Motor Neuron Disease in Ireland: Epidemiology, Risk Factors and Prognostic Indicators

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Thesis submitted for degree of Doctor of Medicine, R.C.S.I. February 2010 under supervision of Professor Orla Hardiman.
Declaration

I declare that this thesis, which I submit to RCSI for examination in consideration of the award of a higher MD degree, 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 breech 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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<tr>
<td>A</td>
<td>Autocrine</td>
</tr>
<tr>
<td>AAN</td>
<td>American Academy of Neurology</td>
</tr>
<tr>
<td>ABG</td>
<td>Arterial blood gas</td>
</tr>
<tr>
<td>AD1</td>
<td>Autosomal dominant</td>
</tr>
<tr>
<td>AD2</td>
<td>Alzheimer's' disease</td>
</tr>
<tr>
<td>AF</td>
<td>Amniotic fluid</td>
</tr>
<tr>
<td>AIDS</td>
<td>Acquired immune deficiency syndrome</td>
</tr>
<tr>
<td>ALS*</td>
<td>Acid related subunit</td>
</tr>
<tr>
<td>ALS</td>
<td>Amyotrophic lateral sclerosis</td>
</tr>
<tr>
<td>ALSbi</td>
<td>Amyotrophic lateral sclerosis with behavioural impairment</td>
</tr>
<tr>
<td>ALSci</td>
<td>Amyotrophic lateral sclerosis with cognitive impairment</td>
</tr>
<tr>
<td>ALS-D</td>
<td>Amyotrophic lateral sclerosis with dementia</td>
</tr>
<tr>
<td>ALSFRSr</td>
<td>ALS functional rating scale (revised)</td>
</tr>
<tr>
<td>ALS-FTD</td>
<td>Amyotrophic lateral sclerosis with FTD meeting Neary diagnostic criteria</td>
</tr>
<tr>
<td>ALS-SSS</td>
<td>Amyotrophic lateral sclerosis swallowing severity scale</td>
</tr>
<tr>
<td>AMPA</td>
<td>A type of glutamate receptor</td>
</tr>
<tr>
<td>ANG</td>
<td>Angiogenin</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>ApoEε4</td>
<td>Gene for apolipoprotein E ε4</td>
</tr>
<tr>
<td>APSS</td>
<td>Aspiration penetration severity scale</td>
</tr>
<tr>
<td>AR</td>
<td>Autosomal recessive</td>
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<tr>
<td>ARF</td>
<td>Acute renal failure</td>
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<tr>
<td>ATP</td>
<td>Adenylate-tri-phosphate</td>
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<tr>
<td>Abbreviation</td>
<td>Definition</td>
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<td>--------------</td>
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<tr>
<td>ATPase</td>
<td>Adenylate-tri-phosphatase</td>
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<tr>
<td>BBB</td>
<td>Blood brain barrier</td>
</tr>
<tr>
<td>BCAA</td>
<td>Branched chain amino acids</td>
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<tr>
<td>BCAT</td>
<td>Branched chain amino transferase</td>
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<td>BCATc</td>
<td>Cytosolic branched chain amino transferase</td>
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<td>Mitochondrial branched chain aminotransferase</td>
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<td>BCKD</td>
<td>Branched chain alfa ketoacid dehydrogenase</td>
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<td>BDNF</td>
<td>Bone derived neurotrophic factor</td>
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<tr>
<td>BiPAP</td>
<td>Bidirectional positive pressure ventilation</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
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<tr>
<td>BO</td>
<td>Bulbar onset</td>
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<tr>
<td>Ca&lt;sup&gt;2+&lt;/sup&gt;</td>
<td>Calcium ion</td>
</tr>
<tr>
<td>CA-1</td>
<td>Cornu Ammonis-1 (region of the hippocampus that projects to the subiculum)</td>
</tr>
<tr>
<td>CCF</td>
<td>Congestive cardiac failure</td>
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<tr>
<td>ChAT</td>
<td>Choline acetyl transferase</td>
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<tr>
<td>CHRNA3</td>
<td>Gene encoding neuronal acetylcholine receptor subunit alpha-3</td>
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<tr>
<td>CHRNA4</td>
<td>Gene encoding neuronal acetylcholine receptor subunit alpha-4</td>
</tr>
<tr>
<td>CHRNβ4</td>
<td>Gene encoding neuronal acetylcholine receptor subunit beta-4</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>CI*</td>
<td>Cumulative incidence</td>
</tr>
<tr>
<td>CIDP</td>
<td>Chronic inflammatory demyelinating polyradiculopathy</td>
</tr>
<tr>
<td>CIR</td>
<td>Crude incidence rate</td>
</tr>
<tr>
<td>Cl&lt;sup&gt;-&lt;/sup&gt;</td>
<td>Chloride ion</td>
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<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>CNS</td>
<td>Central nervous system</td>
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<td>CNTF</td>
<td>Ciliary derived neurotrophic factor</td>
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<td>CPR</td>
<td>Crude prevalence rate</td>
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<tr>
<td>CSO</td>
<td>Central statistics office</td>
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<tr>
<td>CSF</td>
<td>Cerebrospinal fluid</td>
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<td>Cu/Zn</td>
<td>Copper / Zinc</td>
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<tr>
<td>CVA</td>
<td>Cerebrovascular accident (stroke)</td>
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<tr>
<td>Da</td>
<td>Daltons</td>
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<tr>
<td>DCM</td>
<td>Dilated cardiomyopathy</td>
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<tr>
<td>DCM*</td>
<td>Dublin City marathon</td>
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<tr>
<td>DF</td>
<td>Dysexecutive functioning</td>
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<tr>
<td>DNA</td>
<td>Deoxyribo-nucleic-acid</td>
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<td>DOSS</td>
<td>Dysphagia outcome and severity scale</td>
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<td>Gene encoding dipeptidyl aminopeptidase-like protein 6</td>
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<td>Endocrine</td>
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<td>EATT-1</td>
<td>Excitatory amino acid transporter 1</td>
</tr>
<tr>
<td>EATT-2</td>
<td>Excitatory amino acid transporter 2</td>
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<tr>
<td>EDSS</td>
<td>Expanded disability severity scale</td>
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<td>El Escorial diagnostic criteria</td>
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<td>EGF</td>
<td>Epidermal growth factor</td>
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<td>ELP3</td>
<td>Gene encoding elongator protein 3</td>
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<tr>
<td>EMG</td>
<td>Electromyography</td>
</tr>
<tr>
<td>ENT</td>
<td>Ear, nose and throat</td>
</tr>
<tr>
<td>ESR</td>
<td>Erythrocyte sedimentation rate</td>
</tr>
<tr>
<td>EURALS</td>
<td>European ALS Register</td>
</tr>
<tr>
<td>F</td>
<td>Female</td>
</tr>
<tr>
<td>FALS</td>
<td>Familial ALS</td>
</tr>
</tbody>
</table>
FBC  Full blood count
FEES Fibroptic enteroscopic evaluation of swallow
FFA  Free fatty acids
FIG4 Gene encoding FIG4 homolog, SAC1 lipid phosphatase domain containing (S. cerevisiae)
FIM  Functional independence measures
FLJ10986 Gene encoding hypothetical protein FLJ10986
FSP  Familial spastic paraparesis/paraplegia
FTD Frontotemporal dementia
FTD-MND Frontotemporal dementia with motor neuron disease
FTD-PA Frontotemporal dementia with parkinsonism
FTD-SD Frontotemporal dementia-semantic dementia
FTLD Frontotemporal lobar dementia
FUS  Gene encoding FUscd in Sarcoma protein
FVC  Forced vital capacity
fvFTD Frontal variant FTD
G₀  Quiescent phase of the cell cycle
G₁  First growth and normal metabolic phase of the cell cycle
G₂  Growth and preparation for mitosis stage of the cell cycle
GABA Gamma-aminobutyric acid
G93A Type of MND mouse model
GBS Guillain Barre Syndrome
GDNF Glial derived neurotrophic factor
GH  Growth hormone
GLE1 Gene encoding Nucleoporin GLE1 protein
Glu  Glutamate
GluR Glutamate receptor
GO: Generalised onset
GP: General practitioner
GPE: Gly-Pro-Glu (1st 3 N-terminal amino acids of IGF)
GWA: Genome wide association studies
HAQ: Historical activity questionnaire
HD: Huntington’s disease
H63D: Haemochromatosis gene
HIF: Hypoxia inducible factor
HIPE: Hospital inpatient epidemiology
HIV: Human Immunodeficiency Virus
H2O: Water
HPA: Hypothalamic-pituitary axis
HRE: Hypoxia response element
HSP: Hereditary spastic paraparesis
ICD: International classification of diseases
ICV: Intracerebroventricular
IGF: Insulin-like growth factor
IGF-1: Insulin-like growth factor-1
IGF-2: Insulin-like growth factor-2
IGFBP: Insulin like growth factor binding protein
IGFR: Insulin like growth factor receptor
IGFBP-RP: Insulin like growth factor binding protein-related protein
IHD: Ischaemic heart disease
IMNDA: Irish MND Association
Ins: Insulin
IPD: Idiopathic Parkinson’s disease
IR: Incidence rate
IRMA: Immunoradiometric assay
ITPR2  Gene encoding inositol 1, 4, 5 triphosphate receptor 2 protein
IVIG   Intravenous immunoglobulin
JMP    Jump statistical system
KA     Kainite
LDH    Liver dehydrogenase
LFTs   Liver function tests
LMN    Lower motor neuron
M      Male
MBS    Modified barium swallow
MEK    Type of tyrosine kinase
MET    Metabolic equivalent of task
MGF    Mechano growth factor
MI     Myocardial infarction
MMN    Multifocal motor neuropathy
MMNCB  Multifocal motor neuropathy with conduction block
MND    Motor neuron disease
MND-D  Motor neuron disease with dementia
MND-ID Motor neuron disease inclusion disease
mRNA   Messenger ribonucleic acid
MS     Multiple sclerosis
MSA    Multiple systems atrophy
MUNE   Motor unit number estimation
MUP    Motor unit potential
Na⁺    Sodium ion
NADH   Reduced nicotinamide adenine dinucleotide
NBS    Norris bulbar subscale
NCS    Nerve conduction studies
NEFH   Gene encoding neurofilament heavy polypeptide
NF     Neurofilament
NI     Northern Ireland
NIBD   Neurofilament immune reactive inclusion bodies
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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</thead>
<tbody>
<tr>
<td>NIPPV</td>
<td>Non invasive positive pressure ventilation</td>
</tr>
<tr>
<td>NIV</td>
<td>Non invasive ventilation</td>
</tr>
<tr>
<td>NMDA</td>
<td>A type of glutamate receptor</td>
</tr>
<tr>
<td>NMDAR</td>
<td>NMDA receptor</td>
</tr>
<tr>
<td>NHS</td>
<td>UK National Health Service</td>
</tr>
<tr>
<td>NT</td>
<td>Neurotrophic factor</td>
</tr>
<tr>
<td>OR</td>
<td>Odds ratio</td>
</tr>
<tr>
<td>P</td>
<td>Paracrine</td>
</tr>
<tr>
<td>PARALS</td>
<td>Puglia, Piemonte and Valle d’Aosta Register for ALS</td>
</tr>
<tr>
<td>PBP</td>
<td>Progressive bulbar palsy</td>
</tr>
<tr>
<td>PDGF</td>
<td>Platelet derived growth factor</td>
</tr>
<tr>
<td>PEDF</td>
<td>Pigment epithelium-derived factor</td>
</tr>
<tr>
<td>PEG</td>
<td>Percutaneous enteroscopically inserted gastrostomy tube</td>
</tr>
<tr>
<td>PET</td>
<td>Positron emission tomography</td>
</tr>
<tr>
<td>PFTs</td>
<td>Pulmonary function tests</td>
</tr>
<tr>
<td>PI3K</td>
<td>Phosphoinositide 3-kinase</td>
</tr>
<tr>
<td>PLS</td>
<td>Primary lateral sclerosis</td>
</tr>
<tr>
<td>PM</td>
<td>Post mortem</td>
</tr>
<tr>
<td>PMA</td>
<td>Progressive muscular atrophy</td>
</tr>
<tr>
<td>POEMS</td>
<td>Peripheral neuropathy, organomegaly, endocrinopathy and monoclonal gammopathy syndrome</td>
</tr>
<tr>
<td>PON1-3</td>
<td>Genes encoding Paraoxonase enzyme</td>
</tr>
<tr>
<td>PPS</td>
<td>Post polio syndrome</td>
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<tr>
<td>PR</td>
<td>Prevalence rate</td>
</tr>
<tr>
<td>PY</td>
<td>Person years</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
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<tr>
<td>R</td>
<td>Receptor</td>
</tr>
<tr>
<td>RCSI</td>
<td>Royal College of Surgeons of Ireland</td>
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<tr>
<td>RF</td>
<td>Risk factor</td>
</tr>
<tr>
<td>rhIGF</td>
<td>Recombinant Insulin like growth factor</td>
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<tr>
<td>RIG</td>
<td>Radiologically inserted gastrostomy tube</td>
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<tr>
<td>RGD</td>
<td>Arg-Gly-Asp-integrin recognition site on IGF</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribo-nucleic-acid</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
</tr>
<tr>
<td>RR</td>
<td>Relative risk</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SETX</td>
<td>Gene encoding probable helicase senataxin Enzyme</td>
</tr>
<tr>
<td>SFEMG</td>
<td>Single fibre EMG</td>
</tr>
<tr>
<td>SMN-1</td>
<td>Spinal muscular atrophy gene-1</td>
</tr>
<tr>
<td>SMN-2</td>
<td>Spinal muscular atrophy gene-2</td>
</tr>
<tr>
<td>SMR</td>
<td>Standardised mortality rate/ratio</td>
</tr>
<tr>
<td>SNEP</td>
<td>Sniff nasal expiratory pressure</td>
</tr>
<tr>
<td>SNIP</td>
<td>Sniff nasal inspiratory pressure</td>
</tr>
<tr>
<td>SNP</td>
<td>Single nucleotide polymorphism</td>
</tr>
<tr>
<td>SO</td>
<td>Spinal onset</td>
</tr>
<tr>
<td>SOD-1</td>
<td>Superoxide dismutase-1 (can also represent type of mouse model)</td>
</tr>
<tr>
<td>SPECT</td>
<td>Single photon emission computed tomography</td>
</tr>
<tr>
<td>SPEP</td>
<td>Serum protein electrophoresis</td>
</tr>
<tr>
<td>SPMR</td>
<td>Standardised proportionate mortality rate</td>
</tr>
<tr>
<td>SPSS</td>
<td>Statistical package for the social sciences</td>
</tr>
<tr>
<td>SSEP</td>
<td>Somatosensory evoked potentials</td>
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<tr>
<td>T₁/₂</td>
<td>Half life</td>
</tr>
<tr>
<td>TARDBP</td>
<td>Gene encoding TAR DNA-binding protein 43 (TDP-43)</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
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</tr>
<tr>
<td>TDP-43</td>
<td>TAR DNA-binding protein 43</td>
</tr>
<tr>
<td>TFTs</td>
<td>Thyroid function tests</td>
</tr>
<tr>
<td>UBRI</td>
<td>Ubiquitin reactive inclusions</td>
</tr>
<tr>
<td>UK</td>
<td>United Kingdom</td>
</tr>
<tr>
<td>UMN</td>
<td>Upper motor neuron</td>
</tr>
<tr>
<td>UNC13A</td>
<td>Gene encoding a protein that regulates the release of neurotransmitters such as glutamate at neuromuscular synapses.</td>
</tr>
<tr>
<td>USA</td>
<td>United States of America</td>
</tr>
<tr>
<td>UTI</td>
<td>Urinary tract infection</td>
</tr>
<tr>
<td>V</td>
<td>Volts</td>
</tr>
<tr>
<td>VEGF</td>
<td>Vascular endothelial growth factor</td>
</tr>
<tr>
<td>VEGFR</td>
<td>Vascular endothelial growth factor receptor</td>
</tr>
<tr>
<td>VEP</td>
<td>Visual evoked potentials</td>
</tr>
<tr>
<td>VF</td>
<td>Video fluoroscopy</td>
</tr>
<tr>
<td>VF*</td>
<td>Vocal folds</td>
</tr>
<tr>
<td>V^s</td>
<td>Versus</td>
</tr>
<tr>
<td>WFN</td>
<td>World Federation of Neurology</td>
</tr>
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xxiii
Thesis Summary

This thesis describes a series of studies examining aspects of the epidemiology of motor neuron disease (MND). The Republic of Ireland has a unique database of all patients diagnosed with MND which allows detailed study of MND epidemiology over time. Approximately 200-240 patients in Ireland have MND at any one time and 80 patients are diagnosed on a yearly basis. Data on over 1000 patients has been collected since 1993. The Irish population is an ideal study group for analysis as we are a small island population, the patients are well characterised from a clinical perspective and the gene pool has remained relatively homogenous. In addition the number of specialists dealing with MND patients in Ireland is extremely small allowing near 100% capture of the population. Up until recently no data has been available for comparison on the MND population in Northern Ireland (NI) which comes under the jurisdiction of the UK NHS.

The first part of this study aimed to update and reanalyse the Irish MND population data. In particular it was of interest to compare current incidence and prevalence data from a study published in 1999 to examine for changing population trends after introduction of new standards of care in nutritional and respiratory management of MND. In addition we extended advice and support to the NI MND services in the establishment of a sister register in NI such that ‘all Ireland’ MND population data could be calculated, this is part of an MD thesis by Dr Colette Donaghy in NI. We discovered relatively stable epidemiology in our MND population over the last decade with identical figures produced by the NI MND register. It would appear that new standards of care have not yet impacted life expectancy in the Irish MND population but further study is required in the future.

Age, male gender, family history and smoking are the only known risk factors (RFs) for MND to date. Identifying potential RF for MND may allow avoidance of high risk activities in those with other predisposing factors. Athleticism or a history of heavy physical activity is a controversial putative RF for MND. We conducted a prospective case control study to evaluate this hypothesis using a validated lifetime historical activity
questionnaire. We found that a history of exercise did increase the risk of MND, particularly spinal onset disease and that physical activity conducted at work can contribute significantly to calculated figures and should not be omitted. In addition significant differences in energy expenditure defined as ‘vigorous’ were noted between MND cases and controls.

