.29</td>
<td>0.34</td>
<td>0.29</td>
</tr>
<tr>
<td>Superior Temporal gyrus, middle part</td>
<td>1.44</td>
<td>1.60</td>
<td>1.38</td>
</tr>
<tr>
<td>Superior Temporal gyrus, post.</td>
<td>0.50</td>
<td>0.74</td>
<td>0.55</td>
</tr>
<tr>
<td>Middle and Inferior Temporal gyrus ant.</td>
<td>1.34</td>
<td>1.41</td>
<td>1.25</td>
</tr>
<tr>
<td>Middle and Inferior Temporal gyrus post.</td>
<td>1.85</td>
<td>1.85</td>
<td>1.80</td>
</tr>
<tr>
<td>Fusiform gyrus ant.</td>
<td>0.28</td>
<td>0.33</td>
<td>0.25</td>
</tr>
<tr>
<td>Fusiform gyrus post.</td>
<td>0.35</td>
<td>0.42</td>
<td>0.35</td>
</tr>
</tbody>
</table>
Table 22. Fractional regional volumes for the regions of interest (ROI) as per the ALBERT labels. Normal Doppler (ND), Abnormal Doppler (AbD).

<table>
<thead>
<tr>
<th>ROI</th>
<th>Control</th>
<th>ND</th>
<th>AbD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Right</td>
<td>Left</td>
<td>Right</td>
</tr>
<tr>
<td>Posterior Fossa</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cerebellum</td>
<td>4.32</td>
<td>4.24</td>
<td>4.24</td>
</tr>
<tr>
<td>Brainstem</td>
<td>1.81</td>
<td>1.72</td>
<td>1.68</td>
</tr>
<tr>
<td>Insula &amp; Cingulate Gyri</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insula</td>
<td>1.22</td>
<td>1.29</td>
<td>1.21</td>
</tr>
<tr>
<td>Cingulate gyrus anterior part</td>
<td>0.98</td>
<td>0.94</td>
<td>1.14</td>
</tr>
<tr>
<td>Cingulate gyrus posterior part</td>
<td>0.85</td>
<td>0.86</td>
<td>0.85</td>
</tr>
<tr>
<td>Frontal Lobe</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frontal lobe</td>
<td>14.2</td>
<td>14.7</td>
<td>14.89</td>
</tr>
<tr>
<td>Occipital Lobe</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Occipital lobe</td>
<td>5.89</td>
<td>5.60</td>
<td>5.63</td>
</tr>
<tr>
<td>Parietal Lobe</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Parietal lobe</td>
<td>9.89</td>
<td>10.27</td>
<td>9.93</td>
</tr>
</tbody>
</table>

Table 23. Fractional regional volumes for the regions of interest (ROI) as per the ALBERT labels. Normal Doppler (ND), Abnormal Doppler (AbD).

<table>
<thead>
<tr>
<th>ROI</th>
<th>Control</th>
<th>ND</th>
<th>AbD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Right</td>
<td>Left</td>
<td>Right</td>
</tr>
<tr>
<td>Basal Ganglia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caudate nucleus</td>
<td>0.48</td>
<td>0.50</td>
<td>0.45</td>
</tr>
<tr>
<td>Thalamus</td>
<td>1.00</td>
<td>1.06</td>
<td>0.99</td>
</tr>
<tr>
<td>Sub-thalamic nucleus</td>
<td>0.11</td>
<td>0.10</td>
<td>0.98</td>
</tr>
<tr>
<td>Lentiform nucleus</td>
<td>1.17</td>
<td>1.26</td>
<td>1.11</td>
</tr>
<tr>
<td>Corpus Callosum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corpus callosum</td>
<td>0.66</td>
<td>0.70</td>
<td>0.67</td>
</tr>
<tr>
<td>Ventricles</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lateral ventricles</td>
<td>0.45</td>
<td>0.51</td>
<td>0.46</td>
</tr>
</tbody>
</table>
Figure 40. Fractional volume differences between the groups in the medial & inferior temporal gyri – anterior part bilaterally. Control (Ctrl) significantly greater than Abnormal Doppler group (AbD) but not Normal Doppler Group (ND) on the left and both Ctrl and ND significantly greater than AbD on the right but not between Ctrl and ND.
Figure 41. Fractional volume differences between groups. (A) Right Frontal lobe significantly smaller volume in the Control (Ctrl) group compared to the Abnormal Doppler (AbD) group, differences between other groups not significant. (B) Left Parahippocampal & Ambient gyri (posterior part) significantly smaller in the Ctrl group compared to both ND and AbD groups.
Between groups analysis was performed using the CamBA (Cambridge Brain Activation) suite of programs. Regression analyses were performed across voxels to assess neuroanatomical correlates with the 1-year outcome results from the ASQ-3. The 1-year outcome results demonstrated that there were significant differences in the domains of Communication and Personal & Social, with the infants in the Control group achieving higher scores compared to the IUGR infant groups; the infants in the AbD group score lowest. Of the infants that had volumetric analysis performed 1-year outcome and VBM measures were available for 46; 7 Control, 16 Normal Doppler and 23 Abnormal Doppler. Communication scores were positively correlated with an increased volume in two cluster areas (Fig. 42, 43) whereas in the Personal & Social domain scores were negatively correlated with increased volume in one cluster area (Fig. 44).

The Communication cluster 1 includes the brainstem structures of the medulla oblongata and pons as well as the cerebellar peduncles, which are involved in integrating proprioceptive sensory input with motor functions. The second Communication cluster is predominantly based around the right parietal lobe and insula and also includes part of the precentral gyrus of the frontal lobe. The areas included are involved in processing tactile and proprioceptive information (postcentral gyrus, Brodmann Areas 3,1,2) the primary motor cortex (precentral gyrus, Brodmann Area 4) and consciousness, emotions and homeostasis (insula). The highest Communication scores were in the Control group, which also had the highest volumes in the identified clusters. The lowest score was in the ND group and the lowest volume was found in the ND group for cluster 1 and AbD group for cluster 2.

The Personal & Social cluster, cluster 3, is in the right frontal lobe including the right medial frontal gyrus. The medial frontal gyrus is involved in decision-making abilities and executive mechanisms such as planning of complex coordinated movements. The areas highlighted include part of Brodmann Area 6 (which includes the premotor cortex) and Brodmann Area 8 (which includes the Frontal Eye Fields). The highest scores in the Personal & Social domain were in the Control group, which also had the lowest volume for the identified cluster. The Abd group had the lowest scores and the greatest volume for the identified cluster region.
Communication Cluster 2

Figure 43.
Personal and Social Cluster 1

Figure 44.
6.4 Conclusion

The volumetric analysis results have yielded some interesting findings and demonstrate differences between the groups. The first analysis was performed using a study specific template and demonstrated differences based on the study groupings and also by gender. The volumetric differences identified showed that the Control group had significantly greater volume in a number of regions including part of the frontal lobe, brainstem, cerebellum and temporal lobe when compared to the ND and AbD groups. This is in keeping with previous studies where healthy control infants were found to have greater volumes in various cortical and deep white matter regions compared to ex premature infants (282, 388). Differences were also noted based on sex of the infant, with parts of the left frontal lobe greater in the males and elements of the central grey nuclei and occipital lobes greater in female infant brains. The second analysis used the ALBERT neonatal atlas to allow for further clarification of the differences identified. With this analysis the absolute and normalised volumes were assessed and defined regions of interest could be compared. Total brain volume did not differ between the groups. This is in keeping with previous studies that did not demonstrate significant differences in total brain volume between term infants and well preterm infants scanned at term corrected age (380, 389). A difference between hemispheres was noted in the Control group only. An asymmetry in cerebral hemisphere volumes has been reported in many adult and paediatric populations, typically the right side being of larger volume (390). The absence of a significant difference in hemisphere volumes in both IUGR groups may suggest a deviation from the normal growth process, though an asymmetry between hemispheres is not universally presented in the literature (380).