Deficiency of insulin-like growth factor (IGF) has been described in MND patients. Whether this is coincidental or a primary or secondary phenomenon in the disease is unclear. Many other factors can influence IGF levels such as starvation and stress. We conducted both cross sectional and longitudinal studies of IGF in our MND cases and compared them to populations with multiple sclerosis (MS), post-polio syndrome (PPS) and normal controls. In addition nutritional and functional indices were evaluated serially and correlated with IGF data. Our data confirmed perturbations in the IGF system in MND and suggested that IGF-1 levels dip pre-agonally in MND patients.
Chapter One
Motor Neuron Disease

Figure 1: Title page from ‘The Nervous System of the Human Body’ by Charles Bell, 1940. This publication documented the first case of motor neuron disease. (1)

1.1 Introduction

In 1830 Charles Bell (1) reported the case of a middle aged woman with progressive paralysis of her limbs and tongue but preservation of sensation. Post mortem examination revealed degeneration of the anterior portion of her spinal cord. He concluded that "the anterior column of the spinal marrow is for motion, the posterior column is for sensation". (Figure 1) Twenty years later Aran described progressive muscular atrophy (PMA) which was initially thought to be a primary disease of muscle. (2) In 1855 Cruveilhier attributed PMA to atrophy of the anterior horns of the spinal cord. (3) Fritsch and Hitzig pioneered using electrical stimulation to identify the cortical motor strip of the brain. (4) In 1869 Jean-Marie Charcot (Figure 2) used the term ‘Amyotrophic Lateral Sclerosis’ (ALS) for
the first time.\(^5\) By 1874 the clinical and pathological features of the disorder based on the history and examination of twenty similar cases was published, autopsy data was also available on five of the patients.\(^6\)

![Figure 2: Jean Marie Charcot first used the term 'amyotrophic lateral sclerosis'. (Courtesy of http://www.neuromuscular.wustl.edu, 2009 from American Journal of Insanity, October 1893).\(^9\)](image)

Kahler and Pick identified atrophy of the motor cortex in MND patients in 1879.\(^7\) Dejerine related the diseases of Progressive Bulbar Palsy (PBP) and MND in 1883.\(^8\) Subsequently in 1884 Kahler further grouped the diseases of PMA, ALS and PBP together as "primary degenerations of the motor system".\(^9\) The term motor neuron disease (MND) was first used by Brain in 1933 to group the three diseases under one heading recognising that all three pathologically targeted motor neurons for destruction.\(^10\) In practical terms ALS and MND are now used interchangeably to represent the broad spectrum of sporadic motor neuron disease throughout the published works on this subject. To avoid confusion the term MND is preferred throughout this text as ALS can also represent the term acid labile subunit which is relevant to the chapter on IGF. The story of Lou Gehrig, a New York Yankees baseball player encapsulates the physical devastation this condition causes. A champion baseball striker, he died at the age of 38 years, within 2 years of developing his first symptoms. (Figure 3)
**Figure 3:** Legendary American baseball player Lou Gehrig who died from motor neuron disease in 1941 at the age of 38 years. (Courtesy of [http://www.neuromuscular.wustl.edu](http://www.neuromuscular.wustl.edu), 2009 from [http://www.newworldencyclopedia.org/entry/LouGehrig](http://www.newworldencyclopedia.org/entry/LouGehrig))

MND is a relentlessly progressive fatal neurodegenerative condition that causes upper and lower motor neuron loss at multiple levels in the nervous system. It is the commonest neurodegenerative condition affecting young and middle aged adults with an incidence worldwide of approximately 2.6/100,000. 5-8% of cases are familial and 20% of these cases have the well-recognised Cu/Zn superoxide-dismutase-1 genetic mutation on chromosome 21. For the other 90% so called sporadic disease the aetiology remains unknown. The pathophysiological process involves predominantly cortical Betz cells, the corticospinal tracts, brainstem cranial motor neurons and the ventral horns of the spinal cord. Rarely extrapyramidal and spinocerebellar tracts may become involved. A frontotemporal dementia (FTD) has also been described in association with the disease. MND shares many neuropathological and neurobiological features with other neurodegenerative conditions. Treatments for MND are thus likely to have implications for other neurodegenerative conditions such as Alzheimer’s disease (AD), FTD and
idiopathic Parkinson’s disease (IPD). Although the initial severity and anatomical regions affected by the disease may vary, the disease is invariably fatal 2-5 years from diagnosis. Most patients are diagnosed 9-12 months after onset of symptoms. (9, 11)

1.2 Epidemiology

Reported worldwide prevalence and incidence rates of MND vary. The Irish age adjusted incidence and prevalence rates were 2.8 and 6.2 per 100,000 in 1997. (12) There is a particularly high incidence and prevalence of MND in Guam and the Kii Peninsula where MND occurs as part of an MND-dementia-Parkinson’s complex. This disease is felt to be different to sporadic MND elsewhere in the world with a probable environmental/dietary trigger. (9, 11)

The age distribution of MND is interesting with MND more common in males up until 65 years of age, thereafter rates are similar between the sexes. Overall males present with more spinal onset than bulbar onset disease. (11, 12) The study of epidemiology of MND allows us to compare incidence and prevalence rates worldwide and to identify potentially modifiable risk factors for the disease. We may also use epidemiology to investigate the impact of clinical interventions such as expert nutritional management, gastrostomy tube placement, non-invasive ventilation and drug therapy on survival. Ireland is one of the few countries together with Scotland that have established and maintained a national MND register. The data from both Scotland and Ireland are population based which means that all patients with MND in that country are entered on the register on a continuous basis once their diagnosis is confirmed. (12, 13) Population based information is less biased than that obtained from clinic or centre based data which inadvertently selects out certain patient types. Epidemiological records are important when gathering samples of DNA for analysis as although sporadic MND is the most common form of the disease, sporadic MND patients may still have a genetic predisposition to the disease. Sporadic and familial MND are clinically and pathologically similar so any information gleaned from the study of either disease is useful. (9, 11)
Our understanding of the molecular and genetic basis of MND began with the identification by Siddique et al \(^{(14,15)}\) of mutations on the long arm of chromosome 21 that was associated with a familial form of MND. 20% of familial patients have mutations here in the gene for Cu/Zn superoxide dismutase (SOD-1) which can cause a toxic gain of function in the enzyme. Despite a similar genetic cause the resultant disease phenotypes and courses may vary considerably among familial cases. \(^{(16,17)}\) Andersen et al have shown that apparently sporadic MND may also have a truly genetic component i.e. that some cases of sporadic MND may in fact be familial MND with mutations of variable and low penetrance. \(^{(18)}\) MND is thus a complex genetic disorder in which there is an interplay between environment and genetic factors. To date many potential susceptibility and modifier genes have been described in sporadic MND and have helped to further our knowledge about the disease. \(^{(9,19)}\)

## 1.3 Pathology

Grossly MND changes are most evident in the spinal cord though the precentral gyrus (primary motor strip) of the brain may show atrophy. There is loss of the normal cervical and lumbosacral enlargements of the cord. Anterior motor roots are shrunken, spinal cord innervating bowel and bladder is classically spared although it may become involved in prolonged disease.

Microscopically in the precentral motor strip of the cortex Betz cells and other pyramidal cells are lost. In the brainstem there is loss of motor nuclei with sparing of extracocular nuclei. The myelinated axons of the corticospinal tracts are lost with astroglial proliferation. Rarely microglial proliferation has also been described. MND causes shrinkage and loss of cell bodies at the anterior horn cells with apoptosis. Spheroids are seen in proximal axons which represent aggregations of neurofilaments. Bunina bodies (dense granular inclusions) are found in the neuronal cell body cytoplasm. (Figure 4) Lower motor neurons in particular can contain rod shaped inclusion bodies with ubiquinated parallel filaments called Hirano bodies. These inclusion bodies are ubiquitin
and TDP-43 positive and may also been seen in glial cells. Interestingly they are found in MND with FTD but not in SOD-1 FALS. (Figures 4-6) (20, 21) Muscles show numerous grouped angular atrophic muscle fibers of both types indicating denervation. Increased levels of Ceramide C16:0, Ceramide C24:0, Cholesterol esters C16:0 and C18:0 and Sphingomyelin have been described in the spinal fluid of MND cases. Similar changes have also been reported in presymptomatic SOD-1 mice before and after application of oxidative stress. (22)

Figure 4: Marked atrophy of the anterior roots (versus posterior roots) and mild degeneration of the lateral corticospinal tracts. (9)

Figure 5: Marked loss of neurons and gliosis was observed in the anterior horn cell region bilaterally throughout the cord. (9)

Figure 6: Prominent loss of neurons and gliosis in the hypoglossal nucleus. Small intracytoplasmic inclusion bodies, also known as Bunina bodies (arrow) are seen in residual anterior horn cells. (9)
1.4 Aetiology

The aetiology of sporadic MND remains unknown. It is a complex heterogeneous disease which likely results from a combination of genetic susceptibility factors and exposure to environmental toxins or triggers, which together but not in isolation cause the disease. There is evidence to support the following as factors in disease pathogenesis. (Figure 7)

(i) Genetic susceptibility.
(ii) Abnormal RNA Processing.
(iii) Selective vulnerability of motor neurons.
(iv) Disruption of Ubiquitin-proteasome system: Inclusion bodies.
(v) Neurofilament transport disruption.
(vi) Neurotransmitter system disruption.
(vii) Alteration in Neurotrophic factors.
(viii) Oxidative stress.
(ix) Alteration in Hypoxia response.
Pathogenesis of MND

Figure 7: Potential factors involved in the pathogenesis of MND. A number of factors combine to increase motor neuron susceptibility to injury but other factors which may be intrinsic or extrinsic, are required to send the motor neuron finally along the cell death cascade.

1.4.1 Genetic Susceptibility
MND is familial in 5-10% and sporadic in 90-95% cases. Despite this genetic division, clinically and pathologically the two diseases are often indistinguishable. The majority of familial cases are inherited in an autosomal dominant (AD) fashion with few exceptions. (14-18) (Figure 8) As mentioned 20% of familial AD cases show mutations in a gene for SOD-1 on chromosome 21. This region codes for a free radical scavenger enzyme Cu/Zn dismutase. It functions to convert harmful superoxide radicals to molecular hydrogen and oxygen peroxide preventing the generation of reactive oxygen species. (ROS) When mutated the enzyme through ‘gain of function’ acquires excessive toxic capabilities. The exact mechanisms through which this occurs are not yet understood. (16) Andersen et al have demonstrated that a large Swedish kindred previously thought to have sporadic disease actually have an autosomal recessive (AR) D90A SOD-1 mutation resulting in a slowly progressive disease with incomplete penetrance. There was a high frequency of unaffected heterozygotes among the same Scandinavian population. (18) Another autosomal recessive (AR) form of MND in a Tunisian family has been linked to chromosome 2. (17)

![Pedigree for Familial ALS](image)

**Figure 8:** Sample Familial MND Pedigree demonstrating the classic autosomal dominant inheritance pattern. (Courtesy of Dr Orla Hardiman, personal slides 2005)

More recent focus has been assigned to identifying genetic abnormalities in apparently sporadic MND. There are a number of good reasons for doing this:
• The vast majority of cases are sporadic.
• Large databanks of DNA have been collected in MND centres from these patients.
• Sporadic cases can have de novo mutations.
• The phenomenon of ‘Epigenetics’ has been recognised.

Epigenetics refers to changes in gene expression that are stable through cell division but do not involve changes in the underlying DNA sequence unless exposure to certain environmental factors alters their epigenetic programming.\(^{(23)}\) There are two main methods that have been used to evaluate sporadic MND populations genetically to date: Candidate gene association studies and genome wide association studies.

1.4.1.1 Candidate gene association studies:
These studies involved selection of a number of genes for study based on their functions and their potential implications in MND. To date approximately 20 genes of interest have been identified in sporadic MND populations. (Table 1) Researchers have reported deletions or insertions in the genes coding for the neurofilament heavy subunit protein\(^{(24,25)}\), mutations in the gene encoding the cytochrome-c-oxidase subunit 1 (part of the terminal mitochondrial respiratory chain)\(^{(26)}\) and also mutations in the AP endonuclease enzyme which is involved in DNA oxidative damage repair.\(^{(27)}\) The apolipoprotein e4 genotype may also be a risk factor for the development of bulbar onset MND, though this remains controversial.\(^{(28)}\) In the Irish population Greenway et al\(^{(29)}\) have linked a number of their sporadic MND cases to mutations in the genes for the hypoxia inducible factor (HIF) Angiogenin and in the UK examination of the haemochromatosis gene H63D has been of interest.\(^{(30)}\) Mutations in the vascular endothelial factor gene (VEGF) and progranulin have also been implicated.\(^{(19,31,32)}\) It would appear that genetic factors play a role both in familial and sporadic MND. By identifying genetic factors in both familial and sporadic disease we can further the understanding of the molecular processes that malfunction in all MND patients causing disease. We can also create transgenic animal models of disease such as the SOD-1 mice which can be used to rapidly test potential therapeutic agents in human disease.\(^{(33)}\) A disadvantage of this methodology is that when a positive result is identified in one small population it then needs to be replicated in other
populations. Often the second study is negative leading to a third study etc., results have to be interpreted with caution. For example a recent meta-analysis of six positive case-control association studies of the PON1-3 cluster in MND resulted in exclusion of paraoxonase genes as susceptibility factors in MND. 34 Population stratification appears to greatly influence small candidate gene studies resulting in false positive associations. 23
### Table 1: Proposed Susceptibility & Modifier Genes in MND\(^{(19)}\)

<table>
<thead>
<tr>
<th>Gene</th>
<th>Locus</th>
<th>Variant</th>
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<td>19q13</td>
<td>e4 genotype</td>
</tr>
<tr>
<td>CNTF</td>
<td>11q12</td>
<td>Null allele</td>
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<tr>
<td>EAAT2</td>
<td>11p13</td>
<td>Low expression</td>
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<td>GluR2</td>
<td>4q32</td>
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<td>NEFH</td>
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<td>Dynactin</td>
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<td>Progranulin</td>
<td>17q21.3</td>
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<td></td>
<td>9p21.2</td>
<td>Linkage</td>
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</tbody>
</table>

#### 1.4.1.2 Genome-wide association studies:

GWA studies have become an increasingly powerful tool in genetics and have been successful in identifying susceptibility genes for other complex disorders such as type 2 diabetes.\(^{(25)}\) These studies can efficiently process hundreds of thousands of SNPs without requiring candidate genes to be selected first. Results have been disappointing with conflicting results from different populations. It is felt that the heterogeneity of large sporadic populations may dilute out significant findings.\(^{(23)}\)

#### 1.4.2 Abnormal RNA Processing

Recent identification of the involvement of TARDBP, FUS and ELP3 in MND directly links motor neuron degeneration with defective RNA processing pathways. The mechanism by which defects in RNA pathways leads to MND has become a focus of interest for researchers in this area. CNS motor neurons may be more susceptible to RNA-processing defects because the CNS expresses more alternative splicing transcripts than other tissues and so has a lower tolerance for mRNA disturbances.\(^{(26,37)}\) Other MND
genes i.e. SETX, ANG, SMN1 and GLE1 encode RNA processing proteins. SOD-1 may act as an mRNA stabilizer. This is a very exciting area of research at present. (38)

1.4.3 Selective Vulnerability of Motor Neurons

It is an interesting phenomenon that in the majority of patients with motor neuron disease as the name suggests only motor neurons are affected. Rarely patients describe sensory symptoms but this is the exception rather than the rule. Motor neurons subserving eye movement and bladder control are also typically spared or affected very late in the course of the disease. There are a number of features of motor neurons that differentiate them from unaffected motor neurons. Androgen receptors are plentiful on motor neurons affected by the disease but rare on the areas commonly spared i.e. eyes and bladder. (39)

Speculation that androgen containing substances might contribute to the disease has not been supported by research in this area. Excessive exercise has been proposed as a risk factor for MND. Performance enhancing agents containing muscle building androgens are increasingly used by elite sportsmen and women. No data to date has convincingly linked anabolic steroids or other muscle enhancing agents to the pathogenesis of MND. Poliovirus is an infectious agent that like MND preferentially attacks the anterior horn cells causing lower motor neuron degeneration of the affected limb(s). It is known to particularly affect fast twitch muscle fibres in limbs that have been recently exercised suggesting enhanced delivery of polio with exercise. Thus, in theory a similar viral agent could cause MND but no infectious aetiology has ever been proven. (40-43) 1A muscle spindle afferent discharge is increased during physical activity and could contribute to enhanced delivery of a toxin either infectious or Motor neurons lacking 1A afferent input include otherwise to target cells during exercise. Onuf’s nucleus and motor neurons to the eye musculature are typically spared in sporadic MND. (44-47) Spinal motor neurons are also known to lack certain calcium buffering proteins making them particularly vulnerable to calcium related injuries. (11,48-50) In contrast the oculomotor neuron group which are involved in eye movements readily express calcium-buffering proteins and hence are less vulnerable to calcium mediated toxicity. It is plausible that the pathological processes involved in MND involve calcium mediated toxicity at an early stage in the
disease. End stage MND particularly in ventilated patients will eventually result in the absence of voluntary eye movements. (51)

1.4.4 Disruption of Ubiquitin-proteasome System Dysfunction

In 1988 Lowe (52) and colleagues identified ubiquitin positive filamentous inclusion bodies in the anterior horn cells of MND cases but not controls. These inclusion bodies were closely associated with the classical Bunina bodies of MND. These observations linked the protein ubiquitin with MND for the first time. Ubiquitin is a stress protein implicated in the degradation of short-lived and abnormal proteins. In a neuropathological case control study of 43 MND cases the distribution and specificity of Bunina bodies and ubiquitin-reactive inclusions (UBRI) were further characterised. (53) Nerve cell loss was described in the primary motor area in 67% of the MND patients. Bunina bodies were present in the cortical Betz cells in 10% of MND patients while UBRI were present in the small pyramidal cells in 17% of MND cases. Degeneration of anterior horn cells coincided with the presence of Bunina bodies and UBRI in all 43 MND cases.

Motor nuclei of the caudal brain stem were involved to varying extents. The oculomotor nuclei and Onuf's nucleus showed no degeneration although UBRI were found at these sites in 11% and 18% of MND cases respectively explaining why these areas do eventually become involved. Formation of UBRI was not confined to motor nuclei but also involved the brain stem reticular formation, substantia-nigra and Clarke's nucleus. These findings suggest that MND is really a multiple system degeneration favouring motor neuron destruction at certain sites. Two controls had UBRI in the anterior horn cell region and in the hypoglossal nucleus respectively. Thus UBRI are not specific for MND but may have practical value in the supportive neuropathological diagnosis of that disease. (53)
In a separate study Anderson et al\(^{(54)}\) demonstrated ubiquitin inclusions in the amygdala and parahippocampal gyrus in more than 30% of their MND cases. Most of these cases were not known to have significant cognitive impairment at the time of death. The inclusions were unreactive against antibodies to Tau and phosphorylated neurofilaments thus differentiating them from the inclusion bodies seen in Alzheimer's disease and the tauopathies. They also lacked the appearance or immunocytochemical features of cortical Lewy bodies found in Parkinson's disease. Parallel clinical studies found selective cognitive impairment in 25% of their MND patients with typical MND. PET activation studies have also shown impaired activation in the anterior thalamus, parahippocampal gyrus and medial frontal regions of the same patient cohort.\(^{(54)}\)

Estimates of the prevalence of severe cognitive impairment in MND vary but the figure of approximately \(\leq 10\%\) of the total population is felt to be reasonably accurate. A larger proportion may have milder cognitive difficulties.\(^{(55-58)}\) The degree of overlap between FTD and MND is shown in table 2. MND, MND-D, and FTD–MND all represent a spectrum of clinical disease with a common pathological substrate. FTD in association with MND will be discussed further in the section on clinical features of the disease.\(^{(55-58)}\)

There have also been rare case reports of MND patients with classic Alzheimer's disease (AD). Mackenzie et al\(^{(58)}\) found that approximately 30% of MND cases with dementia have AD and that some MND cases without frank dementia have significant AD pathological Features post mortem.\(^{(59)}\) Degeneration and atrophy of the frontal and temporal lobes, which contain ubiquitin-positive neuronal inclusions and dystrophic neuritis in MND patients has been demonstrated by Toyoshima et al.\(^{(60)}\) The authors also noted circumscribed degeneration of the hippocampal CA1-subiculum border zone and significant degeneration in both the upper and lower motor neuron systems, the latter being more severely affected. A few lower motor neurons contained cytoplasmic inclusions characteristic of MND (i.e. Bunina bodies and ubiquitin-positive skeins). Toyoshima et al\(^{(60)}\) suggest that motor neuron disease-inclusion dementia (MND-ID), which has been classified as a pathological subgroup of FTD, is actually a forme fruste of
MND with dementia. In other words, if patients with MND-ID live long enough, they may develop MND. (Table 2)

Table 2: Diagnostic Classification for ALS Cognitive and Behavioural Dysfunction According to Strong, et al. (61)

<table>
<thead>
<tr>
<th>Subheading</th>
<th>Existing terms in the literature</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALS/MNDci</td>
<td></td>
<td>Deficits in frontal cognitive function but insufficient to meet Neary criteria for FTD.</td>
</tr>
<tr>
<td>ALS/MND-FTD</td>
<td>ALS-dementia (ALS-D), FTD-MND</td>
<td>ALS/MND patients meeting criteria for FTD</td>
</tr>
<tr>
<td>ALS/MND-PA</td>
<td></td>
<td>ALS/MND patient meeting criteria for PA.</td>
</tr>
<tr>
<td>ALS/MND-SD</td>
<td></td>
<td>ALS/MND patient meeting criteria for SD.</td>
</tr>
<tr>
<td>FTD-MND-like</td>
<td></td>
<td>Cases of FTLD in which there is neuropathological evidence of motor neuron degeneration, but insufficient to be classified as ALS/MND.</td>
</tr>
<tr>
<td>ALS/MND</td>
<td></td>
<td>ALS/MND with dementia, not typical of FTLD.</td>
</tr>
<tr>
<td>ALS/MND-parkinsonism-dementia complex</td>
<td>Western Pacific variant of ALS/MND</td>
<td>ALS/MND concurrent with dementia and/or parkinsonism occurring in hyperendemic foci of the western pacific.</td>
</tr>
</tbody>
</table>
1.4.5 Neurofilament Transport Disruption

Neurofilament proteins form an integral part of the neural cytoskeleton, maintaining axonal caliber and cell shape. Neurofilament subunits assemble in the perikaryon and travel down the axon by slow axonal transport becoming increasingly phosphorylated along the way. (62) Neurofilament formation and transport has been shown to be faulty in mice models of MND (G93A and SOD-1). (63, 64) Hallmark MND lesions such as fragmentation of the Golgi apparatus and neurofilament (NF)-rich inclusions in the surviving spinal cord motor neurons and selective degeneration of motor neurons were also observed in these animals. The aggregation of neurofilaments in the cell body and proximal axon of the motor neuron, blocks further necessary axonal transport. (64) Whether this is a primary or a secondary phenomenon of MND is not clear. (33, 66) Abnormalities of the neurofilament heavy-subunit genes have been described in sporadic MND. (24, 25, 67, 68) In vivo experiments have shown that disruption of neurofilament assembly results in selective motor neuron injury and death. (68–71) Neurofilament proteins have a long half-life and contain large quantities of lysine, an amino acid derivative particularly susceptible to oxidative modification. (11) An abnormal neurofilament genotype in the presence of other environmental risk factors might combine to disrupt neurofilament production and function in the motor neuron enough to cause neurotoxicity and premature neuronal death.