A number of differences were seen comparing the absolute volumes, however, once normalised a greater number of areas with significant differences were identified. In some regions the healthy term control infants have smaller volumes compared to the IUGR groups, such as in the right frontal lobe and the left parahippocampal and ambient gyri, whereas in other regions the Control group have greater volumes, such as in the anterior part of the medial and inferior temporal gyri bilaterally.
The medial and inferior temporal gyri are involved in cognitive processes, including semantic memory, language, visual perception and sensory integration. These functions are often impaired in children who were born with IUGR. Both lateral ventricles are also smaller in the Control group and largest in the AbD group.

These differences suggest that the IUGR groups’ brain developmental trajectory is altered, compared to the Control group, resulting in failure of regions or areas to achieve the correct volume as a result of the adverse in utero environment. The regression analysis performed correlating the ASQ-3 scores with volumetric analysis identified areas that correlate with scores for the Communication and Personal & Social domains. Communication scores positively correlate with volume in two cluster areas identified; a brainstem region and a region around the central gyrus. Personal & Social scores are negatively correlated with volume in a region in the right frontal lobe. Longer-term psychological assessment is ongoing to compare for differences between groups as they grow up and will be correlated with the volumetric results presented.
Section 3: Results

Chapter 7

Neurodevelopmental and Head Growth Outcome at 1 year
Chapter 7. Results

Neurodevelopmental and Head Growth Outcome at 1 year

7.1 Introduction

Intrauterine growth restriction (IUGR) infants can be defined as babies whose birth weights lie below the 10th percentile for that gestational age, and IUGR affects up to 10% of all pregnancies. Approximately 5 to 10% of pregnancies complicated by IUGR will result in either stillbirth or neonatal death (391, 392). The effects of IUGR continue beyond the neonatal period and may have a profound impact on growth and development. Higher rates of neurodevelopmental issues are seen including poor academic performance, behavioural problems and cognitive impairment (230, 363, 393). IUGR infants born prematurely are also more likely to have behavioural, sensory and cognitive issues as compared to matched AGA premature infants (18, 252). With the advent of more detailed antenatal monitoring of growth restriction, Doppler measurement of umbilical artery (UA) flow has been used to identify those at risk for adverse perinatal and longer-term neurodevelopmental outcomes (394, 395). However, absence of abnormal UA blood flow on Doppler measurement does not necessarily translate to normal developmental outcome. Studies have shown that near term IUGR and SGA with normal Doppler measurements also have increased rates of adverse neurodevelopmental outcome (396-398). In the PORTO study, abnormal Doppler was significantly associated with adverse obstetric and neonatal outcomes, regardless of estimated fetal weight (127).

Neurodevelopmental assessment can be undertaken from birth onwards, the reliability and accuracy improving with advancing age of the child. Whilst an assessment at the age of one year may be too early to be entirely discriminative, it can be predictive of outcome in childhood and later school performance in adolescence (399, 400). The Ages and Stages Questionnaire relies on the parents to observe their child and answer the clear questionnaire regarding the ability of the child.
The ASQ is a well-validated tool and correlates well with the Intelligence Quotient (IQ) and the Bayley scale of infant development. At one year of age it is a useful assessment. The parents complete it and so it is less reliant on performance variables, at a time when reproducing skills can be challenging and observation plays the major part of the assessment. As the parents, have much more information pertaining to their child and their development over time they can more accurately represent the true picture of development. This avoids the situation where the child doesn’t perform a particular task well, or at all, on the day of testing by an independent assessor, resulting in underscoring (396). Assessing IUGR infants with the ASQ has previously yielded interesting results, with IUGR infants with normal antenatal Dopplers displaying significantly lower scores in the problem solving and personal-social domains compared to AGA matched controls (396).

Growth parameters, especially head growth, can also provide valuable information regarding longer-term neurodevelopmental outcome. A close relationship exists between head growth and neurodevelopmental outcome. Head growth is mostly dictated by the development of the brain, the increase reflecting growth of neuronal and neuroglia cells as well as supportive connective tissues. Measuring the occipito-frontal circumference (OFC) is a quick measurement, which is easy to do and provides a valuable surrogate of overall brain growth. Of the anthropometric measurements taken in infancy, head growth, more so than weight or length, appears to be most associated with cognitive outcome. In normal development, it appears that the size of the head at birth and the growth in the first few months of life are more important for longer-term cognitive function than growth after infancy. It also appears that the degree of head growth beyond infancy does not compensate for poor prenatal head growth or growth during infancy and that the period of head growth in first few months is more important than prenatal head growth in children within the normal range at birth (401, 402). Several studies have addressed this relationship and the phenomenon of catch-up growth, where an infant with an OFC on a lower centile than expected for gestational age and weight at birth catches up to a more appropriate centile.
Higher rates of catch-up in head circumference growth are associated with better cognitive outcomes with the majority of the catch-up growth occurring within the period from birth to three months corrected age (403-405). Head growth and its relationship with longer term outcome has been well documented, however, the use of this measure alone as a screening tool may not be reliable on its own and may result in over investigation in many cases (406).

The StOOPS study will also look at the longer-term neurodevelopmental outcome of the cohort at 2.5 – 3 years with formal psychological testing and parent completed assessments as well as continued monitoring of anthropometric measures such as OFC.

We studied the neurodevelopmental outcomes at 1 year as an interim assessment and to identify early differences between the study groups that could facilitate clinical multidisciplinary intervention.

7.2 Materials and Methods

Infants were recruited through participation in the StOOPS study (Short-term Outcome Of infants in the PORTO Study). The PORTO study identified and followed growth-restricted pregnancies recruiting from the largest obstetric units on the island of Ireland. Infants in the cohort were antenatally identified as growth restricted with an estimated fetal weight < 10\textsuperscript{th} centile and detailed serial Doppler ultrasound measurement studies of the umbilical artery performed as per study protocol (127). Eligibility criteria for StOOPS have previously been outlined. There were 74 infants recruited into the StOOPS (Short-term surrogate Outcome Of infants in the PORTO) study from across the participating centres, including 11 healthy term controls. Informed consent was obtained; background data and birth anthropometry measurements were recorded. All 74 infants had a detailed 3T MRI brain at term corrected gestational age (37-44 weeks).
At the time of the MRI scan address and contact details for one year follow up was confirmed and consent for continued participation in follow up was obtained. Prior to reaching the first birthday parents were contacted about completing the questionnaire on infant development and postal address confirmed and instructions on how to complete the questionnaire were discussed. A 12-month Ages and Stages Questionnaire was sent to each infant’s home with a stamped addressed envelope and instructions for completion. This is a parent-completed tool containing 30 developmental items that are categorised in to five domains: communication, gross motor, fine motor, problem solving and personal-social. In each item the parents are required to check “yes” if the child performs the specified behaviour, “sometimes” if they occasionally do or “not yet” if their child has not yet performed the specified task/behaviour. The results are converted to a numerical value and the total score in each domain converted into centiles.

A request for measurement of occipito-frontal head circumference (OFC) was included with the questionnaire and parents were asked to have their GP or public health visitor to perform the measurement at their next visit or vaccination appointment. The vaccination schedule in the Republic of Ireland includes vaccines at 12 months and 13 months of age, parents were advised as to which visit approximated closest to 1 year corrected age and instructed to have the OFC measured at that visit. If parents were not contactable by phone the pack was sent to the last known address on file. A reminder phonecall was placed if there was a delay in returning the documentation and packs were resent if required.

7.3 Results

Of the 74 StOOPS infants, 63 had an EFW <10\textsuperscript{th} centile (IUGR) and 11 infants were term infants (controls) whose weight was appropriate for their gestational age (AGA). The cases included 34 infants with abnormal antenatal ultrasound Doppler’s and 29 with normal Doppler’s. One infant from the abnormal Doppler group was subsequently excluded after the MRI stage due to a grade 3 intraventricular haemorrhage during the neonatal period.
The mean age at completion of ASQ-3 for the entire group was 12 months (+/- 1.3 months) and OFC measurement at 11.5 months (+/- 1.4 months). There was no difference at time of completion of ASQ between the groups (p=0.282) or the time of measurement of head circumference between the groups (p=0.051). Successful 1-year follow up was achieved in 60/73 (82%) of infants for ASQ-3, 61/73 (84%) for OFC with a follow-up rate for both ASQ-3 and OFC combined of 59/73 (81%). There was no difference between the groups in terms of proportions completing the ASQ-3 or having an OFC measurement recorded (Table 24).

Table 24. ASQ-3 and OFC follow up at 1 year. Ages and Stages Questionnaire (ASQ-3), Occipito-frontal circumference (OFC).

<table>
<thead>
<tr>
<th></th>
<th>Controls (n=11)</th>
<th>Normal Doppler (n=29)</th>
<th>Abnormal Doppler (n=33)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASQ-3 Returned</td>
<td>9 (82%)</td>
<td>25 (86%)</td>
<td>27 (82%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OFC Recorded</td>
<td>9 (82%)</td>
<td>24 (83%)</td>
<td>29 (88%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\chi^2 = 0.5024$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$p = 0.777$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\chi^2 = 0.414$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$p = 0.813$</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Prior to analysis of the ASQ-3 results 2 infants from the Normal Doppler group were excluded. One as a result of a diagnosis of Williams Syndrome during the first year of life and the other child was excluded as a result of profound developmental delay under ongoing investigation for other occult diagnoses. The results of the 12-month ASQ-3 did not show a statistically significant difference between the groups for total score, however differences were seen in the domains of communication and personal-social with statistically significant differences seen between the group with Abnormal Dopplers and control. There was no significant difference identified between Normal and Abnormal Doppler groups or between Controls and Normal Doppler groups (Table 25).
Table 25. Comparison of ASQ Scores (Year 1) between Groups. Individual ASQ-3 items reported as median value and total score as mean value. An analysis of variance (ANOVA) was performed for group differences for Total score and Personal-Social and the Kruskall-Wallis test was used for all other analyses. Statistical significance was accepted for an alpha of 0.05.

<table>
<thead>
<tr>
<th>ASQ-3 Item</th>
<th>Control (n=9)</th>
<th>Normal Doppler (n=23)</th>
<th>Abnormal Doppler (n=27)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Communication</td>
<td>60</td>
<td>55</td>
<td>40</td>
<td>0.0334*</td>
</tr>
<tr>
<td>Gross Motor</td>
<td>60</td>
<td>40</td>
<td>30</td>
<td>0.8311</td>
</tr>
<tr>
<td>Fine Motor</td>
<td>50</td>
<td>50</td>
<td>60</td>
<td>0.9185</td>
</tr>
<tr>
<td>Problem Solving</td>
<td>60</td>
<td>60</td>
<td>45</td>
<td>0.1526</td>
</tr>
<tr>
<td>Personal-Social</td>
<td>60</td>
<td>45</td>
<td>40</td>
<td>0.0366*</td>
</tr>
<tr>
<td>Total Score</td>
<td>241</td>
<td>217</td>
<td>206</td>
<td>0.2129</td>
</tr>
</tbody>
</table>

Figures 45-50 show the results of the ASQ-3 for the groups in the various domains in graphical form.
Figure 45.

Figure 46
Figure 49

ASQ-3 Personal-Social

- Control
- Normal Doppler
- Abnormal Doppler

Figure 50

ASQ-3 Communication

- Control
- Normal Doppler
- Abnormal Doppler

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Head circumference and growth trajectory over the first year was measured. A statistically significant difference between groups OFC was seen at delivery (p-value < 0.0001) but not at one year of age (p-value=0.8074) (Fig. 51). Analysis was restricted to those with a 1-year OFC measurement plus either an OFC at delivery or an OFC measurement at MRI (n=182 measurements on 62 cases). The statistical model used was a Linear Mixed Effect model with groups as a factor, time as a continuous covariate, group by time interaction and a random effect for individual cases. The results found a significant interaction (p<0.0001) indicating different trends in growth between groups. The difference between groups OFC was statistically significant at delivery (p<0.0001), but not at 1 year (p=0.8074) and this is the source of the interaction or change in trend i.e. the groups start off different and then normalise to similar levels at one year of age.

Figure 51. OFC trends from delivery to year 1 in the 3 groups
7.4 Conclusion

The difference between groups OFC was statistically significant at delivery; the IUGR groups had lower OFC’s compared to the AGA term controls; however, by 1 year this difference had normalised, displaying catch up head growth. The IUGR infants scored lower in most areas of the assessment, particularly in the communication and personal-social component of the ASQ-3 compared to the controls; this difference was not significant when comparing between the groups with normal and abnormal Doppler’s, but was significant when comparing the Abnormal Doppler group to the Controls. The Problem-Solving domain did not reach statistical significance; however, the trend is in that direction for the infants with abnormal Doppler measurement as compared to the control group. There was no statistically significant difference in results between the control group and the IUGR group with normal Doppler measurement. However, the trend is in that direction in a number of domains. This suggests that whilst there is a significant difference between control and the IUGR group with abnormal Doppler measurement, there may also be a difference between the control and IUGR group with normal Doppler’s, though not quite reaching significance.

Follow-up work to this study with formal psychological assessment at 2.5 – 3 years with the Bayley scale of Infant and Toddler development (third edition) and ongoing anthropometric measures will be correlated with MRI findings at term and outcomes at 1 year of age. As part of the PORTO study all enrolled infants will have a formal psychological assessment beyond 2 years of age, which will be compared with the MRI findings. The 2 year follow up data does not form part of this thesis, but will provide further interesting findings long-term. Funding has been obtained for a paediatric trainee with a specific interest in neurodevelopmental outcome to perform several assessments on the StOOPS cohort as well collection of anthropometric measurements. The tests to be performed include the Bayley scale of Infant and Toddler development (3rd edition), Child Behaviour Checklist (CBCL) 1.5 to 5 years and the Beery-Burktenica Developmental Test of Visual-Motor Integration (VMI) (6th edition).
The Bayley Scales of Infant Development is a standard series of measurements used primarily to assess the motor (fine and gross), language (receptive and expressive), and cognitive development of infants and toddlers, from birth to 3 years. This measure consists of a series of developmental play tasks and takes between 45 - 60 minutes to administer. Raw scores of successfully completed items are converted to scale scores and to composite scores. These scores are used to determine the child’s performance compared with norms taken from typically developing children of their age (in months) (320). The Beery-Buktenica Developmental Test of Visual-Motor Integration is a test of visual-motor coordination. VMI is the degree to which visual perception and finger-hand movements are well coordinated. The test involves the examinees copying increasingly complex designs and assesses visual-motor integration, visual perception and motor coordination skills.

The VMI test includes 24 geometric figures progressing from simple to complex, which the child has to copy. In the Visual Perception test the child has to choose the correct geometric form of two to seven alternatives. In the Motor Coordination test the child traces with a pencil a trail within progressively smaller paths while staying within the confines of a boundary derived from geometric figures. Raw scores are then calculated and converted in to standard scores and compared against normative data.