1.4.6 Neurotransmitter System Disruption

Alterations in the glutamatergic-neurotransmitter system are thought to play a key role in the injury to motor neurones in sporadic MND. Glutamate is the most important known excitatory neurotransmitter in the human CNS. During normal functioning glutamate is released into the synaptic cleft where it can activate several postsynaptic receptor subtypes (NMDA, AMPA, kainite and metabotropic). Excitatory amino acid (glutamate) transporters (EAAT-1 and EAAT-2) located on perisynaptic astrocytes actively remove glutamate from the synaptic cleft. Once within the perisynaptic astrocytes glutamate is converted to glutamine by the action of the enzyme glutamine synthetase. Glutamine is then returned back to the presynaptic neuron where it is converted back to glutamate by the enzyme glutaminase. The presynaptic metabotropic and kainite receptors are thought to have a modulatory effect on this system. Glutaminergic motor neuron inputs arise from
the descending corticospinal pathways \(^{72, 73}\) from collaterals of A-delta fibres innervating muscle spindles and Golgi tendon organs\(^{74, 75}\) and from spinal cord excitatory interneurons.\(^{76}\) Human motor neurons have a high density of glutamate receptors of all types \(^{77-80}\) although there are differences in the relative densities of NMDA and non-NMDA receptor binding sites expressed by motor neuron groups that are susceptible to MND. Human motor neurons express the universal NMDA receptor subunit NMDAR-1 highly, although dendritic staining by immunohistochemical studies is much less than that of the cell body. This is unlike the pattern of staining seen in other groups of neurons elsewhere in the body.\(^{81}\) In non NMDA receptors human spinal motor neurons and surrounding presynaptic processes show positive immunoreactivity for KA-2 and GluR-6/7 subunits\(^{81}\). The expression of AMPA receptor subunits is particularly of interest as they are composed in humans of four protein subunits GluR-1-4, responsible for fast excitatory neurotransmission in the mammalian CNS. The GluR-2 subunit when incorporated into the AMPA receptor complex renders the receptor impermeable to calcium.\(^{82}\) Most human CNS AMPA receptors include the GluR-2 subunit and should therefore be impermeable to calcium.\(^{83}\) However, upper and lower motor neurons studies in the human CNS have shown very low or absent expression of GluR-2 at mRNA and protein level.\(^{84, 85}\) This means that AMPA receptors expressed by motor neurons are atypical and calcium permeable. Calcium plays a crucial role in mediating toxic cellular events following glutamate receptor activation.\(^{86}\) This unusual molecular profile of the AMPA receptors expressed by human motor neurons could be an important factor in rendering the motor system susceptible to glutamate-mediated cellular toxicity. The hypothesis that glutamate excitotoxicity plays an important role in MND is based on evidence from a number of studies:

### 1.4.7 Alteration in Neurotrophic Factors

A number of neurotrophic factors have been investigated as potential therapies in MND after aberrant behaviour or abnormal levels of these peptides were demonstrated in animal models. Initial animal studies of most notably insulin like growth factor-1 (IGF-1), brain derived neurotrophic factor (BDNF), and ciliary neurotrophic factor (CNTF) were followed by disappointing results in human MND populations.\(^{87-91}\) It is possible
that to date the delivery methods in growth factor trials have been suboptimal, incapable of providing therapeutic quantities of the growth factor at their neuronal targets. Administration of growth factors has been either parenteral, with the blood brain barrier limiting the concentration achieved in the CNS, or by intracerebroventricular infusion where significant concentrations could not diffuse beyond the ependymal lining. Growth factor gene therapy introduces genetic information into the cell allowing production of the required growth factors locally, by the target area itself.\(^{(92)}\) With advances in molecular biology we will be able to provide vector systems that can sustain and potentially regulate gene expression and hence growth factor production in vivo at the target site. Neuroprotective factors delivered to the brain via gene therapy may in the future become an acceptable practice for treating neurodegenerative conditions such as MND if concerns about the consequences of long term unregulated production of these factors can be overcome. Some experts believe that trials thus far on growth factors in MND have been somewhat naïve, failing to take into account the role of growth factor receptors and binding proteins in the neurodegenerative process. Growth factors which have been of particular interest to our study group are insulin like growth factor (IGF) and mechano growth factor or MGF (a splice variant of IGF-1), angiogenin and vascular endothelial growth factor (VEGF).\(^{(93)}\)

1.4.7.1 Insulin like Growth Factor (IGF)

IGF has a wide range of biological effects on many different organs. It stimulates bone formation, protein synthesis, glucose uptake in muscle, neuronal survival and myelin synthesis and inhibits protein degradation in muscle. It has trophic effects on the entire motor unit from the body of the motor neuron, along the motor nerve axon to the neuromuscular junction and on the muscle itself. For this reason it has long been of interest to those researching treatments in neuromuscular disorders.\(^{(93)}\) In 1993 Neff et al \(^{(94)}\) showed that IGF-1 could be used to increase embryonic motor neuron survival in in vitro cultures and could reduce naturally occurring embryonic motor neuron death in vivo. Contreras\(^{(95)}\) showed that systemic administration of recombinant IGF-1 enhanced regeneration after sciatic nerve crush in mice. In late onset adult neurodegenerative disease the situation is a little different to animal models of MND, but even in mature
motor neurons IGF-1 is seen to increase motor neuron choline acetyl transferase (ChAT) activity in organotypic spinal cord cultures. In contrast CNTF, BDNF and NT-3 failed to have any impact on motor neuron survival in similar studies.\textsuperscript{(96,97)} Of note addition of GDNF, neurturin (GDNF analog) and PEDF have also shown survival benefit in the same animal model but further research remains to be performed.\textsuperscript{(98-101)}

A number of reports have been published describing the pharmacological effects of rhIGF-1 (recombinant) administration in normal human subjects and in individuals in a catabolic state i.e. post surgery. The recombinant polypeptide was administered for over 12 months in many subjects with few adverse events except hypoglycaemia when given as an IV bolus. This hypoglycaemic effect was reduced by subcutaneous administration after food consumption.\textsuperscript{(102-108)} With encouraging safety data and promising results in animal models of neurodegeneration two large multicentre double blind placebo controlled trials were planned to investigate use of rhIGF-1 in MND. In the North American study patients received placebo, 50 or 100 micrograms/kg rhIGF-1 in a daily subcutaneous injection.\textsuperscript{(90,109)} In the European trial patients received either placebo or the higher dose of IGF-1. The Appel scale was the functional primary outcome measure used. The North American Trial Showed a significant dose responsive slowing of functional decline in their population. The European trial showed a trend in the same direction which was not statistically significant.\textsuperscript{(90,109,110)} A pooled analysis of all patients receiving the higher dose showed unequivocal benefit on muscle strength and bulbar and upper extremity function during disease progression. Effects were more pronounced in those patients with a rapidly progressing disease.\textsuperscript{(111)} The North American trial was not originally designed to measure survival as a primary outcome but further analysis of extended follow up data looking at age and forced vital capacity as prognostic indicators suggested a possible survival benefit from the drug. Less than 4% of patients had to discontinue the recombinant IGF-1 during the trials due to side effects such as hypoglycaemia.\textsuperscript{(112)} Although intravenous administration can be harmful to the cardiovascular system, subcutaneous administration is apparently quite safe in the short term.\textsuperscript{(112,113)} The results of a third study of subcutaneous rhIGF-1 in MND have recently been published and failed to show a significant benefit.\textsuperscript{(91)}
In 2003 Wilczak et al\textsuperscript{(114)} investigated the components of the IGF-1 system in spinal cord sections of ten MND patients compared with ten controls without neurological disease. Total IGF-1 in ventral horns did not differ between patients and controls however free IGF-1 was 53\% lower in MND patients than in controls. Immunoreactivity for IGFBP-2, 5 and 6 was 64\%, 46\% and 33\% higher respectively in MND patients than controls and IGF-1 receptors were upregulated. The authors suggested that free IGF-1 is reduced in the ventral horns because of the increase in IGF binding proteins and the upregulation of IGF-1 receptors. They speculated that these abnormalities might be important in the process of motor neuron death in MND and should be considered when attempting to use IGF-1 as a therapy in MND patients. Compared with IGF-1 there is little known about IGF-2, which actually exerts most of its effects through the IGF-1 receptor.\textsuperscript{(115)} IGF-2 is the prominent species of IGF found in skeletal muscle.\textsuperscript{(116)}

Interestingly when IGF-1 was administered to normal rats via the cerebral intraventricular route in an attempt to directly access the brain and circumvent the blood brain barrier IGF-1 was rapidly cleared from the system. By 180 minutes less than 10\% of the radiolabelled IGF-1 remained in the CSF-brain system. There was also a 20 minute delay between apparent disappearance from the CNS and appearance in the blood which suggests that most of the IGF-1 was in fact removed via the cranial and spinal nerve routes into the blood and lymphatic system rather than via the arachnoid villi and periventricular tissue. IGF-1 penetrated less than 1.25mm into brain tissue. Thus it appears that the entry of IGF-1 into normal brain parenchyma after lateral ventricle administration is limited by rapid clearance from the CSF and brain followed by limited slow diffusion into only the immediate periventricular tissue.\textsuperscript{(117)} Supporters of IGF-1 therapy have argued that in previous trials subcutaneous administration of rhIGF may have prevented IGF-1 crossing the blood brain barrier in adequate quantities to have an effect. If this pattern of distribution and excretion after intraventricular delivery is replicated in humans regardless of brain and spinal cord condition then even intraventricular administration would not have the desired effect. In an injured brain it has been hypothesized that the normal flow of CSF is disrupted which might improve
delivery of growth factors to the diseased or injured tissues. IGF-1 distribution in the
abnormal brain has not yet been studied in detail. (117) Nagano et al have shown
therapeutic benefit of intrathecal injection of insulin-like growth factor-1 in the G93A
mouse model of motor neuron disease. Treated mice showed improved motor
performance, delayed onset of clinical disease and extended survival. (118) Adeno-
associated viral mediated insulin-like growth factor delivery has also been shown to
protect motor neurons in mouse models of motor neuron disease resulting in delayed
disease onset and prolonged survival. (119)

1.4.7.2 Mechano Growth Factor (MGF)
The physiological function of a recently cloned splice variant of insulin-like growth
factor-1 mechano growth factor (MGF) has been studied by Yang et al (120) using an in
vitro cell model. Unlike mature IGF-1, the distinct E domain of MGF inhibits terminal
differentiation whilst increasing myoblast proliferation. When the IGF-1 receptor are
blocked with a specific antibody, MGF continued to act indicating that the MGF-E
domain is mediated via a different receptor. MGF production is markedly affected in the
elderly and in dystrophic conditions resulting in loss of muscle mass. High resistance
exercise results in increased production of MGF mRNA especially in the young. There is
an attenuated MGF response to high resistance exercise in the older subjects, indicative
of age-related desensitivity to mechanical loading. These changes are not seen acutely
with IGF-1Ea splice variants of IGF found in muscle. Hameed et al (121) reported no
observed difference between resting levels of the two isoforms between the young and
old. Their study demonstrated that the MGF and IGF-1Ea isoforms were differentially
regulated in human skeletal muscle.

Studies in animals and human muscle have demonstrated differential splicing of the
insulin-like growth factor-1 gene in response to mechanical strain and damage. In a study
on the expression of insulin-like growth factor-1 splice variants in the levator-ani muscle
after the first vaginal delivery both Insulin-like growth factor splice variants mechano
growth factor and insulin-like growth factor-1Ea were significantly up-regulated in the
delivery population, compared with control subjects. (122)
Muscle satellite cells are mononuclear cells that remain in a quiescent state until activated when they proliferate and fuse with muscle fibres, donating nuclei. This process is necessary for post-embryonic growth, hypertrophy and tissue repair in post-mitotic tissue. These processes have been associated with expression of the insulin-like growth factor (IGF-1) gene which can undergo alternative splicing to generate different gene products with varying functions. Hill et al measured the mRNA transcripts in rat tibialis anterior muscles at different time intervals following either mechanical damage imposed by electrical stimulation of the stretched muscle or damage caused by injection with bupivacaine. Mechano growth factor (MGF) expression rapidly increased initially following both types of damage and then declined within a few days. Systemic IGF-1Ea was more slowly upregulated and its increase was inversely proportional to with the rate of decline in MGF expression. The initial pulse of MGF activates satellite cell. Later IGF-1Ea becomes the main source of mature IGF-1 for upregulation of protein synthesis which is required to complete the tissue repair. (123)

The C-terminal peptide of mechno-growth factor (MGF) functions independently from the rest of the molecule and has shown an independent neuroprotective effect in vivo and in vitro. In a gerbil model of transient brain ischemia, treatment with synthetic MGF C-terminal peptide provided very significant protection to vulnerable neurons. In the same animal model, ischemia resistant neurons in the hippocampus greatly increased their expression of endogenous MGF post insult, suggesting that the endogenous MGF might have an important neuroprotective function. In another study in-vitro organotypic hippocampal culture models of neuro-degeneration, the synthetic MGF C-terminal peptide was as potent as the full-length IGF-1 while its effect lasted significantly longer than that of recombinant IGF-1. The two peptides showed an additive protective effect but the neuroprotective action of the C-terminal MGF was independent from the IGF-1 receptor. Although MGF is mainly known for its regenerative capability in skeletal muscle, these studies also demonstrate a neuroprotective role against ischemia for this specific IGF-1 isoform. (124) Use of recombinant MGF, MGF C-terminal peptide or upregulation of its gene expression may be a future therapeutic option in MND.
1.4.7.3  Vascular Endothelial Growth Factor (VEGF)

Vascular endothelial growth factor (VEGF) was discovered 25 years ago but has only recently been implicated in the pathophysiology of several neurological disorders including IPD, MND, POEMS syndrome, cerebral arteriovenous malformations, MS and Alzheimer's disease. (125-131) During development both blood vessels and nerves are guided to their target locations with the aid of VEGF. Vascular endothelial growth factor (VEGF-A) is a key signalling agent in the induction of vessel growth or angiogenesis. VEGF is now also known to be a neuroprotective cytokine activated by hypoxia. (132-135) Interference with bloodflow to the spinal cord adversely affects the lifespan of spinal motor neurons, which are known to be very sensitive to hypoxia and/or ischaemia. (136) VEGF may also have direct trophic effects on motor neurons and a reduction in VEGF levels may result in excessive motor neuron loss. (137) Genetic studies show that mice with reduced VEGF levels develop an adult-onset motor neuron degeneration, reminiscent of the human neurodegenerative disorder motor neuron disease. (19, 126, 138) VEGF has been shown to have direct effects on neuronal and glial cells through activation of different VEGF receptor (VEGFR) subtypes. (139-140) VEGF is rapidly upregulated by more than 10-fold in response to changes in local oxygen concentration. Hypoxia inducible factors (HIFs) mediate this response by binding to a defined hypoxia response element in the promoter of the VEGF gene. (14-142) In 2001 Oosthuysen et al. (143) developed "knock in" mice by deleting the hypoxia response element (HRE) sequence in the VEGF gene. In mice homozygous for the deletion the hypoxic expression of VEGF in neural tissue was blunted. These mice suffered from severe adult-onset skeletal muscle weakness due to lower motor neuron degeneration and showed clinical and neuropathological changes very similar to those seen in MND. The ventral horns of the spinal cord and the brainstem motor nuclei were most affected. As part of their study the authors demonstrated that the VEGF-165 isoform had direct neuroprotective effects on isolated motor neurons in vitro. They suggest that reduced neural perfusion and insufficient VEGF dependant neuroprotection contributed to the lower motor neuron loss in these mice. This was the first time VEGF had been considered as a possible contributor in the pathogenesis of MND. (144) (Figures 9-11)
Figure 9: The multiple functions of VEGF in the nervous system. VEGF is transported with its receptor Flk intra-axonally both anterogradely and retrogradely. It has direct neurotrophic effects on motor neurons and also affects the vasculature within the brain and the spinal cord, enhancing local oxygen delivery and improving general motor neuron health. VEGF may also have effects on the surrounding glial cells that support motor neurons. (Figure courtesy of Vande Velde C et al, 2005) (145)

Work by Van Den Bosch and colleagues (137) on VEGF in mouse models of MND supported these findings and suggested that shortage of this neurotrophic factor may contribute to the motor neuron death observed in humans and animals with low VEGF expression levels. In 2003 Lambrechts et al had (31) reported that reduced expression of VEGF-A caused MND-like motoneuron degeneration in VEGF-A (delta/delta) mice. In a meta-analysis of over 900 individuals from Sweden and over 1,000 individuals from Belgium and England, subjects homozygous with respect to the haplotypes -2,578A/-1,154A/-634G or -2,578A/-1,154G/-634G in the VEGF promoter/leader sequence had a 1.8 times greater risk of MND (P = 0.00004). These 'at-risk' haplotypes had lower circulating VEGF levels in vivo and reduced VEGF gene transcription, VEGF expression and L-VEGF translation in vivo. (145) SOD-1(G93A) mice crossbred with VEGF-A (δ/δ) mice died earlier than mice with either mutation alone due to more severe motor neuron...
degeneration. VEGF-A ($\delta/\delta$) mice were unusually susceptible to persistent paralysis after spinal cord ischemia, and treatment with VEGF-A protected these mice against ischemic motor neuron death. These findings indicated that VEGF is a potential modifier of motor neuron degeneration in human MND and suggested a therapeutic potential of VEGF-A for stressed motor neurons. Terry et al found a 3-fold increased risk among individuals homozygous for the AAG or AGG VEGF haplotypes consistent with the findings of other studies. These studies demonstrated, for the first time, that vascular endothelial growth factor (VEGF) delays progression of symptoms and prolongs survival in a Cu/Zn superoxide dismutase (SOD1) transgenic mouse model of MND. These observations suggest that VEGF or related compounds might be of value in the treatment of MND patients.

Postmortem spinal cord and serum VEGF from a number of patients with MND and controls has been studied. Nygren et al found no significant difference in VEGF levels in the spinal cord between the MND patients and the controls. Serum VEGF levels were significantly higher in the MND group than in the control group. There was a moderate inverse relation between the duration of the disorder and the serum VEGF levels. These findings indicated the capacity to synthesize VEGF is preserved even in the late stages of MND. The authors felt that the results might also have been consistent with a transient hypoxic component during the course of MND, but not with a persistent spinal hypoxia in the late stages of the disorder. In contrast low VEGF levels in the CSF of MND patients were reported by Devos et al. All samples were during the first year of the disease and abnormalities were independent of VEGF promoter polymorphisms. Ilzecka et al found that in their MND population CSF VEGF levels were significantly increased, especially in patients with long duration of MND and in patients with limb-onset of the disease compared with controls. The site of onset of MND significantly influenced patient CSF VEGF levels. Levels were increased in patients with limb-onset disease compared to those patients with bulbar-onset MND. CSF VEGF levels also differed between patients with long duration of disease compared with short duration. Higher CSF VEGF levels correlated closely with longer duration of disease. It was
postulated that increased VEGF levels in cerebrospinal fluid may represent a protective action against glutamate-mediated toxicity and oxidative damage of motor neurons.\(^{(150)}\)

Expression patterns of VEGFR (receptors) -1, -2 and -3 have been investigated in the spinal cord of controls and MND patients (both familial and sporadic) by Spliet et al.\(^{(151)}\) VEGFR-1, VEGFR-2 but not VEGFR-3 were found to be increased moderately and mildly respectively in the blood vessels and reactive astroglial cells of both white and grey matter. All three receptor subtypes were undetectable in resting glial cells of control spinal cords, although diffuse neuropil staining was observed in the control grey matter for VEGFR-3. VEGFR-3 neuropil expression was reduced and paralleled the distribution of neuronal loss in the ventral horn of MND spinal cord. These findings indicate that VEGFRs have specific distribution patterns, suggesting different physiological functions of VEGF in human spinal cord. The altered expression observed in MND supports a role for these receptors in pathogenesis of MND. Xie et al\(^{(152)}\) found that several pro-inflammatory mediators were up-regulated at asymptomatic and end-stages of MND, whereas VEGF, a neuroprotective factor was down-regulated.

Azzouz et al\(^{(153)}\) reported that a single injection of a VEGF-expressing lentiviral vector into various muscles delayed onset and slowed progression of ALS in mice engineered to overexpress the gene coding for the mutated G93A form of the superoxide dismutase-1 (SOD-1(G93A)), even when treatment was only initiated at the onset of paralysis. VEGF treatment increased the life expectancy of MND mice by 30 per cent without causing toxic side effects, thereby achieving one of the most effective therapies reported in the field of MND so far. Recently intracerebroventricular (ICV) delivery of recombinant vascular endothelial growth factor (VEGF) in a SOD-1 (G93A) rat model of MND has delayed onset of paralysis, improved motor performance and prolonged survival by 22 days, representing the largest effect in animal models of MND achieved by protein delivery. ICV delivery of VEGF was particularly effective in rats with the most severe form of MND with forelimb onset. Using ICV delivery, VEGF is then anterogradely transported, preserving neuromuscular junctions in SOD-1 (G93A) rats. ICV delivery of VEGF in humans has not yet been tried.\(^{(154,155)}\) (Figures 10)
Figure 10: The putative role of VEGF in adult motor neuron survival. Sufficient VEGF secures neural perfusion by acting on the vasculature and exerting direct neuroprotective effects on motor neurons in subjects with high VEGF producing phenotypes (red). Decreased levels of VEGF (green) fail to do so, leading to motor neuron degeneration. Similarly low levels of VEGF in humans may increase risk of ALS in humans. (Figure courtesy of Lambrechts D et al, 2004) (155)

1.4.7.4 Angiogenin

Angiogenin (ANG) is a small polypeptide member of the RNase A superfamily that has been implicated in angiogenesis i.e. formation of new blood vessels in the body. (Figures 11 & 12) It is also an enzyme and has an amino acid sequence with 33% homology to bovine pancreatic ribonuclease (RNase A). Its’ enzymatic activity is essential for many biological functions. It can significantly impact activity of other proteins identified as important in MND pathogenesis such as IGF and VEGF. (Figure 12) The gene for angiogenin lies on chromosome 14q11.2. Analysis of the angiogenin (ANG) gene in the Irish population has demonstrated a significant allelic association with the rs11701 single nucleotide polymorphism (SNP) and has identified a novel mutation in two individuals with sporadic MND that potentially inhibit angiogenin function. The authors proposed a candidate association region for MND on chromosome 14q11.2 and suggested that other genes with similar function to angiogenin and VEGF may be important in the pathogenesis of MND. (29) Most work has been on cultured endothelial cells where it appears to regulate the transcription of other growth factors including VEGF and IGF.
These insights have primed widespread interest in developing VEGF and angiogenin-based therapies for (motor) neuron degenerative disorders, raising new hope for the treatment of MND and other neurodegenerative diseases.\textsuperscript{(154,155)}

**Figure 11:** Angiogenin Structure. (Courtesy of Dr Orla Hardiman, personal slides 2005)

**Figure 12:** Angiogenin is an upstream regulator of IGF and VEGF. (Courtesy of Dr Orla Hardiman, personal slides 2005)
1.4.8 Oxidative Stress

The effects of oxidative stress within neurons may be cumulative and injury by free radical species is a major potential cause of the age-related deterioration in neuronal function seen in several neurodegenerative diseases. There is strong evidence that oxidative stress plays an important role in the pathogenesis of motor neurone disease (MND). Point mutations in the antioxidant enzyme CuZn superoxide dismutase (SOD-1) are found in some pedigrees with the familial form of MND. Rather than resulting in a deficiency of the normal enzyme, the new mutant enzyme is thought to undergo a toxic gain of function resulting in MND. How these mutations cause the selective cell death of specific groups of motor neurones however is not yet clear. (11) A number of hypotheses have been forwarded which include:

(1) the formation of hydroxyl radicals.
(2) the catalysis of reactions of the nitrogen centred oxidant species peroxynitrite.
(3) toxicity of copper or zinc.
(4) protein aggregation.

Experimental support for these hypotheses has been produced by manipulating cells in culture to express the mutant SOD-1 proteins and by generating transgenic mice which over-express mutant SOD-1. Observations in these model systems are, in some cases at least, supported by observations made on pathological material from patients with similar SOD-1 mutations. There are reports of free radical mediated damage to neurones in the sporadic form of MND also. Alterations in the glutamatergic-neurotransmitter system are thought to play a key role in the injury to motor neurones in sporadic MND. Important subcellular targets are preferentially impaired within motor neurones during the process, including neurofilament proteins and mitochondria. (156-168) Mitochondrial energy metabolism dysfunction, free radical production and glutamate receptor activation have all been implicated as key players in MND associated neurodegeneration.

Spinal motor neurones are also known to lack certain calcium buffering proteins making them particularly vulnerable to calcium related injury. (11,48-50) In contrast the oculomotor
neuron group which are involved in eye movement express calcium-buffering proteins and are less vulnerable to calcium mediated toxicity. Mitochondria produce and are targets of reactive oxygen species and are particularly prevalent in neuromuscular tissues. They are a key player in free radical generation within neurons and are an important intracellular calcium storage area. Mitochondrial free radical production increases in conditions of increased calcium and glutamate exposure. Free radicals cause damage at both the protein and DNA levels in mitochondria by inhibiting key mitochondrial enzymes of which NADH dehydrogenase, ATPase and succinate dehydrogenase are most susceptible to damage. Mitochondrial function deteriorates over time and mutations in mitochondrial DNA increase with age, possibly explaining in part the late onset of most neurodegenerative conditions. Reduced respiratory chain complexes I and IV activity (figure 13) during normal neuronal ageing has been shown in both brain tissue and muscle. Reduced mitochondrial efficiency may be apparent until energy production falls below a threshold level, at this stage the neuron is probably more susceptible to excitotoxicity and other injurious processes. In MND mitochondrial abnormalities such as vacuolated and dilated mitochondria with disorganized cristae and inner mitochondrial membrane defects are observed in both sporadic and familial cases.
Figure 13: The Mitochondrial Respiratory chain transports electrons along a series of protein complexes on the inner membrane of the mitochondrion thereby producing energy in the form of ATP. (Courtesy of google images from MIT OpenCourseWare, 2007.)

Excessive glutamate production, calcium alterations and free radical production will all contribute to respiratory chain dysfunction and damage or death to the motor neuron. ‘Excitotoxicity’ was a term developed by Olney\(^{(171)}\) to describe the unregulated action of an excitatory neurotransmitter such as glutamate in the CNS resulting in neuronal cell injury and death. There are many pathways through which this may occur but it is now known that excess glutamate can exert its’ effects both acutely and more chronically.\(^{(172-174)}\) There are two distinct phases involved, firstly a depolarisation mediated influx of Na\(^+\), Cl\(^-\), H\(_2\)O causing neuronal swelling. This phase is reversible.\(^{(175)}\) Secondly there is a Ca\(^{2+}\) influx either directly through calcium permeable NMDA or AMPA receptor ion channels or indirectly via activation of voltage gated calcium channels.\(^{(175)}\) Excessive activation of glutamate receptors results in destabilisation of intracellular calcium homeostasis, triggering a cascade of biochemical events leading to neuronal damage via calcium mediated enzyme systems and free radical generation.\(^{(86)}\) Secondary excitotoxicity may also occur as a result of the compromise of cellular energy processes such as glucose metabolism, mitochondrial function, ATP production and ATPase function. These changes in cellular homeostasis alter the resting membrane potential and trigger excitotoxic injury via NMDA receptor overactivation even in the presence of normal glutamate levels.\(^{(176-178)}\)

Activation of glutamate receptors generates free radicals via calcium dependant activation of the arachidonic acid cascade, calpain and nitric oxide synthase.\(^{(173)}\) Activation of both NMDA and non NMDA receptors has been well documented to produce superoxide free radicals.\(^{(174,179)}\) Resultant oxidative stress causes dysfunction directly of essential cell organelles and macromolecules. Glutamate itself can reduce ability of neurons to survive oxidative stress by depleting glutathione levels.\(^{(180)}\) Post mortem specimens from MND patients show reduced CNS levels of glutamate in spinal
cord and brain samples. Although elevations in glutamate levels in CSF samples from MND patients have also been demonstrated by several groups, although other studies have failed to replicate these findings. It appears that CSF glutamate levels can vary widely among MND patients and even controls and according to age and gender. CSF samples taken from MND patients have been shown to be toxic to cultured motor neurons via activation of non-NMDA glutamate receptors. Reports on fasting serum glutamate levels have been conflicting.

1.4.9 Alteration in Hypoxia Response

Angiogenesis describes a process of endothelial cell stimulation to form new blood vessels and is critical for maintenance of tissues and support of growth. Angiogenin is a 123 amino acid protein similar in structure to pancreatic ribonucleases. Both VEGF and Angiogenin mRNA are present in nervous tissue and are potent stimulators of angiogenesis, vascular permeability as well as neuroprotective agents. Knockout mice deficient in VEGF develop a syndrome similar to adult onset motor neuron disease. A major inducer of Angiogenin and VEGF is tissue hypoxia hence the use of the term hypoxic inducible factors to encompass angiogenin, VEGF and several other peptides with similar functions. Analysis of the angiogenin (ANG) gene on chromosome 14q11.2 in the Irish population had demonstrated a significant allelic association with the rs11701 single nucleotide polymorphism (SNP) and has identified a novel mutation in two individuals with sporadic MND that potentially inhibit angiogenin function. The authors proposed that other genes with similar function to VEGF may be important in the pathogenesis of MND. These insights have primed widespread interest in developing VEGF and angiogenin-based therapies for (motor) neuron degenerative disorders, raising new hope for the treatment of MND and other neurodegenerative diseases.

1.5 Clinical Features of MND

MND typically involves a combination of upper and lower motor neuron symptoms and signs presenting in an asymmetric and progressive fashion. (Table 3)
Table 3: Features of Upper and Lower Motor Neuron Dysfunction

<table>
<thead>
<tr>
<th>Lower motor neuron dysfunction</th>
<th>Upper motor neuron dysfunction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Symptoms</td>
<td>Symptoms</td>
</tr>
<tr>
<td>Weakness</td>
<td>Stiffness</td>
</tr>
<tr>
<td>Cramps</td>
<td>Poor coordination</td>
</tr>
<tr>
<td>Muscle twitching</td>
<td>Slowing down</td>
</tr>
<tr>
<td>Fatigue</td>
<td>Weakness</td>
</tr>
<tr>
<td>Poor coordination</td>
<td></td>
</tr>
<tr>
<td>Signs</td>
<td>Signs</td>
</tr>
<tr>
<td>Weakness</td>
<td>Spasticity</td>
</tr>
<tr>
<td>Muscle atrophy</td>
<td>Hyperreflexia</td>
</tr>
<tr>
<td>Fasciculations</td>
<td>Weakness</td>
</tr>
<tr>
<td>Hyporeflexia/areflexia</td>
<td>Babinski sign</td>
</tr>
<tr>
<td>Hypotonia</td>
<td>Hoffman sign</td>
</tr>
</tbody>
</table>

The El Escorial diagnostic criteria\(^{(199)}\) aid in the diagnosis of MND and will be discussed in more detail in a later section. (Table 4)
Table 4: El Escorial Diagnostic Criteria for MND (including Arlie House Revisions)

<table>
<thead>
<tr>
<th>Clinically Definite MND</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Presence of UMN and LMN signs in the bulbar region and at least two spinal regions, or</td>
</tr>
<tr>
<td>• Presence of UMN signs in two spinal regions and LMN signs in three spinal regions.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Clinically Probable MND</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Presence of UMN and LMN signs in at least two regions with some UMN signs rostral to (above) the LMN signs.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Probable MND-Laboratory supported</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Clinical evidence of UMN and LMN signs in one region only, or</td>
</tr>
<tr>
<td>• UMN signs in one region only and LMN signs defined by EMG criteria in at least two limbs, but only when neuroimaging and clinical laboratory evidence for other causes is lacking.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Clinically Possible MND</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Presence of UMN and LMN signs in one region only.</td>
</tr>
<tr>
<td>• Presence of UMN signs alone in two or more regions.</td>
</tr>
<tr>
<td>• Presence of LMN signs found rostral to UMN signs.</td>
</tr>
</tbody>
</table>

Patients may present initially with predominantly bulbar or predominantly limb symptoms & signs. (Figure 14) Rarely a patients’ first presentation is with early respiratory failure.\(^{(194)}\) One of the most important features required to diagnose MND is the progression of disease with time. In a patient who fails to progress the diagnosis should be reviewed.

The disease may be divided into two subgroups according to site of onset of disease or first symptom: spinal onset (SO) or bulbar onset (BO). Rarely it is not possible to differentiate site of onset or both sites occur together and this is referred to as ‘generalised onset’ (GO). The subdivision between SO and BO disease has important management and prognostic implications for the patient. Approximately 2/3 of patients develop spinal onset disease which in general has a better prognosis. The common
presenting symptom for these patients is distal, asymmetric weakness or incoordination i.e. tripping, dragging one foot, difficulty with closing buttons, turning keys, performing fine motor tasks. Occasionally cramping or twitching of muscles or loss of muscle mass, especially the first dorsal interosseus muscle brings the patient to see a physician. Absence of sensory symptoms and sparing of oculomotor and bladder function are supportive of a diagnosis of MND and aid the physician in ruling out other lower motor neuron diseases.

Approximately 1/3 of patients present with bulbar symptoms such as dysarthria, hypophonia or dysphagia, nasal regurgitation or drooling. Dysfunction may be predominantly upper or lower or a mixture of both. Pseudobulbar palsy (upper motor neuron) is characterised by a spastic dysarthria, dysphagia and spontaneous and often inappropriate laughing or crying known as “pseudobulbar affect”. On examination these patients have a small spastic tongue, slow tongue movements, exaggerated gag reflex and a brisk jaw jerk. Pure bulbar dysfunction (lower motor neuron) is rarer, these patients have a flaccid, fasciculating tongue, diminished or absent gag reflex and normal or absent jaw jerk. Patients with bulbar onset disease have a worse prognosis than spinal onset disease although they may conversely benefit more from riluzole therapy.  

Patients with upper limb or bulbar involvement often develop weak respiratory musculature resulting in breathlessness especially when supine (if there is diaphragmatic weakness). Symptoms of early respiratory decline include poor sleep pattern, headaches on awakening, excessive daytime somnolence and poor cough. Over time MND progresses to involve contiguous body parts resulting in increasing disability and eventually many patients become bedbound. Death occurs within 2-5 years from pneumonia related to neuromuscular respiratory failure.
Figure 14: Classic Amyotrophy of Forearm and Intrinsic Hand Muscles seen in MND. (Courtesy of Anonymous www.neuromuscular.wustl.edu, 2008.) (9)

1.5.1 Cognitive Impairment in MND

Traditionally it was believed that MND patients did not have cognitive problems, we now know that this is not the case. A spectrum of executive dysfunction exists from mild to a severe fronto-temporal dementia syndrome. This may precede, occur concurrently or follow diagnosis with MND. There is considerable overlap in pathological findings between MND and FTD which have been mentioned in the section on pathology. Estimated prevalence of cognitive impairment in MND varies greatly. Frank FTD is found in 5-10% of cases. (55, 197-99) (Table 2)

FTD/FTLD was previously called Pick’s disease and is the second most common of all the dementias. Three forms of FTD were defined by the Lund and Manchester consensus criteria in 1994 and the Neary criteria in 1998. (200, 201) The form most frequently described in patients with MND is frontal variant frontotemporal dementia (fvFTD). The other two variants of FTD are non-fluent progressive aphasia (characterised by progressive loss of
vocabulary and language impairment) and semantic dementia (characterised by progressive loss of conceptual knowledge). FvFTD causes predominantly behavioural changes or altered personality, other features vary depending on the specific areas of the brain involved. Patients may variably become disinhibited, fatuous, purposely overactive, easily distracted, socially inappropriate or exhibit an apparently callous disregard for others. In contrast other patients become apathetic and mentally rigid, lacking in volition and mental effort.\(^{55}\) The nomenclature for this area is still developing and can be quite confusing. A new classification system for MND in association with cognitive impairment was proposed in 2007 in Canada by a panel of experts because of the increasingly complex crossover: between clinical, genetic and pathological findings reported in the literature. (Table 2)\(^{61}\)

Ubiquitin inclusions, the typical pathological finding in MND, have also been described in cases of FTD associated with MND and a proportion of pure FTD patients at autopsy (not previously known to have MND).\(^{201-204}\) These cases are labelled ‘motor neuron disease inclusion dementia’ (MND-ID).\(^{205}\) An FTD with tau negative neurofilament immune reactive inclusion bodies (NIBD) has also been described.\(^{206}\) In addition, a family with MND/ALS, Parkinsonism and FTD has been linked to chromosome 17q21-22 with a mutation on the tau gene. This has led to the concept that FTD, parkinsonism and MND may all be part of a group of disorders called ‘tau-opathies’.\(^{207-208}\) A clinically similar but otherwise distinct disease complex of dementia, parkinsonism and MND has been well documented on the island of Guam and in the Kii peninsula in Japan. This western pacific variant of MND was first reported after World War II and is the prototypic example of MND with cognitive impairment. In this condition 50% of the siblings of the proband cases developed parkinsonism and dementia, 25% developed MND and 5% develop parkinsonism, dementia and ALS/MND together.\(^{209-213}\) A further kindred with FTD associated motor neuron disease has also been described and localized to chromosome 9q21-q22.\(^{214}\) These cases did not have parkinsonism and were referred to as FTD-ALS cases. Frontally predominant pathologically proven Alzheimer’s’ type dementia preceding MND (mimicking FTD clinically) has also been reported in a Japanese MND cohort. It is not clear whether this is merely a chance association given
the increased prevalence of both MND and Alzheimer’s disease in older populations. These complex cases with mixed signs are rare and account for only a very small proportion of MND patients. FTD in association with corticospinal disturbances, muscle wasting and fasciculations is a rare phenomenon in the general population. \(^{(59)}\)

1.6 Diagnostic Criteria

The diagnosis of MND is made clinically using the revised El Escorial Diagnostic Criteria (EEDC) \(^{(192)}\). (Table 4) The EEDC were initially devised in 1994 by the World Federation of Neurology Research Group on Neuromuscular Diseases and Subcommittee on MND/ALS and revised in 1998 at a meeting in Arlie House, Virginia. Standardised diagnostic criteria are necessary to improve accuracy of diagnosis and epidemiological studies of the disease. Early and accurate diagnosis allows the patient and their family to adjust to the diagnosis and to have the option of potentially disease modifying procedures or drugs at a time when they may exert the greatest benefit. Standardised criteria also allow clinical and genetic comparison between groups of patients from different countries and backgrounds. Because the diagnostic criteria were only standardised in 1994 it is difficult to interpret studies prior to this which may have included patients with MND mimic syndromes. \(^{(123,216)}\)

The diagnosis of MND requires evidence of LMN degeneration by clinical, electrophysiological or neuropathological examination and evidence of UMN degeneration by clinical examination with progressive spread of symptoms or signs within a region or to other regions by history or examination. There must be absence of electrophysiological and pathological evidence of another disease process which could explain the clinical and electrophysiological findings. Neuroimaging in particular should be performed of the brain and cervical spine to outrule an alternative disease process.