The CBCL covers an empirical range of behavioural, emotional and social function problems and is comprised of 100 problem items: 99 closed items and an open-ended item, which requests respondents to add any additional problems not previously listed. It is completed by the parents or guardian living with the child and they are asked to rate each item, based on the preceding two months, as 0 for ‘not true’, 1, for ‘somewhat or sometimes true’ and 2 for ‘very true or very often’. The questions are grouped to assess children into a number of DSM (Diagnostic and Statistical Manual of mental disorders) classified areas such as emotionally reactive, anxious/depressed, somatic complaints, withdrawn, attention problems and aggressive behaviour. There are parallel Internalising, Externalising and Total Problem scales.
Section 4: Discussion

Chapter 8

Discussion
Chapter 8. Discussion

8.1 Discussion

Intra-uterine growth restriction (IUGR) is commonly seen in obstetric practice with placental insufficiency as one of the commonest causes. As such efforts, have concentrated on identifying this at the earliest instant in the pregnancy to optimise management or delivery. Antenatal Doppler ultrasound of the umbilical artery has been shown to be the most effective way of monitoring such a pregnancy once concern has been raised, usually because of a weight < 10th centile at fetal assessment. The neurodevelopmental consequences of IUGR are well described. Lower nonverbal and verbal IQ than controls, as well as a reduction persisting in to later stages in childhood, have been reported (407). The visuomotor development, learning and behavioural problems associated with IUGR are also significant with the detrimental impact of these issues affecting school performance and social integration (231, 232). Individual differences in capacity to orient to sensory inputs and sustain attention are known to correlate with later attentional development and development of other cognitive and behavioural abilities such as early language, achievement at school and incidence of hyperactive/impulsive behaviours (408-411). A great degree of confusion exists relating to the definition of intra-uterine growth restriction and how it differs from small for gestational age. Therefore, many studies reporting on measures of IUGR have included infants who are small for gestational age (SGA) or have used the term SGA when describing IUGR. If the definition used for IUGR is not precise the results produced are not robust, and analysing outcomes becomes difficult secondary to the inclusion of infants who have no pathological cause for their small size.

The infants in this study had antenatal concerns about IUGR prompted by poor growth with subsequent serial Doppler measurement as well as a placental examination. Evidence of chronic uteroplacental insufficiency on histological examination of the placenta provides definitive evidence for growth restriction.
The infants in the ND group show similar rates of chronic uteroplacental insufficiency on histological examination with the AbD group, thus ensuring both groups were IUGR as opposed to comparing an IUGR group to a SGA group. The infants in the AbD group are lighter and born more prematurely than the ND group. Obstetric intervention may explain this as evidence of abnormal Doppler’s warranted admission and close observation with early delivery more likely as a result. Matching for gestational age is difficult as ND fetuses are deemed to be safe in utero until either Doppler status changes or they achieve a gestational age where the risks of an early delivery outweigh the risk of an in utero fetal demise. As such the AbD infants are generally delivered earlier than an IUGR infant with ND identified at the same time. The statistical models used corrected for the unavoidable differences in gestational age between the groups. Despite this, the results should be interpreted acknowledging this element. We used resting state functional MRI and volumetric analysis to investigate the hypothesis that differences exist when comparing between infants affected with IUGR based on Doppler status at term corrected gestational age.

The resting state fMRI study investigated for changes in neural connectivity between the groups using seed correlation techniques. Intra-network and network-to-brain connectivity in several key network systems were studied. Firstly, the default mode network (DMN) - an interconnected and anatomically defined brain system that preferentially activates when individuals are attending to internal stimuli. Secondly, the dorsal attention network (DAN) - important in directing attention and salience processing of external stimuli (353, 356). In addition, sensory network systems and thalamic connectivity were also studied. Clear evidence of the existence of these networks was found and the results obtained are consistent with other studies where, in early infancy, both the DMN and DAN were somewhat primitive and incomplete (310, 412, 413). The main difference seen in these infants compared to that observed in older children and adults is reduced connectivity strength between different parts of a network (310).
Such findings are consistent with increasing functional segregation and specialisation of adjacent brain regions and increasing integration of distributed brain regions into common networks as the brain matures (414-417). In this study, the DMN network appears separated into two compartments, one involving the hippocampal formations and the other encompassing the remaining hubs. This is consistent with evidence that different parts of the DMN perform different functions with different connectivity patterns (342). In this context, the hippocampal formations may not be ‘core’ DMN regions and thus may only become associated with DMN function as the system matures and more ‘specialised’ functions (like memory functions) become relevant to DMN processes (418). The differences that were identified between the groups were in the DAN and sensory networks. In the DAN system, the left frontal eye field (FEF) and the right intra-parietal sulcus (IPS) demonstrated reduced connectivity to other brain regions in the AbD infants. The IPS and FEF, in conjunction with primary visual cortices, have been implicated in a network involved in salience evaluation, maintenance of attentional set and suppression of distractors (419). The current findings could therefore suggest deficiencies in oculomotor attentional networks in the AbD infants. Visual attentional deficits have previously been identified in IUGR infants (363, 420). The connectivity changes noted within the sensory networks included change within the auditory system, where the data initially suggested increased connectivity of the left auditory cortex to the striatal subcortical structures and the left superior parietal lobule (SPL) in the AbD infants. However, by comparing correlation values between groups it was clear that the increased connectivity to the left superior parietal lobule in the AbD infants actually reflected a loss of an anticorrelation between this region and the auditory cortex. The SPL forms part of attentional control systems within the parietal cortex and is particularly associated with attentional set shifting (356, 421, 422). It is difficult to predict the consequences of this altered connectivity to the limbic system and striatum in IUGR. However, as the areas concerned are implicated in making reward choices and goal related decisions the processes by which information salience is determined might be altered in the AbD infants (423, 424).
A limitation in this aspect of the study was that there was no rs-fMRI data for term healthy controls. Recruitment of controls proved challenging, as the MRI scan was not performed in a maternity hospital and as such required an unnecessary trip to a research imaging facility that many parents were not interested in. The parents of infants with IUGR were more interested in having the MRI performed, as there had been some antenatal concern raised and were invested in the PORTO study. Some parents initially consented to be involved as controls whilst inpatient, but changed their minds once home. Of the Control group that were scanned, a number woke during the fMRI sequence (the loudest sequence) resulting in uninterpretable data, which left an insufficient number of infants to generate legitimate control data. Another potential limitation is that a paediatric template was not used, however, as the data from both IUGR groups was handled in the same fashion the differences identified are robust. It has been previously reported that preterm birth does not seem to have a detrimental influence on the resting-state patterns in the infant brain, as such these differences may indeed be related to IUGR and the severity of it (311).

The results of the volumetric analyses have provided some interesting findings. The first analysis was performed on the study specific template. The significant volumetric differences identified showed the Control group to have a greater volume in a number of regions – Region 1 (including the lower diencephalon and elements of the frontal lobes), parts of the temporal lobes bilaterally and the cerebellum. The finding of the Control group having the greatest volume in these regions is in keeping with previous studies where term controls demonstrate larger volumes in certain cortical and deeper structures (282, 389). For Region 1 the Control group was significantly greater than both ND and AbD groups and the ND group was significantly greater than the AbD group. The functions that these areas are involved in include language comprehension, response inhibition (inhibitory control over motor response), sensory integration and adaptive learning through decision-making and expectation (425-427). There was no difference between the Control group and the ND group in the temporal regions where differences were identified, only between Control and AbD groups.
The region of the cerebellum where differences were identified showed that the volume in the Control group was greater than both IUGR groups and that the AbD group had a greater volume than the ND group, though not significant. Interestingly, this is at odds with a recent study that found that birth weight and gestational age were not associated with brain volumes later in childhood, apart from the cerebellum, where a shorter gestational age was associated with a relatively smaller cerebellar volume (428). That study found that the effect of gestational age on cerebellar volume was not limited to children with very premature birth or very low birth weight, but was also present in children born >32 weeks of gestation and with birth weight >1500 g, however, a relationship with IUGR was not investigated. Some differences were noted based on gender; an increase in volume in the occipital lobe/visual cortex in female infants has previously been reported (429). Though detecting sexual dimorphisms in neonatal brains has not been consistent when identified (429, 430). The second volumetric analysis using the ALBERT atlas showed that there was no difference between the groups in terms of total brain volume, however, the Control group was unexpectedly shown to have the smallest brain volume. The left hemisphere was larger than the right, which has been previously demonstrated in neonates and the lateral ventricles were largest in the AbD group, who were also the most premature, as previously demonstrated (380, 431).

The Control group was recruited from the postnatal ward of the Rotunda hospital and were infants born at term without any antenatal concerns about IUGR. A potential limitation is that as there was no concern regarding fetal growth, they did not have antenatal Doppler monitoring so the Doppler status is assumed to be normal and placental histology was not assessed in this group. The demographics of the Control group show that the mean birth weight centile was 32 and mean head circumference was 26. These infants were therefore well grown, but not on the 50th centile or above. As described previously, recruiting control subjects was difficult as the research imaging facility was in another hospital and required a trip with a newborn that many parents were not interested in or subsequently changed their minds.
The number of control infants included is double that included in the ALBERT protocol and despite the marginally smaller total brain volume noted during the volumetric component of the study, the one-year outcomes of the Control group suggest that they were indeed an appropriate control.

The power calculations undertaken prior to initiation of the study determined 30 infants would be required for each group. As outlined previously, recruitment of controls proved challenging and resulted in a number far less than originally sought. The study groups were closer to the number set out with 34 in the Abnormal Doppler group and 29 in the Normal Doppler group, however for the fMRI analysis only 15 data sets in each group were suitable for analysis and in the volumetric study there were 28 and 22 respectively. The failure to achieve sufficient numbers of usable data sets for the various analyses undertaken may impact on the results produced. The results presented herein may be more robustly demonstrated with greater numbers and may explain some of the unexpected findings in the control group in the volumetric study.

One-year outcome data provided further interesting results and allowed analysis with some of the MRI data obtained at birth. Catch up growth was demonstrated so that by one-year corrected there was no significant difference in OFC between the groups. Catch up growth improves outcome, but does not completely ameliorate the effects of the hostile in utero environment on both brain growth and neurodevelopmental outcome (432). It was not anticipated that significant findings would be present at this early stage of development. The correlations identified between the domains with significant differences and volumetric changes on the MRI scan are interesting and will be further explored with future psychological assessments at more discriminating ages. More extensive robust psychological assessments were planned for when the infants were older than 2.5 years and at time of submission of this thesis approximately half of the cohort had been assessed.
The effects of intra-uterine growth restriction secondary to placental insufficiency on the developing brain are complex; hypoxia and poor blood supply impair the normal neurodevelopmental trajectory. The results of this study demonstrate that differences are present in the brains of infants affected with intra-uterine growth restriction on assessment at term corrected age based on Doppler status. Differences in resting state connectivity patterns and volumetric differences have been demonstrated. Infants with abnormal flow measured by antenatal umbilical arterial Doppler are generally delivered earlier than those with evidence of intra-uterine growth restriction and normal Doppler measurements, however, both groups are at risk for longer-term neurodevelopmental sequelae. The differences demonstrated have been correlated with early outcome measures at one year of age and show that even at such an early stage, the infants with abnormal Dopplers have poorer scores than those with normal Doppler’s in many domains.

Those infants with normal Dopplers also demonstrate impairments and should not be discharged from follow-up based on Doppler status and catch up growth. These imaging findings and the ongoing psychological assessment form the foundation for a risk stratification model for neurodevelopmental issues for infants born with IUGR. This will help clinicians in arranging appropriate developmental supports at the earliest time frame to maximise the longer-term outcome.
References

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Appendix

Parent Information Leaflet and Consent

Ages and Stages Questionnaire

Growth Charts

Letters

Output Arising from this Work
  Presentations / Prizes / Abstracts
  Submissions in Progress
Magnetic Resonance Imaging (MRI) of the Brain

Comparison of brain imaging findings in infants with different Doppler flows
(Short-term Outcome Of the PORTO Study)

Information Sheet for Parents of Infants in the StOOPS MRI study

You will be given a copy of this Information Sheet

We are seeking your permission to involve your baby in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve for your baby. Please take time to read the following information carefully and discuss it with relatives as you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

What is the purpose of the study?

We are using an MRI scanner, which uses magnetic fields to take pictures of the brain of babies who have been born smaller than would have been expected. The scanner gives us detailed pictures of the brain that cannot be obtained in any other way; it does not use X-rays or ionising radiation. It is not possible to get these types of pictures from other scanners. We wish to see if there are any differences in the pictures between two groups of babies and use this information to determine the best approach to managing follow up of infants who are not growing properly in the womb.
Why has my baby been chosen?

Your baby has been chosen, as you have had a child who was born smaller than would have been expected for his/her gestation.

Does my baby have to take part?

It is up to you to decide whether or not you wish your baby to take part. If you do allow your child to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. This will not affect the standard of care your baby receives.

What will happen to my baby if he/she takes part?

For magnetic resonance (MR) imaging your baby will be placed in a scanner in the Centre for Advanced Medical Imaging in St James's Hospital. MR imaging has been used safely in newborns for decades. The scan takes around one hour. The technique is considered completely safe, but we will continue to monitor your baby’s oxygen levels and heart rate throughout, just as it is done in the neonatal unit. Your baby will be wrapped up and special earplugs and earmuffs will be used so that he/she remains asleep through the scanning. Some of the sequences we use to acquire images are quite noisy. A picture of the scanner is shown below.

An MRI scanner
It is possible to perform these scans just after a baby has been fed and wrapped up. In this state your baby is likely to sleep through the scan.

**What are the side effects of taking part?**

The MR scanner makes a knocking noise during the scan, but there are no known hazards with the technique, although care is necessary to keep metallic objects away from the magnet. Certain types of pictures taken with this scanner make noise and so we will protect your baby from the noise with the use of ear muffs/plugs.

**What are the possible disadvantages and risks of taking part?**

There are no known risks from MR imaging. Your baby will be moved into the scanner only when he/she is stable.

**What are the possible benefits of taking part?**

A benefit of having the scan done is that term corrected MRI in preterm babies is a good way of predicting if there is a strong likelihood of your baby having difficulty crawling or walking.

However, it is important to stress that we are carrying out this study to help us learn better ways to help babies in the future, rather than specifically to help your baby. While it is possible that the information gained may be helpful in guiding your baby’s care we would expect that there would only be rare occasions when this was the case.

**What if there is a problem with my baby’s brain?**

In the event of our uncovering a problem we will talk to you about it, and arrange for your baby to have any treatment or follow up that is required.
Will my baby taking part in this study be kept confidential?

All information collected about you and your baby during the course of the research will be kept strictly confidential. This will include information about the length of your pregnancy and type of delivery. Information from your baby’s time in the neonatal unit will also be collected. Any information about you or your baby that leaves the hospital will have names and addresses removed so that neither you nor your baby can be recognised from it.

What will happen to the results of the research study?

The research results will be published in international journals and presented at scientific meetings. Neither you nor your baby will be identified in any report or publication.

Who has reviewed the study?

Rotunda Hospital Research Ethics Committee, National Maternity Research Ethics Committee, Research Ethic Committee of Coombe Women and Infants University Hospital, The Clinical Research Ethics Committee of UCC, Galway University Hospitals Research Ethics Committee, The Mid-Western Regional Hospital Ethics and Research Committee of Limerick, The Children’s University Hospital Research Ethics Committee and the St. James’s Hospital Research Ethics Committee.