According to the EEDC patients are initially classified according to the likelihood of diagnosis of MND, which may increase as the disease progresses. These criteria were validated by Chaudhuri et al in 1995. \(^{(217)}\) Lambert and Mulder in 1957 had formulated
electrophysiological study criteria used to diagnose MND, in practice these are used as supportive evidence for clinical findings rather than alone to make the diagnosis. (218) (Table 5) In the revised EEDC these criteria were modified to exclude those patients with motor conduction block as suggested by Cornblath in 1992. (219) More recently the proposed Awaji criteria allow more emphasis to be placed on the importance of spontaneous activity. As a cautionary note however although this method improves sensitivity and allows earlier diagnosis, specificity may be reduced as a consequence. These proposed criteria have not yet been widely accepted by neurophysiologists. (220)

Table 5: Electrophysiological Evidence in the Diagnosis of MND (Arlie House, 1998)

<table>
<thead>
<tr>
<th>Signs of active denervation</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Fibrillation potentials.</td>
</tr>
<tr>
<td>• Positive sharp waves.</td>
</tr>
</tbody>
</table>

Fasciculations

<table>
<thead>
<tr>
<th>Signs of chronic partial denervation</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Motor unit potentials of increased duration and amplitude with high proportion of polyphasia.</td>
</tr>
<tr>
<td>• Reduced interference pattern, usually with high firing rates, e.g. higher than 10Hz.</td>
</tr>
<tr>
<td>• Unstable motor unit potentials.</td>
</tr>
<tr>
<td>• Chronic partial denervation could also be demonstrated by other techniques, e.g. SFEMG, macro EMG, turns-amplitude analysis, quantitative MUP analysis and MUNE.</td>
</tr>
</tbody>
</table>

Features compatible with UMN involvement

| • >/=30% increase in central motor conduction time. |
| • Low firing rates of motor unit potentials on effort. |

Features suggesting other disease processes

| • Evidence of motor conduction block. |
| • Motor conduction velocity lower than 70% of lower limit of normal. |
| • Distal motor latency greater than 30% above the upper limit of normal. |
| • Abnormal sensory nerve conduction studies except in the presence of entrapment syndromes or co-existing peripheral nerve disease. |
| • F-wave or H-reflex latencies more than 30% above established normal values. |
| • Decrements greater than 20% on repetitive stimulation. |
| • SSEP latency greater than 20% of established normal value. |
| • Significant abnormalities in autonomic function or electronystagmography. |
| • Full motor unit potential interference pattern in a clinically weak muscle. |
EMG signs of LMN dysfunction should be demonstrated in at least two of the four body regions in MND: bulbar/cranial, cervical, thoracic and lumbosacral. EMG changes may antedate clinical weakness and atrophy, a function of the balance between denervation and reinnervation in a region after onset of the disease process. Spread of disease to contiguous spinal segments results in electrophysiological evidence of muscle involvement within these segments, often before clinically evident on exam. (221) Beasley (222) estimated that by the time physical weakness is detected clinically at least 50% of the motor neurons in that segment have died. EMG studies are particularly useful in aiding early diagnosis in MND and also in outruling the known MND mimic syndromes, some of which are treatable and do not carry such a grave prognosis. (223) (Table 6)

Table 6: MND Mimic Syndromes

- Spinal Muscular Atrophies - adult onset proximal SMA, Kennedy’s syndrome.
- Endocrine disease – thyrotoxicosis and hyperparathyroidism.
- Immune mediated – paraproteinaemic and multifocal motor neuropathies with conduction block.
- Exogenous Toxins – lead and mercury.
- Tumours – lymphoma and paraneoplastic neuromuscular syndromes.
- Inherited Metabolic disease – hexosaminidase deficiency.
- Autoimmune – Sjogren’s syndrome.
- Post infectious – postpolio syndrome.

At least two proximal and two distal muscles. should be tested in all four limbs by concentric needle EMG testing. The presence of fasciculations in the tongue either grossly or by EMG studies has a high specificity for the diagnosis of MND if other features are suggestive. (221-225) Denervation in trapezius muscle may also be highly supportive. (224) Important tests in the workup for MND include FBC, LFTs, ESR, SPEP, Immunoglobulins, LDH, PTH, Thyroid function testing, Autoimmune screen, appropriate
genetic/metabolic screen, lumbar puncture and MRI of brain and spinal cord. A useful algorithm is presented as figure 15 to aid in avoiding mimic syndromes as MND. (Figure 15)
1.7 Disease Variants

There are two variants of MND that deserve mention. They can make the diagnosis more challenging and the prognosis differs from common MND. PMA or primary muscular atrophy is a pure lower motor neuron form of the disease. It presents with asymmetrical weakness that may be proximal or distal often involving paraspinal or respiratory muscles early. Bulbar musculature is usually spared. Nocturnal cramping, fasciculations and pain due to immobility are common. There are no upper motor neuron signs. Course may be more rapid, identical to or slower than classic MND although in clinical studies PMA is reserved for cases with a disease duration >4 years. (227-229) Interestingly a recent small PM study of 4 MND patients and 2 PMA patients showed the presence of TDP-43 protein in the neural tissue of all of these patients and only 1/13 controls supporting the idea that these two conditions are not distinct entities but form part of a spectrum of disease. (229)

Figure 16: Primary muscular atrophy, the lower motor neuron variant of MND with prominent paraspinal and upper extremity wasting. (Courtesy of www.neuromuscular.edu, 2008, from A Manual of Diseases of the Nervous System, Gowers WR, 1888.) (9)
Patients with a pure lower motor neuron syndrome in particular should be checked for anti-GM1 antibodies (seen in treatable autoimmune mediated neuropathies such as Guillain Barre Syndrome (GBS), chronic inflammatory demyelinating polyneuropathy (CIDP) and multifocal motor neuropathy (MMN)). In the latter condition conduction block (CB) on EMG/NCS may be found, but is not always present. All of the above conditions respond variably to intravenous immunoglobulin (IVIG) and other immunosuppressive therapies. MMN with CB should always be considered in relatively young men (45-55 years) presenting with a pure lower motor neuron syndrome. It is reasonable to give a trial of treatment with IVIG if there will be delay in getting investigations. (11,223)

Primary Lateral Sclerosis (PLS) is another MND variant first described by Erb in 1875. (230) Pringle proposed diagnostic criteria in 1992 that are still relevant today. (231) It is a rare variant of MND with upper motor neuron findings only on clinical exam. It accounts for less than 2% of all MND. Occurrence in 5th decade is typical. Legs are classically affected before arms with symmetrical or asymmetrical spasticity, brisk reflexes and upgoing planters. Pseudobulbar palsy is common. There are no lower motor neuron signs present but muscle atrophy can occur late in the disease. (230-236) The sensory system, bladder and oculomotor systems are usually spared but can be affected, Pringle, le Forestier, Gordon and Chio et al all described rare involvement of all of these systems in addition to minor cramping and fasciculations in their cohorts. (231-236) Frontal cognitive deficits are relatively common but frank FTD is exceedingly rare. (237,238) The presence of a family history should be a major red flag to consider alternatives such as hereditary spastic paraparesis (HSP). (239) Imaging of brain and spine may be normal or show atrophy of the motor strip with corresponding reduced PET glucose consumption in the region of the premotor strip. (237,238) Transcranial magnetic stimulation often shows prolonged or absent cortical motor evoked latencies and central motor conduction times early in the disease. SSEPs and VEPs are typically but not always normal. (232) In Le Forestier’s study 9/20 patients had abnormal VEPs and 11/20 had abnormal SSEPs. (234) The disease is slowly progressive with survival up to 3 decades described. (232-237) The prognosis of PLS is usually better than MND therefore the diagnosis should only be made after the disease has been present for at least
3 years and with the exclusion of other more typical MND features.\(^{(233-237)}\) Even after the
diagnosis is reached periodic reassessment is advised. The development of LMN signs
(MND) has been described remotely even 27 years after onset of UMN signs and for this
reason PLS should always be classed as a variant of MND. The patient should be made
aware of the possibility of the transformation to MND at a later date.\(^{(234)}\) Pathologically
PLS demonstrates striking loss of frontal and prefrontal cortical layer 5 Betz cells with
gliosis of layers 3 and 5. Associated corticospinal tract degeneration is typical though
spinal anterior hom cells are spared.\(^{(229,238)}\)

### 1.8 Management of Disease

Disease management is aimed largely at symptom control and improving quality of life.
Riluzole, a glutamate antagonist is the only licensed treatment. It prolongs life in the
order of months.\(^{(195,239)}\) Other important treatments involve regular nutritional
assessments, early speech and language intervention, respiratory assessments,
physiotherapy, occupational assessments and counselling and support. Loss of > 10%
body weight indicates appropriate timing for considerations of gastrostomy insertion.
Gastrostomy feeding improves quality rather than quantity of life and does not prevent
aspiration. Nutritional supplementation targets dehydration, reduced energy due to poor
oral intake and rapid weight loss. Many patients spend hours trying to eat a meal because
they are aware of the important relationship between nutrition and survival. Gastrostomy
tubes free up this time to concentrate on things the patient enjoys doing but does not alter
the risk of aspiration. Speech and language therapy is extremely important with regards to
maintaining a communication pathway which needs constant reassessment. In addition
the speech and language specialist will work closely with the dietician and physician to
decide when gastrostomy feeding should be offered and to whom. Not all patients want
or are suitable for gastrostomy insertion which can be high risk in rapidly progressing
patients or those with significant respiratory compromise. Choosing who not to insert a
RIG/PEG device in is equally important and the decision often comes down to patients’
wishes and the expertise/acumen of the team involved. Alternatives are NG feeding and
palliative care. Ideally both the SALT and dietician should attend an MND specific clinic held on a regular basis approximately 2-3 monthly. (195, 241, 242) (Figure 17).

Figure 17: American Academy of Neurology Practice Parameter Update: the care of the patients with ALS; drug nutritional and respiratory therapies, 2009: Nutrition management algorithm. *E.g., bulbar questions in the Amyotrophic Lateral Sclerosis Functional Rating Scale, or other instrument. † Percutaneous endoscopic gastrostomy: rule out contraindications. ‡ Prolonged meal time; ending meal prematurely because of fatigue; accelerated weight loss due to poor caloric intake; family concern about feeding difficulties. (195)
The survival of patients' with respiratory compromise post PEG insertion is improved by periprocedural NIV. For this reason patients should all have a respiratory workup prior to insertion. A forced vital capacity (FVC) < 50%, supine FVC < 75% or a sniff inspiratory pressure (SNIP) of < 40cm H₂O is a particularly sensitive marker for respiratory failure that requires attention. (194, 195, 242) A respiratory physician should help with the provision of non invasive ventilation in this instance, though not all patients will tolerate it. Early feedback from NIV trials suggests that 4 hours a day of NIV can increase lifespan by up to a year or more in those patients who tolerate it. (194) Unfortunately those patients with bulbar disease or poor arm function can feel quite claustrophobic because of the tight mask required and tend to tolerate it poorly. It has been suggested that early use of NIV even presymptomatically may improve tolerance later in the disease. Respiratory infections should be treated in the normal manner, except with regards to intubation which should be discussed with patient and family. (195, 241, 242) (Figure 18)

Regular physiotherapy and occupational therapy review is important both for moral and to assess changing equipment requirements. The aim is to keep the patient mobile and active for as long as possible while maintaining their quality of life. Social workers and counselors are an important part of the team providing support, financial and emotional to both the family and patient. Some patients can develop low mood during the course of their illness and may require psychiatry review, medication and/or psychotherapy. (241) It is important to broach the issue of resuscitation wishes earlier rather than later to avoid inadvertent ventilation against the patients’ wishes during a crisis. It is also preferable to involve palliative care relatively early as they provide an excellent additional service with regards to pain management, symptom management and respite. Most patients would prefer to die at home, in Ireland currently many die in hospital without the involvement of palliative services. A recent international review of MND patient palliative care guidelines by an MND expert panel advocates early rather than late involvement of palliative care specialists in the management of MND. (243)
Figure 18: American Academy of Neurology Practice Parameter Update: the care of the patients with amyotrophic lateral sclerosis: drug, nutritional and respiratory therapies: Respiratory management algorithm. *Symptoms suggestive of nocturnal hypoventilation: frequent arousals, morning headaches, excessive daytime sleepiness, vivid dreams. † If NIV is not tolerated or accepted in the setting of advancing respiratory compromise, consider invasive ventilation or referral to hospice. PFTs=pulmonary function tests, PCEF=peak cough expiratory flow, NIV=noninvasive ventilation, SNP=sniff nasal pressure, MIP=maximal inspiratory pressure, FVC=forced vital capacity (supine or erect), Abnl. nocturnal oximetry=pO₂<4% from baseline. (105)
1.9 Drug Trials in MND

There have been many disappointing drug trials in MND. Agents such as gabapentin, topiramate and minocycline produced exciting effects in mouse models of disease only to fail when tried on human MND populations. More recently Lithium carbonate produced some positive results in a small human trial of 28 MND patients. There were a number of problems with the trial methods however and this data has not yet been replicated elsewhere. (Table 7)

In the USA there is a fast-tracking initiative in place to try and identify potentially therapeutic agents in MND, particularly among already existing drugs. It is debatable whether mouse models of disease have been of great benefit in this regard. Although they allow faster and safer testing of medications than if we were to use human populations we now know there are agents that appear to be effective in mice which subsequently fail in humans. It is plausible that the converse is also true, agents which are apparently useless in mice might actually be of benefit in humans. To further confound matters different trials of the same agents have produced conflicting results. IGF-1 was found to be of benefit in the USA but did not significantly alter life expectancy in the European population. A recent third trial definitively showed that subcutaneous IGF-1 is not effective in the treatment of MND. This will be discussed further in the chapter on IGF. (244) Table 7 contains a list of some recent trials that have been conducted or are ongoing at present, it is not all exhaustive and does not include therapies such as stem cells or gene therapy.
### Table 7: Drug Trials in MND (Negative trials to date = Red) (data courtesy of www.neuromuscular.wustl.edu/, 2011) (9)

<table>
<thead>
<tr>
<th>Recent Drugs trials in MND</th>
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</thead>
<tbody>
<tr>
<td>Arimoclomel</td>
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<td>Buspirone</td>
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<tr>
<td>Ceftriaxone</td>
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<tr>
<td><strong>Celebrex</strong></td>
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<tr>
<td>CL201</td>
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<tr>
<td><strong>CoQ10</strong></td>
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<tr>
<td>Creatine</td>
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<tr>
<td><strong>IGF-1</strong></td>
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<tr>
<td>Indinavir</td>
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<td>Lithium</td>
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<td>Memantine</td>
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<td><strong>Minocycline</strong></td>
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<td>Myotrophin</td>
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<td>Neurodex</td>
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<td>Oxandrolone</td>
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<td>Sodium Phenybutyrate</td>
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<td>Thrombopoietin</td>
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<td>Tretinoin &amp; Pioglitazone</td>
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<tr>
<td><strong>Vitamin E</strong></td>
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<td>Xaliproden</td>
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</table>
1.10 Future Treatment Strategies

Sporadic MND is a multifactorial disease with both genetic susceptibility factors and environmental exposures likely contributing to disease development. It seems likely therefore that in the future MND will be treated with a cocktail of neuroprotective medications rather than one curative agent. Likewise current interventions such as symptomatic management, aggressive nutritional management, gastrostomy feeding and non-invasive ventilation will continue to help improve quality of life and for some patients’ survival. Both stem cell therapy and provision of neurotrophic agents via gene transfection are still under development and offer the hope of a potential cure in the future.

1.11 Summary

MND is a disorder affecting approximately 6.2/100,000 people over the age of 15 years in Ireland at any one time. (12) The incidence of MND was 2.8/100,000 when last calculated for Ireland. (12) This is an incidence rate comparable with diseases such as Guillain Barre syndrome and Myasthenia Gravis but these disorders do not have the high mortality that is associated with MND. (245) Despite intensive research few causes or risk factors have been identified to date. There have however been significant advances in the management of MND. Whether all of the current treatment strategies under use are advantageous to the patient in advancing quality or quantity of life or both is not always clear, some treatments may even prove to be detrimental. (246) Any significant treatment advance in prolonging life should be reflected in the survival epidemiology of the Irish MND population over time. Ireland has the advantage of an established population based MND register which is highly regarded in the international MND community and is considered the gold standard for epidemiological studies in MND. The last update and review of the Irish MND population was ten years ago, a further review on this topic is therefore timely. In addition evaluation of risk factors at this juncture may provide valuable clues as to the aetio/pathogenesis of this complex disease. By identifying modifiable risk factors we may be able to alter or avoid occurrence of disease in certain
risk factors to date (male gender and smoking)\(^{246}\) reflects the need for further study in this area. New prognostic factors in MND are also highly desireable so that we may best predict the disease course of different individuals diagnosed with this heterogenous but terminal condition. In particular exercise as a potential risk factor for MND and IGF-1 levels as either a risk factor (when low) or a potential prognostic indicator were of particular interest in this study. The aims of this study were to:

- Provide a detailed overview of MND from its’ initial description by Charcot to current theories on aetiology and treatment strategies in chapter one.

- Evaluate and discuss the current epidemiology of MND in Ireland including demographics, disease subtypes, incidence, prevalence and survival in chapter two.

- Discuss current knowledge regarding risk factors for MND and prognostic indicators in this disease also in chapter two.

- Evaluate and discuss in detail exercise in MND and IGF-1 in MND in chapters three and four.

- Summarise the key findings of the studies undertaken in this M.D thesis in chapter five.
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Rothstein JD, Martin IJ, Kuncio RW. Decreased glutamate transport by the brain and spinal cord in amyotrophic lateral sclerosis. NEJM 1992;326(22):1464-8.


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Chapter Two
Epidemiology of Motor Neuron Disease

Figure 1: Geographical Map of the Republic of Ireland. Courtesy of Google Images, 2010 from Ireland-map.co.uk, (1)

2.1 Introduction

The Republic of Ireland (figure 1) is an island in Northern Europe with a population of approximately 4 million inhabitants. There are also 1.5 million inhabitants in the North of the island that form the country of Northern Ireland, which is part of the United Kingdom and has its own health system (part of the NHS) and its own government. From ancient times the entire island of Ireland was divided into 32 counties but only 26 of these remain part of the Republic of Ireland. (2) Although we will allude to some epidemiological research ongoing in Northern Ireland, the majority of the work below deals exclusively with the Republic of Ireland. Motor neuron disease (MND) also known as Amyotrophic Lateral Sclerosis (ALS) is the commonest neurodegenerative disorder of young and middle aged adults with a reported worldwide incidence of approximately 2.4/100,000 population (3). The incidence and prevalence of sporadic MND has traditionally been
described as similar in all races and all countries worldwide. Over the past decade we have realized that this may not in fact be the case. There may be wide regional variations in incidence and prevalence and in some areas such as Africa we have very little data available. (4-6) Because AIDS and famine has devastated most countries in sub-Saharan Africa many generations of Africans die at a young age before reaching the age group that MND typically occurs in. The importance of conducting accurate epidemiological studies cannot be underestimated. They allow us to establish the incidence and prevalence rates of disease so that they may be tracked over time. Improvements in prevalence without change in incidence provides supportive evidence of improved survival with interventional treatments. Changes in incidence may suggest important environmental factors that may predispose to disease. Alterations in the environment may reduce the incidence of disease as with smoking and lung cancer. Epidemiology also helps health services to plan and budget with regards to future monetary needs in different diseases. A positive change in collection of epidemiological data over the last decade has been the establishment of a number of population based prospective registers predominantly in European populations. Ireland was second only to Scotland in establishing an MND population based register. These registers have produced interesting data suggesting that the frequency of disease may actually vary between regions and from male to female according to the population being examined and confirm that MND is more heterogeneous worldwide than we first thought. (7-12) The accuracy of data has also been greatly improved by the establishment of internationally recognized diagnostic criteria however even with the EEDC there are a number of mimic syndromes that still make the accurate diagnosis of MND challenging. (13-15)

2.2 Aims

The aims of our studies were as follows:

2.2.1 Study 1

1. To examine whether there has been any significant change in the Irish MND incidence and prevalence figures over the last 10 years.
2. To describe the current demographic details of the Irish MND population.
3. To examine whether there has been any early change in survival since the institution of percutaneous gastrostomy and non invasive ventilation as standards of care.
4. To examine what other factors influence survival in our population.
5. To ascertain the percentage of patients with frontotemporal dementia and familial MND documented on the register.

2.2.2 **Study 2**

1. To ascertain what percentage of death certificates were available for deceased MND patients over the specified periods and to ascertain if MND was noted on the death certificate and if not what was the recorded cause of death.
2. To estimate the accuracy of death certificates in documenting MND.
3. To examine location of death of patients: at home, in a hospice, a hospital or in a nursing home and to establish if there has been a shift in location of death over time in tandem with improvements in hospice services nationally.
4. To ascertain if there is was a difference between males and females, married, unmarried, separated, divorced and widowed patients with regard to place of death.
5. To compare annual death rates released by the central statistics office with annual death figures based on the Irish MND register.
6. To aid in the establishment of a sister register in Northern Ireland and to compare early incidence figures with the Republic of Ireland figures.
7. To investigate exercise as a risk factor in MND. (This will be described in a separate chapter later)

2.3 **Methods**

2.3.1 **Study 1: Study Population**

Full details of the Irish MND register have been published by Traynor et al.\(^{(10)}\)

Established in 1993 by Professor Orla Hardiman, Dr Brian Traynor, Bernie Corr RN and the founder of the Irish MND association Eithne Frost, the first published figures from the Register appeared in 1999. The Irish MND Register has prospectively collected
observational demographic data on Republic of Ireland residents diagnosed with suspected, possible, probable or definite sporadic MND according to the EEDC \(^{13, 14}\) since 1993. Ascertainment was only considered to be acceptably high for study by 1995. Relevant data on mimic syndromes such as Kennedy’s disease and also familial MND with or without cognitive disturbance are also now being stored in the register, although this is a relatively recent adaptation. \(^{10}\) Since 1999 the Register has been the source of numerous publications on the epidemiology, genetics, psychology, endocrinology of MND and also the availability and quality of health care resources for MND patients.

The MND Register database is stored on a locked computer in Beaumont hospital research facility. Each year a nominated person, usually the research fellow or Nurse is responsible for collecting and updating the information. Data is identified from multiple intersecting sources. Most neurologists and support care services such as physiotherapy, speech and language therapy and occupational therapy services in Ireland are aware of either the MND register or the Irish MND association. Referrals to the Register come directly to the Register via email, telephone or letter and via the Irish MND association. The Irish MND Register data included in this study came from seven main sources:

1. The patient themselves or a family member.
2. General practitioner.
3. Hospital Consultant including other neurologists.
5. Paramedical staff i.e. occupational therapy, physiotherapy, speech and language therapy, dieticians, social workers.
6. The Irish Motor Neuron disease association.

Once a patient is flagged as potentially having MND that patient is reviewed at the MND specialty clinic in Beaumont Hospital, Dublin to confirm and classify the certainty of diagnosis. If this is geographically not possible the patient is preferably reviewed in the community by a neurology Specialist Registrar or Specialist Nurse and/or their medical
records are also reviewed at the local hospital. The patient is assigned a certainty of
diagnosis and followed over time until they are either deceased or placed on a ventilator.
Demographic and clinical data is entered into the Register database on a regular basis by
the delegated Research Fellow or Specialist Nurse. If a patient exhibits atypical symptoms
or signs, investigations are repeated until all mimic syndromes have been excluded.
Because the Republic of Ireland is an island with less than thirty neurologists and less
than five neurophysiologists case under-ascertainment was felt to be minimal using this
population based technique. (10) The methods used for data collection are time consuming
and labour intensive but without a centralised computerised patient record service this is
currently the best method available. Hospital inpatient data is frequently inaccurate and
unreliable and only accounts for those patients who are admitted to public hospitals, it
could not be used as a stand alone record of MND cases in Ireland. In addition there is
currently no equivalent Irish health service initiative with regards to outpatient services.
The Irish MND Register is based on a sister Register in Scotland established by Professor
Swingler in 1989. Using similar methods of collection and using capture recapture
techniques they have reported an ascertainment rate of 98%. (16)

At time of data analysis there were more than 1000 patients on the Irish Register. Case
ascertainment was estimated to be close to 100% although this had never been formally
assessed. Sister population based MND Registers in Scotland and Northern Ireland using
capture recapture techniques have reported very high ascertainment rates of 98-99%. (17, 
18) Due to close link up of services at the time of analysis and lack of independent
resources/lists of patients we were unable to perform the capture recapture technique to
confirm high case ascertainment formally. The high inter-reliability of the services and
their patient lists became apparent during this study and as a result steps have now been
taken to make separate service lists independent in the future so that capture recapture
techniques may be used at the next analysis of the Irish MND population. Data produced
from the register at the time of this study has been used to define incidence and
prevalence figures, to analyse subtypes of disease and survival times over the last decade.
At the time of study smoking status and exposure to previous trauma were not completed
consistently on the majority of patients in the database.
MND epidemiological data from the three year period January 1st 1995 to December 31st 1997 has previously been published.\(^{(10)}\) Because a decade has elapsed since this study and new treatments and management strategies for MND have been developed since this time, a review of the Irish MND population is timely. The main register-based studies of MND incidence that have been published to date are based on the Caucasian populations of Europe and North America.\(^{(7,9-10,19-23)}\) A recent new initiative to pool European register data into one large register called EURALS was commenced approximately four years ago. This will include register data from Italy, Scotland, Ireland and England and clinic based data from Russia, Serbia, London, Madrid, Limongen and Israel.\(^{(24)}\)

The Northern Ireland MND Register commenced on 1\(^{st}\) August 2004 by Dr Victor Patterson and Dr Colette Donaghy with cooperation from the Republic of Ireland Register and using identical computer programs. The two registers are kept separately given that they represent two different countries with differences in demographics, governance and health systems (NHS in Northern Ireland). There is however the potential to produce combined figures for the entire island of Ireland and comparative figures once the Northern Ireland register has been established for a prolonged period. Initial results from this new register will briefly be presented but are the subject of another MD thesis.\(^{(17,25)}\)

Statistical Analysis
We collected and examined epidemiological data on Irish MND patients during the three year period from January 1\(^{st}\) 2002 to December 31\(^{st}\) 2004 and compared this to the previously published data. Census values from the year 2002 were provided by the Irish Central Statistics Office. SPSS version 15.0 was used for statistical calculations. Crude incidence rates were calculated as observed number of new cases during the six years of study (1995-1997 and 2002-2004) divided by the total Irish population during the same periods. Crude prevalence rates were calculated as the observed number of cases with MND during the six years of study divided by the total population during the same periods. Prevalence rates were assessed on the 31\(^{st}\) December at midnight. 95%
confidence intervals were calculated for both populations. (CSO, National Census 1997 & 2003) (26, 27) As the EEDC specifically excludes patients < 15 years from a diagnosis of MND, figures were calculated using both entire population and population > 15 years as the denominator. To do this we subtracted the number of people < 15 years in the population from the total population and used this as the new denominator. Age specific rates are always higher than general population or crude rates because the denominator is smaller. Age adjusted rates are important to calculate in MND not only because of the diagnostic criteria excluding children, but also because the population in Ireland is under immense change. If we compare the population distribution by age over the last decade we see a population bulge developing among young people in their 30s and 40s. (Figure 2) This age group make up the main percentage of the population and therefore the denominator when calculating the age adjusted MND incidence and prevalence rates. This young group are not the typical age group that get motor neuron disease. This could inadvertently cause artificially low crude incidence and prevalence rates of disease.

Figure 2: Population bulge in Irish population. There has been a significant change in the Irish population since the ‘baby boom’ of the 1950s. This will result in an increasing
elderly population over the next 3 decades, the population most at risk of MND. Courtesy of the Irish Central Statistics Office, 2008.(2)

In addition because of significant immigration to Ireland over the last decade (226,100 in the last 9 years, 2002 population = 3,917,203) in order to analyze the temporal trend of MND in the face of shifting demographics, incidence rates for the 2002-2004 cohort were age- and gender-adjusted to the 1996 Irish population using the direct method. In addition age specific average rates of disease over the two periods studied were compared. The denominator calculation of prevalence rates were the Irish population on December 31st, 1996 and 2003 taken from Irish census data. Comparisons were made between both total and age specific groups using the Student T-test. Survival was estimated by the Kaplan Meier method using time from date of diagnosis to death or tracheostomy (1995-1997, n = 2; 2002-2004, n = 2) or to last follow up and results were compared using Univariate analysis. We also evaluated the entire MND population over both periods as incident and prevalent cohorts by age, gender and site of onset of disease: bulbar or spinal. The prevalent cohort includes everyone in the study population with MND (both new and existing cases) during both periods, the incident population includes only those persons who were newly diagnosed with MND during the period of study. Incident and prevalent populations included cases that were neither dependent nor independent making statistical analysis difficult. The data produced is therefore largely descriptive apart from Kaplan Meier curves which are plotted together for comparison.

2.3.2 Study 2: Study population
This was a retrospective observational study. Death certificates were sought on all MND cases on the Irish MND Register as of Jan 1st 2006 from the Irish Register of Births, Deaths and Marriages. This included cases from 1995-2004 and results were compared between 1995-1999 and 2000-2004 hereafter called period 1 and period 2 respectively. The Central statistics office (CSO) was able to provide anonymised statistics on deaths from MND from the period 2002-2004 for comparison.

Statistical Analysis
Microsoft excel 2007 statistical packages was used for all statistical calculations and illustrative graphs/charts.

2.4 Results

2.4.1 Study 1

Over the ten year period and in particular during the six years of study (1995-1997 and 2002-2004) 465 Irish residents were diagnosed with suspected, possible, probable or definite MND. Of these 29 (6.2%) had familial MND based on a detailed family history and 32 cases (6.9%) had clearly documented frontotemporal dementia according to the Manchester-Lund criteria. (1994) Demographics and clinical features of Irish patients with MND did not change significantly between the 1995-1997 and the 2002-2004 cohorts. (Table 1) Demographics of 'incident' and 'prevalent' cohorts did demonstrate some significant differences. (Table 2) The median ages of onset of MND were 65.7 and 66.1 years between 1995-1997 and 2002-2004 respectively. Male to female sex ratios were 1.4:1 in both eras. Peak incidence of MND in females was the 65-70 year age group whereas in males peak incidence was 75-80 years of age. (Figure 3)
<table>
<thead>
<tr>
<th></th>
<th>1995-1997 (n = 231)</th>
<th>95% CI</th>
<th>2002-2004 (n = 465)</th>
<th>95% CI</th>
</tr>
</thead>
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<tr>
<td>Median Age</td>
<td>65.7</td>
<td></td>
<td>66.1</td>
<td></td>
</tr>
<tr>
<td>Male:Female</td>
<td>133:98</td>
<td></td>
<td>135:99</td>
<td></td>
</tr>
<tr>
<td>Limb:bulbar:GO</td>
<td>129:80:22</td>
<td></td>
<td>139:84:11</td>
<td></td>
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<tr>
<td>Family Hx MND</td>
<td>9</td>
<td></td>
<td>20</td>
<td></td>
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<tr>
<td>+ FTD</td>
<td>4*</td>
<td></td>
<td>28</td>
<td></td>
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<tr>
<td>Mean diagnostic delay(mths)</td>
<td>♥10.5</td>
<td></td>
<td>15.4</td>
<td></td>
</tr>
<tr>
<td>Median Survival(mths)</td>
<td>17.2</td>
<td></td>
<td>16.1</td>
<td></td>
</tr>
<tr>
<td>Incidence per 100,000 py</td>
<td>2.1</td>
<td>1.8,2.4</td>
<td>1.9*</td>
<td>1.5,2.4</td>
</tr>
<tr>
<td>Incidence per 100,000 py &gt;15 years</td>
<td>2.8</td>
<td>2.4,3.1</td>
<td>2.5*</td>
<td>2.2,2.8</td>
</tr>
<tr>
<td>Female incidence per 100,000 py</td>
<td>1.8</td>
<td>1.4,2.1</td>
<td>1.6</td>
<td>1.3,2.0</td>
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<tr>
<td>Male incidence per 100,000 py</td>
<td>2.5</td>
<td>2.0,2.9</td>
<td>2.3</td>
<td>1.9,2.7</td>
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<tr>
<td>Bulbar incidence per 100,000 py</td>
<td>0.9</td>
<td>0.8,1.1</td>
<td>0.8</td>
<td>0.6,1.0</td>
</tr>
<tr>
<td>Limb incidence per 100,000 py</td>
<td>1.2</td>
<td>1.0,1.4</td>
<td>1.2</td>
<td>1.0,1.4</td>
</tr>
</tbody>
</table>

Py = person years  GO = generalised onset

*Age & gender adjusted to the Irish population

† The Irish MND Register did not routinely collect cognitive data until 2000

♥ t Test statistic = -3.2, p = 0.001
Table 2: Demographics and Clinical Features of Irish Patients Diagnosed with MND in Incident and Prevalent Cohorts

<table>
<thead>
<tr>
<th></th>
<th>Incident cohort (n = 465)*</th>
<th>Prevalent cohort (n = 280) ♦</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age (years)</td>
<td>66.0</td>
<td>61.8</td>
</tr>
<tr>
<td>Male:Female (%)</td>
<td>268(57.6):197(42.4)</td>
<td>120(60.0):80(40.0)</td>
</tr>
<tr>
<td>Limb:bulbar:GO(%)</td>
<td>268(57.6):164(35.3):33(7.1)</td>
<td>137(68.5):63(31.5):0</td>
</tr>
<tr>
<td>Patients &lt; 55 years</td>
<td>93(20.0)</td>
<td>67(33.5)</td>
</tr>
<tr>
<td>Patients &gt; 65 years</td>
<td>244(52.5)</td>
<td>74(37.0)</td>
</tr>
<tr>
<td>Fam Hx MND</td>
<td>29(6.2)</td>
<td>11(10.5)</td>
</tr>
<tr>
<td>+ FTD</td>
<td>32(6.9)</td>
<td>12(6.0)</td>
</tr>
<tr>
<td>Mean diagnostic delay</td>
<td>13.0</td>
<td>22.7</td>
</tr>
<tr>
<td>Median survival (mths)</td>
<td>16.4</td>
<td>120.9</td>
</tr>
</tbody>
</table>

♦ on December 31st 2003

Figure 3: Age and gender specific incidence rates of ALS/MND in Ireland, 1995-1997, demonstrating male > female incidence and differing peak incidences between sexes. (2002-2004 graph is almost identical, see below)
Figure 4: Total age specific incidence rates of ALS/MND in Ireland, 1995-1997 and 2002-2004, demonstrating virtually identical graphs with a peak in the 65-80 year age groups, as would be expected in MND.

The peak incidence of total MND and both bulbar- and limb-onset disease is between 65-80 years. The total MND age specific rates (average incidence rate over each 3 year period) are virtually identical. (Figure 4) Examining subtypes of disease bulbar-onset MND peaked at approximately 65-70 years whereas limb onset in females peaked at 65-70 years compared with males at 75-80 years. (Figure 5) Limb: bulbar: generalized onset ratios were 5.9: 3.6: 1 and 12.6: 7.6: 1 respectively. Mean times from onset of symptoms to diagnosis of MND were 10.5 and 15.4 months respectively. Only 9 patients in 1995-1997 compared with 20 patients in 2002-2004 had a family history of MND.

Based on 465 newly diagnosed cases the crude annual incidence rate of MND in Ireland was 2.1 (95% CI: 1.8-2.4) and 1.9 (95% CI: 1.5-2.4) per 100,000 persons from 1995-1997 and 2002-2004, the average over the entire period being 2.0 cases per 100,000 person years. (95% CI: 1.9-2.2) The incidence rates for the population older than 15 years were 2.8 (95% CI: 2.4-3.1) and 2.5 (95% CI: 2.2-2.8) per 100,000 person years, the average value was 2.6 per 100,000 person-years (95% CI: 2.4-2.9). MND incidence remained relatively stable over the decade 1995-2004.
Figure 5: Age- and gender-specific incidence rates of bulbar- and limb-onset MND in Ireland, demonstrating male preponderance for both bulbar and spinal onset disease and later peak age among males for both subtypes, 2002-2004.

Incidence figures differ among the sexes. During the initial period of study female incidence was 1.8 (95% CI: 1.4-2.1) and the later period 1.6 (95% CI: 1.3-2.0) per 100,000. Males consistently have higher incidence figures with the 1995-1997 figures at 2.5 (95% CI: 2.0-2.9) and the 2002-2004 figures at 2.3 (95% CI: 1.9-2.7) per 100,000 respectively. The incidence of bulbar-onset disease was identical among males and females (0.9 per 1000,000 person years, 95% CI: 0.7-1.0) during both periods. In contrast, the male incidence of limb-onset disease was twice that of females (1.5 per 100,000 person years (95% CI: 1.3-1.7) versus 0.8 per 100,000 person years (95% CI: 0.7-1.0). Women had similar incidence rates for both limb-onset and bulbar-onset disease. These findings were consistent throughout all age groups.

Regardless of gender the incidence of bulbar-onset disease was lower than that of spinal-onset disease. During the period 1995-1997 overall bulbar incidence was 0.9 (95% CI: 0.8-1.1) per 100,000, later from 2002-2004 the incidence was virtually identical at 0.8 (95% CI: 0.6-1.0) per 100,000. Corresponding overall limb-onset MND figures were 1.2 (95% CI: 1.0-1.4) for both eras. (Figure 5)
The prevalence rate of MND on December 31st, 1996 was estimated as 6.2 per 100,000 population over the age of 15 years (n= 172, 95% CI: 5.3-7.1). The prevalence rate on December 31st 2003 was 6.4 per 100,000 population (n = 200, 95% CI: 5.5-7.2) The clinical features of the prevalent and incident cohorts differ considerably. (Table 2) For example, bulbar-onset disease accounted for less than one third of overall prevalence rate (2.0/6.6 per 100,000 i.e. 31.3%), but formed a larger percentage of incidence rate (1.1/2.6 per 100,000 i.e. 42.3%).

Median survival among Irish MND patients was 16.4 months from the time of diagnosis. Incident and prevalent population survival times were different (16.4 Vs 120 months). (Figure 6) Prognosis did not alter significantly over the decade despite the introduction of gastrostomy and non invasive ventilation use (figure 7, logrank = 0.376, p = 0.54) and regardless of site of symptom onset although there was a trend towards shorter survival with bulbar onset disease. (figure 8, logrank = 0.281, p = 0.60)

Figure 6: Diagrammatic representation of incident and prevalent population Kaplan Meier curve survival over time, survival times in prevalent population were greater than incident population.
Figure 7: Kaplan Meier survival curves for MND cohorts (1995-1997) and (2002-2004), there has been little change in survival between the 2 periods studied.

Figure 8: Kaplan-Meier survival curves comparing survival in the 1995 - 1997 and 2002 – 2004 cohorts of Irish patients with bulbar-onset and limb-onset MND. Limb onset disease has a better prognosis than bulbar onset disease in both time periods.