Contact for Further Information

Chief Investigators: Dr. Adrienne Foran, Prof. Naomi McCallion
Lead Researcher: Dr. Michael Boyle
Rotunda Hospital
Parnell Square
Dublin 1
PH: 01 8171700
Email – michaelboyle@physicians.ie

You will be given a copy of the information sheet and a signed consent form to keep.
Magnetic Resonance Imaging (MRI) of the Brain in Babies enrolled in the PORTO trial

Chief Investigators: Dr. Adrienne Foran, Prof. Naomi McCallion.
Lead Researcher: Dr. Michael Boyle
Rotunda Hospital
Royal College of Surgeons in Ireland
Dublin 1
PH: 01 8730700
e-mail: michaelboyle@physicians.ie

Consent for Parents/Carers

The relative/carer should complete this sheet him/herself.

<table>
<thead>
<tr>
<th>Patient’s Name:</th>
</tr>
</thead>
</table>

Circle as appropriate

1. I confirm that I have read and fully understood all the information provided in the accompanying information leaflet and each of my inquiries about the study has been answered.

   Yes/ No

2. I fully understand that my participation is completely voluntary and that I am free to withdraw at any given time (prior to publication) without providing a reason and it will not affect my care in any way.

   Yes/ No

3. I understand that the researchers involved in this study will hold in confidence and securely all collected data and other relevant information. Additionally, I understand that my baby will not be identified as a participant in this study (unless a legal requirement) and that the researchers may hold my child’s information for a 20-year duration.

   Yes/ No

4. I understand that responsible individuals from amongst our research collaborators or from regulatory authorities may look at sections of my child’s medical notes and images, which are relevant to his/her participation in research. I give permission for these individuals to have access to my child’s records and images.

   Yes/ No
5. I give permission for the information collected about my baby to be stored and used for possible future research but only if a Research Ethics Committee approves the research without the need for further consent from myself.

   Yes/ No

6. I agree that I will not restrict the use to which the results of this study may be put. I give my approval that unidentifiable data concerning my baby may be stored or electronically processed for the purpose of scientific research and may be used in related or other studies in the future. This would be subject to approval by an independent body, which safeguards the welfare and rights of people in biomedical research studies.

   Yes/ No

7. I consent to being contacted in the future for follow-up studies.

   Yes/ No

8. I agree to participate in the above research study.

   Yes/ No


<table>
<thead>
<tr>
<th>Participant Name</th>
<th>Date</th>
<th>Signature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Researcher Name</td>
<td>Date</td>
<td>Signature</td>
</tr>
</tbody>
</table>

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Ages & Stages Questionnaires®

12 Month Questionnaire

11 months 0 days through 12 months 30 days

Please provide the following information. Use black or blue ink only and print legibly when completing this form:

Date ASQ completed:

Baby’s information

Baby’s first name: [ ]
Middle initial: [ ]
Baby’s last name: [ ]

If baby was born 3 or more weeks prematurely, # of weeks premature: [ ]

Baby’s gender: [ ] Male [ ] Female

Baby’s date of birth: [ ]

Person filling out questionnaire

First name: [ ]
Middle initial: [ ]
Last name: [ ]

Street address: [ ]

City: [ ]
State/Province: [ ]
ZIP/Postal code: [ ]

Country: [ ]

Home telephone number: [ ]
Other telephone number: [ ]

E-mail address: [ ]
Names of people assisting in questionnaire completion: [ ]

Program Information

Baby ID #: [ ]
Age at administration in months and days: [ ]

Program ID #: [ ]
If premature, adjusted age in months and days: [ ]

Program name: [ ]

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P10120100

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COMMUNICATION

1. Does your baby make two similar sounds, such as "ba-ba," "da-da," or "ga-ga"? (The sounds do not need to mean anything.)
   YES  SOMETIMES  NOT YET

2. If you ask your baby to, does he play at least one nursery game even if you don't show him the activity yourself (such as "bye-bye," "Peekaboo," "clap your hands," "So Big"?)
   YES  SOMETIMES  NOT YET

3. Does your baby follow one simple command, such as "Come here," "Give it to me," or "Put it back," without using gestures?
   YES  SOMETIMES  NOT YET

4. Does your baby say three words, such as "Mama," "Dada," and "Baba"? (A "word" is a sound or sounds your baby says consistently to mean someone or something.)
   YES  SOMETIMES  NOT YET

5. When you ask, "Where is the ball (hat, shoe, etc.)?" does your baby look at the object? (Make sure the object is present. Mark "yes" if she knows one object.)
   YES  SOMETIMES  NOT YET

6. When your baby wants something, does he tell you by pointing to it?
   YES  SOMETIMES  NOT YET

COMMUNICATION TOTAL

GROSS MOTOR

1. While holding onto furniture, does your baby bend down and pick up a toy from the floor and then return to a standing position?
   YES  SOMETIMES  NOT YET

2. While holding onto furniture, does your baby lower herself with control (without falling or flopping down)?
   YES  SOMETIMES  NOT YET

3. Does your baby walk beside furniture while holding on with only one hand?
   YES  SOMETIMES  NOT YET
GROSS MOTOR (continued)

4. If you hold both hands just to balance your baby, does he take several steps without tripping or falling? (If your baby already walks alone, mark "yes" for this item.)

5. When you hold one hand just to balance your baby, does she take several steps forward? (If your baby already walks alone, mark "yes" for this item.)

6. Does your baby stand up in the middle of the floor by himself and take several steps forward?

FINE MOTOR

1. After one or two tries, does your baby pick up a piece of string with his first finger and thumb? (The string may be attached to a toy)

2. Does your baby pick up a crumb or Cheerio with the tips of her thumb and a finger? She may rest her arm or hand on the table while doing it.

3. Does your baby put a small toy down, without dropping it, and then take his hand off the toy?

4. Without resting her arm or hand on the table, does your baby pick up a crumb or Cheerio with the tips of her thumb and a finger?

5. Does your baby throw a small ball with a forward arm motion? (If he simply drops the ball, mark "not yet" for this item.)

6. Does your baby help turn the pages of a book? (You may lift a page for him to grasp.)

FINE MOTOR TOTAL

"If Fine Motor item 4 is marked "yes" or "sometimes," mark Fine Motor item 2 "yes."
PROBLEM SOLVING

1. When holding a small toy in each hand, does your baby clap the toys together (like "Pat-a-cake")?

2. Does your baby poke at or try to get a crumb or Cheerio that is inside a clear bottle (such as a plastic soda-pop bottle or baby bottle)?

3. After watching you hide a small toy under a piece of paper or cloth, does your baby find it? (Be sure the toy is completely hidden.)

4. If you put a small toy into a bowl or box, does your baby copy you by putting in a toy, although she may not yet go of it? (If she already lets go of the toy into a bowl or box, mark "yes" for this item.)

5. Does your baby drop two small toys, one after the other, into a container like a bowl or box? (You may show him how to do it.)

6. After you scribble back and forth on paper with a crayon (or a pencil or pen), does your baby copy you by scribbling? (If she already scribbles on her own, mark "yes" for this item.)

PERSONAL-SOCIAL

1. When you hold out your hand and ask for his toy, does your baby offer it to you even if he doesn't let go of it? (If he already lets go of the toy into your hand, mark "yes" for this item.)

2. When you dress your baby, does she push her arm through a sleeve once her arm is started in the hole of the sleeve?

3. When you hold out your hand and ask for his toy, does your baby let go of it into your hand?

4. When you dress your baby, does she lift her foot for her shoe, sock, or pant leg?

5. Does your baby roll or throw a ball back to you so that you can return it to him?

6. Does your baby play with a doll or stuffed animal by hugging it?

E101220460
OVERALL

Parents and providers may use the space below for additional comments.

1. Does your baby use both hands and both legs equally well? If no, explain:

   ○ YES  ○ NO

2. Does your baby play with sounds or seem to make words? If no, explain:

   ○ YES  ○ NO

3. When your baby is standing, are her feet flat on the surface most of the time?
   If no, explain:

   ○ YES  ○ NO

4. Do you have concerns that your baby is too quiet or does not make sounds like other babies do? If yes, explain:

   ○ YES  ○ NO

5. Does either parent have a family history of childhood deafness or hearing impairment? If yes, explain:

   ○ YES  ○ NO
OVERALL (continued)

6. Do you have concerns about your baby's vision? If yes, explain:     YES  NO

7. Has your baby had any medical problems in the last several months? If yes, explain:     YES  NO

8. Do you have any concerns about your baby's behavior? If yes, explain:     YES  NO

9. Does anything about your baby worry you? If yes, explain:     YES  NO
Baby’s name: ___________________________ Date ASQ completed: ___________________________
Baby’s ID #: ___________________________ Date of birth: ___________________________
Administrating program/provider: ___________________________ Was age adjusted for prematurity when selecting questionnaire? Yes No

1. SCORE AND TRANSFER TOTALS TO CHART BELOW: See ASQ-3 User’s Guide for details, including how to adjust scores if item responses are missing. Score each item (YES = 10, SOMETIMES = 5, NOT YET = 0). Add item scores, and record each area total. In the chart below, transfer the total scores, and fill in the circles corresponding with the total scores.

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1. Uses both hands and both legs equally well? Yes NO Comments: ___________________________________________
2. Plays with sounds or seems to make words? Yes NO Comments: ___________________________________________
3. Feet are flat on the surface most of the time? Yes NO Comments: ___________________________________________
4. Concerns about not making sounds? YES No Comments: ___________________________________________
5. Family history of hearing impairment? YES No Comments: ___________________________________________
6. Concerns about vision? YES No Comments: ___________________________________________
7. Any medical problems? YES No Comments: ___________________________________________
8. Concerns about behavior? YES No Comments: ___________________________________________
9. Other concerns? YES No Comments: ___________________________________________

3. ASQ SCORE INTERPRETATION AND RECOMMENDATION FOR FOLLOW-UP: You must consider total area scores, overall responses, and other considerations, such as opportunities to practice skills, to determine appropriate follow-up.

If the baby’s total score is in the area, it is above the cutoff, and the baby’s development appears to be on schedule.
If the baby’s total score is in the area, it is close to the cutoff. Provide learning activities and monitor.
If the baby’s total score is in the area, it is below the cutoff. Further assessment with a professional may be needed.

4. FOLLOW-UP ACTION TAKEN: Check all that apply.

______ Provide activities and rescreen in ___ months.
______ Share results with primary health care provider.
______ Refer for (circle all that apply) hearing, vision, and/or behavioral screening.
______ Refer to primary health care provider or other community agency (specify reason):
______ Refer to early intervention/early childhood special education.
______ No further intervention/treatment taken at this time.
______ Other (specify):

5. OPTIONAL: Transfer item responses (Y = YES, S = SOMETIMES, N = NOT YET, X = response missing).

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GIRLS
FOUR-IN-ONE
(DUODECIMAL)
GROWTH CHARTS
(BIRTH - 20 YRS)

United Kingdom cross-sectional reference data: 1996/1
updated in 2002 for use with 95% & 99% thrive lines.

INDEX
(see back and inside pages for instructions)

Chart 1: PRE-TERM [20wks - 37 completed wks]. Draw a vertical birthline at appropriate week.
Do not initiate growth plots in shaded area. Week 38-40 deliveries should be plotted from the EDD line.

Chart 2: PRE-TERM [30wks] - 1yr

Chart 3: 1yr - 5yrs

Chart 4: EDD - 20yrs

MEASUREMENT RECORDING BOXES (complete as appropriate)

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Surname: 
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D.O.B. ...
NHS no: 
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H.V. 

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(Charity Reg. No 274325)
2 Mayfield Avenue, London W4 1PW

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CHILD GROWTH FOUNDATION 1996/1
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2 Mayfield Avenue, London W4 1PW

Printed and distributed by
HARLOW PRINTING LIMITED
Maxwell Street South Shields Tyne & Wear NE33 4PU
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PLOTTING
Plot each measure on the relevant grid with a well defined dot. Trace the growth curve with a line but leave the dots clearly visible. A normal growth curve is one that always runs roughly on/parallel to one of the printed lines.

Footnotes:
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BOYS FOUR-IN-ONE (DUODECIMAL) GROWTH CHARTS
(BIRTH - 20 YRS)

United Kingdom cross-sectional reference data: 1996/1
[updated in 2002 for use with 5% & 95% thrive lines]

INDEX
(see back and inside pages for instructions)
Chart 1: PRE-TERM (26wks - 37 completed wks); Draw a vertical birthline at appropriate week.
Do not initiate growth plots in shaded area. Week 38-40 deliveries should be plotted from the EDD line.
Chart 2: PRE-TERM (30wks) - 1yr
Chart 3: 1yr - 5yrs
Chart 4: EDD - 20yrs

MEASUREMENT RECORDING BOXES (complete as appropriate)

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<th>LENGTH/HEIGHT (cm)</th>
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263
10th June 2011

Dr Adrienne Foran, Dr Michael Boyle, Dr Stephanie Ryan & Prof Naomi McCallion
Children’s University Hospital
Temple Street

Dear Adrienne

Further to your recent application to the PAC for Research funding, we have approved the following:

- 3.0 tesla MRI of the Brain and Neuro-developmental exam at Term in growth restricted newborns. (PAC 11-53)

This approval is subject to:
- Prior written confirmation of Scientific Committee Approval
- Prior written confirmation of Ethics Committee Approval
- All Intellectual property rights to remain with Childrens Fund for Health Ltd

Please quote PAC Number on all correspondence in relation to this application.

I trust you will find this to your satisfaction – any queries, please feel free to give me a call on 4382 or 086-8264544.

Yours sincerely

Denise Fitzgerald
Chief Executive

CC: Dr Alec Blayney
22nd March 2011

Dr. Adrienne Foran
Consultant Neonatologist
Children's University Hospital
Temple St.
Dublin 1

Dear Adrienne

11.008 3.0 Tesla MRI of the Brain at Term and neurodevelopmental exam of infants with intrauterine growth restriction

Thank you and Michael Boyle for attending the Ethics Committee meeting held on Tuesday 15th February. You addressed satisfactorily a number of queries raised by the Committee in relation to the identification of unsuspected MRI findings in the control group, transport by the NNTP and Garda clearance.

Following further discussion, the Committee approved the study subject to the Patient Information sheet being amended. It should be written in plain, simple English suitable for an individual with a reading age of 12 years. The Committee had particular issue with the sentence on page 3 paragraph 3 under the heading "What are the possible benefits of taking part?" The Committee request that you review and re-write the first sentence.

Yours sincerely

Prof Philip D Mayne
Medical Secretary to the Ethics Committee
MCRN: 06935
11th February 2011.

Mr. Michael Boyle,
Paediatric SpR
Children's University Hospital,
Temple Street,
Dublin 1.

Proposal: 3.0 tesla MRI of the Brain at Term and neurodevelopmental exam of infants with intrauterine growth restriction.

Dear Michael,

The Scientific Committee have reviewed your proposal: ‘3.0 tesla MRI of the Brain at Term and neurodevelopmental exam of infants with intrauterine growth restriction.

We are very happy to accept it. It is now being passed on to the Ethics Committee for their approval.