The initial incidence & prevalence figures from the sister Northern Ireland (NI)MND register have been virtually identical to the current republic of Ireland figures. From 2004-2005 there were 78 cases of MND in NI. The crude prevalence rate was estimated
at 4.6 (95% CI: 3.7-5.8) per 100,000 population. The number of new prevalent cases estimated by the capture recapture method was 23.3. The adjusted prevalence rate was therefore 6.0 per 100,000 population (95% CI: 4.9-7.3). The crude incidence rate was 1.9 per 100,000 (95% CI: 1.6-3.3) and was higher in females. Peak incidence was noted in the 70-74 year old age group in females and in the 80-84 year age group in males. 42% of incident females had bulbar onset disease as compared with only 15% of males. 80% of bulbar onset disease patients were female. Standardised incidence ratios were calculated for the 26 local government districts in NI and although the findings were non significant, there was a peak incidence in the Ards peninsula and the middle of the province next to Lough Neagh.\(^{17,25}\)

![Deaths due to MND from 2 sources](image)

**Figure 9:** No. MND related deaths by CSO and Irish MND Register data, data is consistently similar. (1999-2003)

### 2.4.2 Study 2

The sensitivity of the MND register for documenting the deaths of persons with MND as compared with the CSO records was examined from 1999-2003. (Figure 9) The Irish MND register numbers were similar to official CSO mortality data with a trend towards increased pick up by the Irish MND register in most years excepting 2002. Death
certificates were sought on all MND deaths (according to the Irish MND register) from 1995-2004 and results were compared between 1995-1999 and 2000-2004 hereafter called period 1 and period 2 respectively. There were 706 deaths in total from 1995-2004 inclusive recorded by the MND register. Death certificates were available on 540/706 patients (77%). Speculated reasons as to why death certificates were unavailable were death not registered, death abroad, death registered under a different name, lag time between death and registration of death. Of those available 262/540 (49%) patients died during period 1 and 278/540 (51%) patients’ deaths occurred during period 2. The majority of deaths from MND occurred in Leinster and Munster. (Figure 10)

![Death by province graph](image)

Figure 10: Deaths by province, the majority of MND deaths occur in Leinster and Munster

In 506/540 certificates obtained (94%) MND +/- bronchopneumonia was recorded either as the main cause of death or antecedent cause of death. In 34/540 cases (6%) the main cause of death and/or antecedent causes of death did not include MND. In 20 of these cases the main causes of death was bronchopneumonia +/- respiratory failure, with no mention of MND. Table 3 shows all the registered causes of death where MND was omitted. It should be mentioned that in many cases mechanisms rather than a condition or disease causing death have been used, which is not considered acceptable according to the death certification guidelines.
Table 3: Main Causes of Death and Locations of Death in those MND Patients without MND on Death Cert

<table>
<thead>
<tr>
<th>Cause of death</th>
<th>No. Patients</th>
<th>% Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute renal failure*</td>
<td>2</td>
<td>0.4</td>
</tr>
<tr>
<td>Alcoholism</td>
<td>1</td>
<td>0.2</td>
</tr>
<tr>
<td>CCF</td>
<td>1</td>
<td>0.2</td>
</tr>
<tr>
<td>Cerebellar atrophy</td>
<td>1</td>
<td>0.2</td>
</tr>
<tr>
<td>Cerebral anoxia*</td>
<td>1</td>
<td>0.2</td>
</tr>
<tr>
<td>Cerebral atrophy*</td>
<td>1</td>
<td>0.2</td>
</tr>
<tr>
<td>Cervical myelopathy</td>
<td>1</td>
<td>0.2</td>
</tr>
<tr>
<td>Cor pulmonale</td>
<td>1</td>
<td>0.2</td>
</tr>
<tr>
<td>CVA/stroke</td>
<td>1</td>
<td>0.2</td>
</tr>
<tr>
<td>Dementia</td>
<td>1</td>
<td>0.2</td>
</tr>
<tr>
<td>Dilated cardiomyopathy (DCM)</td>
<td>1</td>
<td>0.2</td>
</tr>
<tr>
<td>Ischaemic heart disease (IHD)</td>
<td>1</td>
<td>0.2</td>
</tr>
<tr>
<td>Left hemiparesis*</td>
<td>1</td>
<td>0.2</td>
</tr>
<tr>
<td>Multiple sclerosis (MS)</td>
<td>1</td>
<td>0.2</td>
</tr>
<tr>
<td>Multiple systems atrophy (MSA)</td>
<td>1</td>
<td>0.2</td>
</tr>
<tr>
<td>Myocardial infarction (MI)</td>
<td>4</td>
<td>0.8</td>
</tr>
<tr>
<td>Neurological insult*</td>
<td>1</td>
<td>0.2</td>
</tr>
<tr>
<td>Oesophageal carcinoma</td>
<td>1</td>
<td>0.2</td>
</tr>
<tr>
<td>Idiopathic Parkinson’s disease (IPD)</td>
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<td>0.2</td>
</tr>
<tr>
<td>Peritonitis</td>
<td>1</td>
<td>0.2</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>15</td>
<td>3</td>
</tr>
<tr>
<td>Pseudobulbar palsy</td>
<td>1</td>
<td>0.2</td>
</tr>
<tr>
<td>Respiratory failure*</td>
<td>11</td>
<td>2.2</td>
</tr>
<tr>
<td>Septicaemia</td>
<td>2</td>
<td>0.4</td>
</tr>
<tr>
<td>Septic shock</td>
<td>1</td>
<td>0.2</td>
</tr>
<tr>
<td>Urinary tract infection (UTI)</td>
<td>1</td>
<td>0.2</td>
</tr>
</tbody>
</table>

* denotes mechanisms of death rather than diseases or descriptive terms that should not be used on death certificates.

Overall the accuracy of death certificates for documenting MND as cause or antecedent cause for death in the death certificates that were available (77%) was 94%. In patients for whom a location of death was completed, there was no significant difference over the two periods 1995-1999 and 2000-2004 (p = 0.9). (Figure 11) 179/540 (33%) patients died at home. The numbers of patients who died at home overall during the two periods were 85/261 (32%) Vs 94/ 279 (34%). 257/540 (47%) patients died in acute hospital beds from 1995-2004, 130/261 (50%) during the first period and 127/279 (46%) during the latter period. In 2003 & 2004 for the first time the number of patients dying at home surpassed those dying in hospital (20 Vs 21 patients in 2003 and 26 patients each in
Hospice inpatient deaths have remained stable. 63/540 (12%) deaths occurred in the hospice inpatient setting from 1995-2004, 31/261 (12%) during period 1 and 32/279 (11%) during period 2. The minority of patients passed away in nursing homes. A total of 40/540 (7%) patients died in a nursing home representing 15/261 (6%) and 25/279 (9%) respectively during periods 1 and 2. (Figures 11 & 12) The influence of marital status on location of death is shown in figure 13.

Most MND deaths occur in hospital or at home. There has been little change in location over the time periods studied
Figure 12: Location of patient deaths by year. Most MND deaths occur in hospital or at home. There has been little change in location over the years studied.

Figure 13: Location of death and marital status. Marital status does not appear to influence location of death.

Deaths in hospices and nursing homes have been evenly matched between the sexes over the decade. Consistently more males died in hospital or at home than females, although the number of females dying at home has been increasing. (Figure 14)
Figure 14: Location of death by year and sex, sex does not appear to influence location of death

2.5 Discussion

2.5.1 MND Accuracy of Diagnosis

Prior to the establishment of international diagnostic criteria in 1994 (13) under ICD-9 all chronic progressive motor neuron disorders were grouped under one code (335.20). (28) This would have included ALS/MND, immune mediated motor neuropathies, multifocal motor neuropathies, poliomyelitis sequelae (post-polio syndrome) and other MND mimic syndromes. Beghi et al in 2001 demonstrated that using hospital discharge coding (based on ICD-9) up to 40% apparent cases of MND actually had mimic syndromes or other disorders. The overall incidence rate of MND in Lombardy for that period using hospital based data was 2.1 per 100,000 but dropped to 0.8 per 100,000 when false positives were excluded and false negatives included. By using the local MND register which relied on data from multiple sources in addition to hospital data the actual incidence rate was estimated at 1.6 per 100,000. Thus hospital based data alone using ICD-9 had a tended to overestimate incidence. (29) This problem was partially but not completely rectified by segregation of many different motor neuron disorders under ICD-10. Epidemiological
studies prior to the 1990s included a heterogeneous mix of cases labeled MND and so must be interpreted with caution.

From the 1994 onwards the EEDC made MND diagnosis more accurate and standardized. (13) Later at Arlie House in 1998 supportive electrophysiological criteria for MND were further defined. (14,30) Awajii modification of Arlie House criteria have lately been proposed but are not yet widely adopted. (31) Clinicians still rely heavily on the EEDC whose sensitivity, specificity and reliability have been well documented. (32-33) Neuropathological features and exclusion criteria for MND are criteria for the diagnosis of MND kindreds have also been published. (34) Failure to completely outrule other causes of MND before applying EEDC leads to approximately 7% of cases being rediagnosed later with MND mimic syndromes. (15) Beghi et al demonstrated the importance of clinical examination of the patient and familiarity with applying the EEDC in making an accurate diagnosis of MND. (35) Makki et al, recently assessed the usefulness of electromyographic MND diagnostic criteria in the revised EEDC criteria. They reported the highest sensitivity and specificity for diagnosis required EMG changes in two segments with abnormalities in a single muscle in the cranial and thoracic segments and abnormalities in two muscles in the cervical and lumbar sacral regions. These findings reaffirmed use of the revised EEDC EMG criteria to support accurate and early diagnosis of MND. (36) In the future addition of other specialized imaging techniques such as MRI diffusion tensor imaging, PET and SPECT may improve accuracy and timeliness of diagnosis. (37) In the case of our data, accuracy of diagnosis of MND was assessed by Traynor et al in 2000. At that time 7% of patients in the MND Register were felt to have questionable or alternative diagnoses. There have been no universally accepted adjustments to the diagnostic criteria from 1998 onwards it follows that potentially up to 7% of the current Irish MND population may have erroneous diagnoses, except that a diagnosis by a neurologist significantly improves accuracy of diagnosis. (15,38) Since the time of 'raynors' audit of the register every effort is made on a regular basis to re-evaluate patients with atypical features (i.e. non progression) and on rare occasions patients are removed from the register under the guidance of Professor Orla Hardiman.
2.5.2  Neurological Epidemiology

Neurological diseases present certain unique problems to epidemiologic investigations. (39) As previously mentioned the definition of neurological disorders may vary between studies or the certainty of diagnosis may change with disease progression as is the case in MND. If early diagnoses are included in a study the number of false positive diagnoses is likely to be higher, but if only advanced diseases are included then the numbers will be smaller and their survival usually shorter. Time of onset of disease is often not clear and this may complicate risk factor determination. In MND some patients may also have frontotemporal dementia which could prevent them recalling accurately past events that may have contributed to the development of the disease. Similarly if the patient has difficulty with communication the history may come from a partner, relative or friend who may not give an accurate unbiased account of the disease. Deterioration in condition is quite heterogeneous in MND and difficult to quantitate. This provides a challenge to those attempting to study or identify factors that may influence the development or the rate of progression of disease. All of the above factors must be considered when studying a degenerative neurological disease such as MND.

The following areas are of particular interest in the epidemiology of MND and are relevent to the studies included in this thesis and will be briefly discussed:

- Disease frequency calculations
  - incidence
  - prevalence

- Risk Factors
  - relative risk
  - attributable risk
  - odds ratio

- Study designs
  - cohort studies
  - case-control studies

- Bias and confounders
  - bias
  - recall bias
2.5.2.1  Disease Frequency

Disease frequency encompasses incidence, prevalence and mortality figures. As mentioned in the methods section the Irish incidence of MND is the number of new cases of MND occurring in a defined population (the Republic of Ireland) in a specified time period. It can be expressed as cumulative incidence (CI) or incidence rate (IR) which are both equally valid. In our study we calculated the incidence rate.

\[
\text{IR} = \frac{\text{Number of new cases of MND during a given time period}}{\text{Total person-time of observation}}
\]

According to the EEDC the diagnosis of MND should only be made in cases over 15 years of age. The corrected total population at risk of MND is therefore all those alive over 15 years of age in Ireland at the time of data analysis (1st January every year).\(^{(40)}\) In our study we have shown incidence figures using both the total and corrected (> 15 years) population to demonstrate the difference that this can make to study results (i.e 2002-2004 incidence rate: 1.9 becomes 2.5/100,000 corrected). In addition figures are expressed as total incidence, gender related incidence rate (2002-2004: Male:female = 2.3:1.6/100,000) and disease subtype incidence rate (2002-2004: bulbar: limb onset incidence = 0.8:1.2/100,000). To make meaningful comparisons between different populations age- and sex-adjusted rates were standardized to another large population (the USA) using the direct method. It should be noted that not all other studies in the literature standardise their results or report age corrected incidence and prevalence rates, therefore while comparisons between figures reported in Ireland and these populations can be crudely made, it is not as accurate as if they were all standardised to the same population. The majority of large MND population studies do standardise to the USA population figures of the time and do report age appropriate figures. Where the same population is compared over time as in our study, the 2002-2004 population was standardised to the 1996 Irish population. \(^{(41)}\) The prevalence rate measures the
instantaneous number of MND cases in a population at a given point in time, again in our case 1st January each year.

\[
\text{Number of cases of MND at a given point in time} = \frac{\text{Prevalence}}{\text{Total population}}
\]

This may also be expressed as a crude or age-adjusted rate but MND diagnostic criteria again require that only the population > 15 years of age at the time of evaluation be included in calculations. The date and time used for prevalence was midnight on December 31st for both populations.\(^{(40-42)}\) In addition in our study we introduced the idea of an incident and a prevalent population. This is of importance because studies in Europe are often designed using incident populations (i.e. usually only cases of < 2 years diagnoses are allowed into trials, in comparison trials in the USA are often prevalent population based (i.e. any person with a diagnosis of MND regardless of time of diagnosis is allowed to participate). The prevalent cohort often contains atypical or variant MND cases with for example already prolonged survival. It can be argued that patients in this cohort often are not typical of the MND population as a whole and may therefore erroneously skew data such as survival results. In addition it becomes difficult to honestly compare results from MND trials between Europe and the USA. (Prevalent Vs Incident survival Irish population: 16.4 months Vs 120.9 months. Also generally, as was in this study bulbar patients are under represented by the prevalent group because of their poorer prognosis that limb onset disease (Incident Vs Prevalent % Bulbar onset cases = 35.3% Vs 31.5%). Excluding patients from trials based on an above average length of survival for MND may be statistically preferred in future but raises interesting and difficult ethical issues.\(^{(40)}\) Population sources is also an interesting confounding difference when comparing disease frequency rates. For example patients gathered from neurology clinics rather than from the general population tend to be younger, of higher socioeconomic class and with slower disease progression. The results of clinic or hospital based studies cannot always be extrapolated to represent the entire population or to be
compared to other populations. Population based studies as in the case of the Irish MND population are less biased but difficult to control with regards to ascertainment and accuracy of diagnosis in larger countries. \(^{(8,10,16,43)}\)

In MND Mortality rates should approximate incidence rates because of the standardized EEDC, the high case fatality rate and short median survival (in our population 16-17 months). \(^{(40)}\) In our study we concentrated on survival times and prognosis over mortality and morbidity data because MND is relatively uncommon and theses values are of more significance clinically to the patient, the carer and the physician. Traditionally these are also the figures that other populations have published which again facilitates comparison between Ireland and other populations, however it should noted that some populations define survival from the time of onset of symptoms as opposed to from the time of diagnosis of disease which is our definition of survival and the definition accepted by most large international MND groups. \(^{(40)}\)

Differences in the definition of disease, denominators for incidence and prevalence calculations, definitions of survival and methods of data collection possibly account for the wide ranges in MND incidence and prevalence figures reported over the last 2-3 decades, particularly earlier studies. \(^{(6)}\) Using the EEDC from 1994 and 1998 and population based registers should enhance the accuracy of MND figures henceforth. The relative stability of incidence and prevalence figures in the Irish population and the similarity to independently produced figures from our closest neighbours Northern Ireland suggest that our figures are reasonably accurate and reflective of our population. Any ongoing distinctive epidemiological differences between Ireland and other European or American populations where statistical methods are considered sound, may be due to social, genetic or cultural factors and might provide clues to the aetiology of the disease. Once a difference in disease frequency is identified in a previously stable population is identified it is important to attempt to reason out why this is the case. Increasing incidence in MND has been reported among the elderly for example. This could be due to a changing environmental risk or more likely reflects increasing recognition of the disease in this cohort, increasing survival of patients into old age and improved
accessibility to health services of the elderly. \(^{(11)}\) Some ongoing differences between the Western world and first world countries might be expected, Ireland and other European countries have an increasingly large elderly population for example who are at greatest risk of developing MND. In contrast African countries ravaged by famine, war and AIDS have a much shorter citizen life expectancy, never achieving the ages where MND typically occurs.

2.5.2.2 Risk Factors

There are several methods used to determine risk factors (RF)s for disease. \(^{(44)}\) Risk factors may be suggested by population studies but should then be further evaluated by for example examining exposed and non exposed groups or conducting a case control study. Unfortunately the Irish MND Register did not collect information consistently about potential risk factors for MND such as trauma, exercise, smoking until recently. Quantifying exposure to a possible RF involves comparison of the frequency of disease in an exposed and unexposed group. The odds of developing disease is defined as risk/1 − risk. If the risk of disease is small the corresponding odds of disease are also small. The odds ratio (OR) is defined as:

\[
\text{OR} = \frac{O_{\text{exposed}}}{O_{\text{unexposed}}}
\]

ORs are commonly seen in case-control studies as was calculated in our exercise study in a later chapter, where the risk of disease associated with exposure cannot be directly calculated. This is the case with most studies of MND where study subjects are chosen by presence or absence of a disease and not by exposure or non-exposure to a risk factor. Case-control studies are often small especially in diseases such as MND and making assumptions and extrapolations about the general population from these studies can be difficult. Risk factors for MND will be discussed in more detail in the next chapter.
2.5.2.3  Study Designs

Most MND studies are cohort and case-control studies, both of which have been used in this thesis. Cronin et al conducted a review of ethnic variability in MND in 2007 which found a wide disparity between methods of population study in Europe and North America vs the rest of the world. Prospective descriptive studies with multiple case ascertainment sources such as the Irish MND Register provide higher estimates of disease frequency than retrospective studies in the same populations. A brief description of the study types used in this thesis are included below.

2.5.2.3.1  Cohort Studies

Classic cohort studies are ones where patients are classified according to the presence or absence of exposure to a RF for the disease. Population based descriptive cohorts such as the Irish or Scottish MND Registers are another useful method for collecting data not only on risk factor exposures but also to enable calculation of incidence and prevalence of diseases such as MND. The is especially true in small defined populations such as on an island such as Ireland, where the majority of patients pass through a public health service. Most MND patients will access public health services in Ireland because services such as occupational therapy, speech and language therapy, nutritional evaluation and physiotherapy are better resourced in the public health system. In the USA where healthcare depends on health insurance, this is not possible because only a selected proportion of the population (those who can afford it) will attend and receive timely treatment for their disease. For very rare disorders a centre based cohort or study that recruits subjects with the illness from the centre can be useful though prone to bias. Specialist centres see the most severe, atypical or young cases of a disease. These centres may also give above average care for the disease or be involved in clinical trials. Incidence and prevalence figures cannot be accurately produced from such centers alone. Specialist centres may however be useful in monitoring disease progression and factors that influence the disease such as new therapies as long as there are objective parameters that can be measured serially i.e. in MND these would be ALSFRSr, SNIP, FVC and BMI. Cohort studies may be either retrospective or prospective. In retrospective studies all of the information is gleaned from historical records. The information
available is limited and may be incomplete on some subjects. Retrospective cohort studies are commonly done because they are inexpensive, involve data collection that can be done by a student or junior staff and take less time. Quality of data is reliant on the person doing the chart search. Often they serve as a preliminary study which helps decide whether a topic is worth pursuing with a more time-consuming and expensive prospective study.