Best wishes,

Yours sincerely,

[Signature]
Dr. Adrienne Foran (Chair)
Scientific Committee
Dr. Michael Boyle,
Neonatal Department,
Rotunda Hospital.

Re: 3.0 tesla Magnetic Resonance Imaging (MRI) of the Brain at Term. A short-term surrogate outcome of infants enrolled in the Perinatal Ireland Porto Trial

Dear Michael,

Thank you for attending the Research Ethics Committee meeting again on 28th July 2010. The issues raised at the previous meeting have been addressed and the Committee have no ethical concerns about the study. They did however suggest that you might put in a bit about the 'PORTO Study' at the beginning of the Information Sheet to refresh parents' memory about this study.

We wish you well with this work.

Kind regards.

Yours sincerely,

[Signature]

Dr. Michael Geary.
Chairman.
Research Ethics Committee.

c.c. Dr. Adrienne Foran

[Handwritten note: or alternatively include the PORTO info leaflet]
17th September 2010

Dr Adrienne Foran,
Neonatal Department,
Rotunda Hospital,
Parnell Sq.,
Dublin 1


Dear Dr Foran,

Thank you for submitting the above protocol to the Ethics Committee. I am pleased to inform you that it has received Ethical approval.

We wish you success with the study.

Kind regards

Yours sincerely

______________________________
Dr. John Murphy
Consultant Paediatrician
Chairman of Ethics Committee

Cc Dr. Michael Boyle, 225 Loreto Abbey Rathfarnham Dublin 14
24 March 2011

Dr Adrienne Foran  
Consultant Neonatologist  
Rotunda Hospital  
Parnell Street  
DUBLIN 1

Re.: Study of 3.0 tesla Magnetic Resonance Imaging (MRI) of the brain at term. A short-term surrogate outcome of infants enrolled in The Perinatal Ireland PORTO Study (STOOPS)

Dear Dr Foran

This is to acknowledge your letter of 17th February 2011, and the contents therein. This study is now approved.

Yours sincerely

[Signature]

Dr Michael Carey  
Chairman
29th July 2011

Dr Adrienne Foran
Consultant Neonatologist
Rotunda Hospital
Parnell Square
Dublin 1

Re: 3.0 Tesla Magnetic Resonance Imaging (MRI) of the Brain at Term A Short-Term Surrogate Outcome of Infants Enrolled in The Perinatal Ireland PORTO Study (STOOPS).

Dear Dr Foran

Expeditied approval is granted to carry out the above study in:

➢ Cork University Maternity Hospital.

The following documents have been approved:

➢ Application Form
➢ Study Protocol
➢ Parent Information Leaflet
➢ Consent Form
➢ Ethics Approval Letters
➢ PORTO Study Summary
➢ Neonatal Neurological Examination Preforma.

The co-investigators involved in this study will be:

➢ Dr Michael Boyle, Dr Stephanie Ryan, Dr Ailbhe Tarrant, Dr James Meaney and Professor Fergal Malone.

Yours sincerely

Dr Michael Hyland
Chairman
Clinical Research Ethics Committee of the Cork Teaching Hospitals

The Clinical Research Ethics Committee of the Cork Teaching Hospitals, UCC, is a recognised Ethics Committee under Regulation 7 of the European Communities (Clinical Trials on Medicinal Products for Human Use) Regulations 2004, and is authorised by the Department of Health and Children to carry out the ethical review of clinical trials of investigational medicinal products. The Committee is fully compliant with the Regulations as they relate to Ethics Committees and the conditions and principles of Good Clinical Practice.

Ollscoil na hUíreann, Corcaigh - National University of Ireland, Cork.
Dr. Mike Boyle  
Specialist Paediatric Registrar  
C/o The PORTO Trial  
Perinatal Ireland  
The Centre for Advanced Medical Imaging (CAMI)  
St James' Hospital  
Dublin 2.  


Re: Utilisation of the NNTP to Transport a Neonate as part of the PORTO Trial.

Dear Dr Boyle,

In response to your query regarding the availability of the NNTP for transport in the unlikely event of a neonatal collapse while undergoing an MRI Scan as part of the PORTO trial at the Centre for Advanced Medical Imaging (CAMI) on the St James' Hospital site, I can confirm that it would be appropriate to utilise the NNTP for such an event. The NNTP is not however a frontline emergency ambulance service but rather a critical-care inter-hospital service and as such cannot be guaranteed to be available to respond. The usual process for calling and utilising the NNTP will still apply. This would mean that you could not book the team to be on standby but could however call them on 0818300188 (if not already engaged with another call) when needed. As is the system at present, you would need to arrange the receiving hospital yourselves also.

I trust this information provides clarity regarding this matter.

Kind regards,

Ann Bowden,  
NNTP Coordinator
Presentations

Placental volume & weight differ in intra-uterine growth restricted infants according to antenatal Doppler measurements.
Boyle M, Doyle E, Malone F, Foran A.
Irish & American Pediatric Society Annual Meeting, Belfast; Sept 2012. (Oral Presentation)

Functional MRI (fMRI) analysis of the intra-uterine growth restricted infant brain – interim analysis of the StOOPS study.
Boyle M, Watson D, Meaney J, McGinnity M, Foran A.
- Inaugural North Dublin Hospital Group Meeting, Dublin; Nov 2012. (Oral Presentation)
  - Irish Congress of Obstetrics, Gynaecology & Perinatal Medicine, Wicklow; Dec 2012. (Oral Presentation)
  - Temple Street Children’s University Hospital Research Update Meeting, Dublin; Apr 2013. (Oral Presentation)

Placental volume & weight differ in intra-uterine growth restricted infants according to antenatal Doppler measurements.
Boyle M, Doyle E, Malone F, Foran A.
Irish Congress of Obstetrics, Gynaecology & Perinatal Medicine, Wicklow; Dec 2012. (Oral Presentation)

Resting state fMRI connectivity analysis of the intra-uterine growth restricted infant brain.
- Irish Paediatric Association Meeting, Dublin; Nov 2013. (Oral Presentation)
  - Irish Neonatal Research Symposium, Dublin: Nov 2013. (Oral Presentation)
Head growth and neurodevelopmental outcome at 1 year following fetal growth restriction.

Boyle M, Pinnamaneni R, Unterscheider J, Malone F, McCallion N, Foran A. 
1st Congress of joint European Neonatal Societies (jENS), Budapest; Sept 2015 (Oral Poster Presentation).

Papers and Published Abstracts

Boyle M, Watson D, Meaney J, McGinnity M, Foran A. 
Resting-state fMRI (rs-fMRI) connectivity analysis of the intrauterine growth restricted (IUGR) infant brain—An interim analysis of the StOOPS study 
Neuropediatrics. 2013 May; 44(S1):A20

Boyle M, Pinnamaneni R, Unterscheider J, Malone F, McCallion N, Foran A. 
Head growth and neurodevelopmental outcome at 1 year following fetal growth restriction 
Selected abstracts of the 1st Congress of joint European Neonatal Societies (jENS) 2015 

Prizes

2nd Prize, The Children’s University Hospital Annual Research Day 
The Children’s University Hospital, Dublin; June 2012 - Oral Presentation 
‘A Short-term surrogate Outcome Of infants enrolled in the PORTO Study (StOOPS)’.

Best Investigator Award, Irish Paediatric Association Meeting 
Moran Hotel, Dublin; November 2013 - Oral Presentation 
‘Resting state fMRI connectivity analysis of the intra-uterine growth restricted infant brain’. 
Research Bursary €1,000
Len and Arlean Fries Travel Award, Irish & American Paediatric Society Annual Meeting
Nashville, Tennessee, September 2015
‘Head growth and neurodevelopmental outcome at 1 year following fetal growth restriction’
Travel Grant $2,000
*Award to R. Pinnamaneni who presented this work.