Prospective studies are planned before information is collected. As much information as is required can be then gathered from all the subjects at the start and throughout the duration of the study. Prospective cohort studies look at the natural history of disease, examine outcomes over short periods of time or after rare exposures. Prognostic studies are more useful to study disease outcomes and prognostic indicators of disease. They are not useful studying rare outcomes or orphan diseases because of the extensive time and expense involved. The Irish MND Register is prospective from when it was commenced in 1993 but there have been adjustments to the structure and information collected over time.

2.5.2.3.2 Case-control Studies

Case-control studies are often used to study rare diseases, diseases with a short lifespan and limited prevalence such as MND. In these studies the outcome of interest i.e. MND diagnosis is identified and the exposure of cases to a possible risk factor such as physical activity is compared to that of the exposure of controls without the disease. The exposure in question should not be rare and should be relatively common among known cases. Selection bias is inevitable because the proposed association is studied after exposure has occurred; the subjects of study are those with the outcome of interest. Most case-control studies do not give precise estimates of cumulative incidence of disease under study in the population. Several design features are key in undertaking such a study:

- Selection of cases and controls.
- Sources of information on the exposures.
- Multiple hypotheses testing.
- Power of the study.
2.5.2.3.2.1 Selection of Cases and Controls

A clear-cut distinction between cases and controls is required i.e. a definition of the
disease. The EEDC enables us to identify relatively accurately MND in a fashion that is
not overly restrictive, so that a reasonable sample size is possible, while attempting to
avoid excess mimic syndromes which would decrease the relative risk of the disease.\(^{40}\)
Controls are defined as individuals who do not have the disease. As MND is
predominantly sporadic it is not possible to guarantee that some controls will not later
develop the disease. Subjects who have a family history of MND have a higher than
normal chance of developing the disease and should be excluded from sporadic MND
studies as was the case in our study on physical activity and MND described in a later
chapter. Cases and controls should however come from the same population source i.e.
same country. Cases chosen should reflect the homogeneity normally seen within the
MND population. An increased ratio of controls to cases is often employed to increase
the power of the study where case numbers may be small. This method is commonly used
in MND studies but reaches a maximal effect once the ratio has reached 4:1. We were
unable to achieve a higher than 1:1 ratio in our MND and exercise study.\(^{40}\)

2.5.2.3.2.2 Sources of Information on Exposures

Case-control studies and cohort studies often rely on informants or medical records for
information. If data is inaccurate or not complete then estimates of effects of exposures
on the likelihood of developing the disease can be biased. The cognitive status of some
MND patients may be questionable (both a fronto-temporal dementia (FTD) and a subtle
dysexecutive functioning (DF) have been described) which may be of relevance with
regards to information sources for the MND Register and other studies. Every effort was
made in the case of the Irish MND Register to confirm most data obtained with a second
source, either family member or chart information. Figures for the occurrence of these
cognitive phenomena in MND vary widely.\(^{47}\)

2.5.2.3.2.3 Multiple Hypotheses Testing
The more hypotheses to be tested, the more complicated the statistical analysis. There are however methods designed to perform multiple hypotheses testing such as the Bonferroni method, the Bayesian method, all of which are beyond the scope of this thesis. (40-41)

2.5.2.3.2.4 Power of the Study
The power of a study is the probability of correctly identifying a difference between the two groups in the study sample when one genuinely exists. In an ideal study the power is high. Power depends on sample size, a larger sample size usually ensures higher power. This is a problem when studying MND. At any one time the living MND population in Ireland is around 220. Many of these patients will be significantly infirm, immobile, unable to communicate without great difficulty and a small percentage will also be dementing. If too few subjects are enlisted into a study this results in large P values and wider confidence intervals than would be seen in larger studies. (48) In a study of randomized controlled trials accepted to well recognize journals with high impact factors Moher et al found that only approximately 40% of studies had > 80% power to detect a relative difference of 50% and many studies did not include sample size calculations. An excessively large trial chasing a high power is not ideal either resulting in much resource and time wastage particularly in a terminal disease such as MND. The P value is most commonly set at 0.05 and power will typically be approximately 80%. Once these three values have been decide on then there are formulae and tables to work out the appropriate sample size. In the case of MND case control studies 60 cases and 120 controls should be powered enough (>/=80%) to appreciate a 15% difference between cases. (49)
<table>
<thead>
<tr>
<th>Factor</th>
<th>Magnitude</th>
<th>Impact on identification of effect</th>
<th>Required Sample size</th>
</tr>
</thead>
<tbody>
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<td>P value</td>
<td>Small</td>
<td>Strict criteria make 'significance' difficult to achieve</td>
<td>Large</td>
</tr>
<tr>
<td></td>
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</tr>
</tbody>
</table>
2.5.2.4 Bias and Confounders

Whether a cause-effect relationship is true or simply due to chance is determined using statistical tests and establishing confidence intervals. The alternative explanation is that the association is due to bias or confounding of the data. Bias is any systematic error in an epidemiological study resulting in incorrect estimation of the association between exposure and risk of disease. Confounding occurs when the observed association is due to the effects of differences between the study groups rather than the exposure under study. For example in the case of our examination of physical activity and MND in order to avoid confounders controls were age, sex and geographically matched. In addition although it might have been easier we avoided using controls that were friends or family of the MND cases because they may have led a similar lifestyle to the cases. It is possible that the cases may have been biased toward limb onset disease (less communication difficulties) and also towards younger and more complex cases because the majority of cases were interviewed at the Beaumont Hospital MND clinic. There are many different types of bias and confounding which will be discussed in more detail below including:

- Case definition.
- Case ascertainment.
- Capture-recapture techniques.
- Selection and information bias.
- Competing causes of mortality in the older population.
- Referral bias.
- Earlier diagnosis of disease.
- Time of onset of disease.
- Date of diagnosis.
- Disease subtypes.
- Disease progression.
- Effect modification.
- Unknown familial disease.
- Other confounders.
2.5.2.4.1  Case Definition

It is critical to define the disease under study precisely.\(^{(50)}\) The EEDC define cases of MND as definite, probable, possible and suspected. Case definition in our population was defined by a neurologist. Patients were thereafter serially reviewed at each visit. Every effort was made to outrule any MND mimic syndromes and investigations were repeated if required before MND was diagnosed. We know from previous studies in Ireland and Scotland that using the EEDC without outruling all possibilities of mimic syndromes first, there was approximately a 7-8% rate of misdiagnosis respectively. In those studies diagnoses were made by both neurologists and other specialists such as geriatricians and internal medicine physicians.\(^{(15,38)}\) However, when the EEDC are applied exclusively by a consultant neurologist they have a 98.5% specificity for correct MND diagnosis.\(^{(38)}\) As supportive evidence of the importance of a neurologist making the diagnosis, in 44 post mortem studies on MND patients in Scotland diagnosed by a neurologist, MND was pathologically confirmed in all cases at autopsy. However autopsy cannot be relied on to confirm the diagnosis in all cases as only approximately 4% MND cases undergo autopsy.\(^{(18,51)}\) In 2007 new Awaji criteria for the diagnosis of MND combining both EMG and clinical features of disease were proposed by Nodera et al.\(^{(52)}\) The sensitivity of these criteria have been tested and they appear to be more sensitive than the EEDC, particularly in bulbar onset patients and ‘clinically possible MND’ by EEDC criteria. It has been proposed that the Awaji criteria be ‘superimposed’ on the EEDC criteria for future clinical trials but this has not yet been widely accepted.\(^{(31,52)}\) Since the Irish MND Register has been established all cases entered into the MND database have their diagnosis confirmed by a neurologist therefore it follows that the number of mimic syndromes in the Register probably falls between 1.5-7%. There has been no follow up to the landmark 2000 paper by Traynor et al on this topic.\(^{(15)}\)

2.5.2.4.2  Case Ascertainment

Every effort is made to ascertain all MND cases occurring within the Republic of Ireland during register data collection. Occasionally some patients are missed or die before a final diagnosis is made, however because of the small number of neurologists and
neurophysiologists in Ireland and the widespread knowledge regarding the Irish MND Register we feel that these numbers are likely to be low. Most patients with MND experience progressive disability before they die, requiring frequent assessment and intervention by occupational therapy, speech and language therapy or physiotherapy. These paramedical specialities often have little experience with MND and contact our multidisciplinary clinic in Beaumont Hospital or the MND association regularly for expert advice regarding equipment ascertainment and patient management. This is another source of referral for us. Our epidemiological results have been relatively consistent over time and our similar to our Northern Ireland colleagues who were able to use capture-recapture methodology to calculate a 99% ascertainment in their population, supporting the theory of a similar high ascertainment rate for the Irish MND register. \(^{(17)}\)

2.5.2.4.3 Capture-recapture Techniques

Use of the capture-recapture to estimate case ascertainment has been adapted from zoology to estimate accurate population sizes from multiple (potentially incomplete) sources. By identifying matched patients between sources, one can estimate the number of cases not identified by each source, the estimated total number of cases of the disease and hence the sensitivity of each source. We contemplated using this method to statistically prove the high ascertainment of the Irish MND register. However in order to perform this calculation ideally 6 conditions needed to be satisfied:

1. All cases identified by each source are true cases.
2. All cases occurred during the time under study and in the geographical area studied.
3. The study population is closed.
4. All matches are true matches.
5. Sources are independent.
6. There is no heterogeneous catchability within sources.

In the case of our MND population in Ireland at the time of this study it was difficult to satisfy many of these criteria. Although all identified cases should be true cases if sources used are non medical or non-neurological then the possibility of contamination with non
MND cases exists. We know already that up to 7% of the Irish MND population in the past have been mimic syndromes rather than true MND. The second criterion is easier to fulfill although as much MND register data is filled in retrospectively, a certain amount of time must pass before ascertainment for a particular time period can be felt to be as complete as possible. What this time period should be is unclear however in our study we examined the periods 1995-1997 and 2002-2004 with final statistics produced in 2006 which should have allowed enough time for potentially complete inclusion of the MND population onto the MND Register. The MND Register population is not closed as data is continually being added to, however the time period elapsed for alterations to the 2002-2004 population in particular to be made before analysis should make this less likely. Also with advancements in log linear modeling open systems are now permitted using the capture-recapture technique. To the best of our knowledge all matches included would be true matches. The biggest problem is that the sources of information with regards to MND patients up until recently were not independent. The main aim in the past was to avoid missing patients which meant sharing data. Once it became apparent that this was an issue significant changes have been made to keep the Irish MND register separate from other sources of data such as the Irish MND Association. In the future we should be able to statistically measure case ascertainment using the capture-recapture method. As an illustration when a two source capture-recapture analysis was applied to Huntington’s disease (HD) in the UK in 1992 Hook et al estimated that disease prevalence was underreported by as much as 25% compared to other studies. The most commonly applied method of the capture-recapture technique is Frischers’ three-source model which is beyond the scope of this thesis.

Swingler was the first to apply the capture recapture technique to an MND register. He recognized several problems with using this method for neurological disorders in the UK health services. In particular interdependence among sources is common and produces a downwards bias in any maximum likelihood estimate. One way around this is to perform analysis several times using different numbers of sources and see whether the conclusion is similar. Laporte et al contended that the capture-recapture technique is only of use in two situations: when data is difficult to obtain (‘difficult to catch’ population) or
when all that is required is a rough estimate of the numbers with a disease in a certain area. \(^{(53)}\) A study of cancer registries by Schouten et al \(^{(62)}\) demonstrated that the technique could accurately identify under ascertainment from a source but only after the registration process was complete. Capture recapture has also used by Murphy et al in their New Zealand (South island) population in 2008. \(^{(63)}\) The Northern Ireland Register has reported an ascertainment rate of 99% using capture recapture methodology on their population and have a similar incidence and prevalence of MND to Ireland which is reassuring. \(^{(17)}\)

2.5.2.4.4 Selection and Information Bias

There are two main types of bias, selection and information. Selection bias refers to a systematic error in the identification of a study population i.e. when subjects chosen for study are different from those who are eligible but not included in the study. Case-control studies more commonly have selection bias because we know about exposure and occurrence of disease before the study begins increasing the likelihood of a patient being chosen as a case rather than a control. For example in the MND and physical activity study many of the patients were interviewed in the Beaumont Hospital clinic which sees younger, more complex and perhaps healthier patients who are able to travel. In addition if the Irish MND register was based on a single hospital i.e. Beaumont Hospital only rather than being population based, this might also lead to patient selection bias. In fact to avoid this the register keeps in touch with patients all over Ireland throughout the course of their disease. There is evidence that patients with MND attending a specialized centre do better. The authors were able to look at this because of the information recorded in the Irish MND Register from both patients attending and not attending Beaumont MND clinic. \(^{(64)}\)

Information bias occurs when there is a systematic error in the measurement of data on exposure or outcome, there are several subtypes. Misclassification bias is due to inaccurate detailing of exposure or disease status. \(^{(40)}\) In neurological diseases such as MND where over 50% of motor neurons are lost or dying by the onset of symptoms it is difficult to ascertain when the disease actually started. In these cases it is often difficult to
decide how far back an exposure history should go. Also, if a study is looking at subcategories of disease such as spinal or bulbar onset MND and attempting to show a difference between them, if someone has misclassified cases into the wrong group this can impact on the final results. In most cases disease does start either in the bulbar or limb regions. Occasionally patients report onset of limb and bulbar symptoms at the same time, in the Irish Register every effort is made to try and ascertain the very first symptom to avoid misclassifying too many cases as 'both' inaccurately.\(^{(65)}\)

Recall bias occurs when subjects with and without disease report exposures with differing accuracy. Neurological disorders such as MND may be associated with cognitive decline. The major cognitive findings in MND patients usually impact executive functioning rather than the ability to recall past events. Nevertheless this is a real problem when assessing validity of data in these populations. Follow up examinations can help in retrospective studies to check intra-rater reliability (the patient and the tester) but the optimal time between information gathering sessions is not clear especially in a disease such as MND where survival is relatively short. Is data less valid if it hasn’t been rechecked before death or another significant event?\(^{(66)}\) Disorders where communication is a problem such as the bulbar type of MND may require use of a relative or spouse (a proxy-respondent) to gather information. Depending on who this is they may not have known the patient through their entire life or the period that you are interested in looking at, or they may selectively recall exposures they think are helpful but not necessarily with great accuracy. Because most MND patients are elderly there is often a long delay between potential exposures and development of the disease which is also problematic.\(^{(66)}\) The MND Register has not been successful at accurately and consistently reporting smoking status, exposure to potential risks such as trauma or exercise in the past. Adjustments have now been made to the Register so that the frequency of exposure to these factors may be properly assessed but cannot be included in this current study.

2.5.2.4.5  Competing Causes of Mortality in Older Population
MND occurs predominantly in an older group of patients who have co-morbidities. Occasionally this may lead to a more premature death than that expected with MND.
Conversely as medicine improves and survival of the general population to an age where MND is more likely occurs in the developed world there may be changes in the incidence and prevalence of the disease. In Ireland there was a ‘baby boom’ in the 1950s such that we expect to see an increase in the number of elderly patients approximately 60-70 years later. That bulge in addition to improvements in the care of other major medical conditions such as cancer, heart disease and lung disease should also result in more cases of MND in the near future. The timing of our study is important because it establishes stable rates of disease thusfar to compare to figures in the next 1-2 decades when the change is expected. Not all countries will experienced the same population shifts so it is important to recognise their effects when comparing Ireland to other countries in the future. Age specific rates will be particularly important in this regard. As death certificates become computerised, this will be an additional important resource to capture information with regards to MND, currently anonymised figures of deaths from MND are released by the Central Statistics Office on a yearly basis, but the accuracy of their figures which predominantly use HIPE data have not been assessed. For this reason we reviewed all death certificates of known deceased MND patients on the Irish MND register to assess how frequently MND was documented on the death certificate and compared these figures to CSO figures for the same years. These statistical agencies form a key reference point for health services when planning their upcoming budgets, any discrepancy in their MND figures would have an important impact on public services allocated for this disease in the future.

2.5.2.4.6 Referral Bias
Referral bias is generally not a problem with our population based data in contrast to centre based data. All patients with MND whether they attend our specialist clinic or not are included in the MND register once the diagnosis is confirmed.

2.5.2.4.7 Earlier Diagnosis of Disease
As we start to pick up MND cases earlier in the course of the disease, survival times will increase without any true change in the biological pattern of the disease. Earlier diagnosis may be due to a pro-active patient, an MND savvy doctor and/or improved education
regarding the disease. Currently we do not have any means of picking up earlier diagnoses other than through the usual means: history taking, physical examination and supportive investigations. By the time a person is symptomatic from MND 50% of their motor neurons are already dead or dying. In a study by Traynor it was shown that attendance at a specialist MND clinic improves survival of the MND patient. It has been argued by skeptics that these patients were simply diagnosed earlier because of the experience of the team and hence appeared to have a prolonged survival time, however in comparison to patients attending elsewhere no difference was noted in the distribution of “certainty of diagnosis” at first presentation between the specialist centre and elsewhere. There has also since been an Italian study which reported similar findings. As our database includes all patients as long as they fulfill EEDC criteria for MND, regardless of stage of disease this is not an issue here.

2.5.2.4.8 Time of Onset of Disease

There is no way to accurately estimate exact time of onset of disease except by clinical symptoms. Some patients may have a difficult protracted course where back pain and concurrent cervical spondylosis delay diagnosis and where the onset of MND is unclear. Other patients develop or notice MND symptoms after a trauma or post operatively (i.e. when a patient has shoulder surgery for weakness in their arm presumed to be related to a muscular tear/injury and fails to recover afterwards). In some patients the time their arm or leg became weak or their speech became slurred is clear but in reality the disease has already been actively destroying motor neurons for quite a while. This complicates accurate determination of RFs for MND, for this reason in our MND and physical activity study we chose a questionnaire that evaluated physical activity since childhood. Until we have a way to identify people at risk of developing MND or a method of diagnosing subclinical disease onset we will continue to use first symptom to define onset of disease.

2.5.2.4.9 Date of Diagnosis

Variability in interpretation of date of diagnosis may also be problematic. Is the date of diagnosis the date MND was first considered, the date the patient was first told about
the possibility of MND or the date the diagnosis was confirmed? With patients who request a second opinion is it the first or second consultation that is considered the diagnostic date? In the Irish MND register we consider the time a neurologist saw the patient and discussed MND as the likely diagnosis, after all other diagnoses were outruled by appropriate investigations as the time of diagnosis of disease.

2.5.2.4.10  Disease Subtypes

MND is a heterogeneous disease. It is more common in males than females (3:1), patients may present with either bulbar or spinal/limb onset symptoms first. In Europe it is believed that these may be two different diseases perhaps with different aetiologies but a similar common final pathway. In the USA studies divide disease into bulbar, upper limb and lower limb onset for similar reasons. Bulbar onset MND has a worse prognosis but receives most survival benefit from Riluzole. (69) By subdividing the disease in this way it is felt that we may identify RFs and prognostic indicators that are more useful in one subtype than another. In the Irish MND Register patients were analysed as a group and also by sex and disease subtype.
2.5.2.4.11 Disease Progression

In MND disease progression can be quite variable, the majority of patients survive between 2-5 years after diagnosis. In fact the average survival time in our study was 16-17 months. There are however outliers who rapidly decline and die within days to weeks of diagnosis and others who survive beyond 10 years. Similarly young patients with non familial MND have a more prolonged survival time. This is demonstrated nicely by the difference in survival times between incident and prevalent cohorts in our study (16.4 months Vs 120.9 months). The ALSFRSr is a universally accepted questionnaire to define functional status, it is heavily biased towards physical deficits leaving out many of the symptoms of patients with predominantly bulbar disease, despite this it is still considered the most appropriate tool for assessing MND progression.\(^{(70)}\) In our IGF in MND study in a later chapter patients showed an expected serial decline in ALSFRSr over time. We know from studies by Desport that loss of weight is a negative prognostic indicator therefore serial weights or BMIs (body mass indexes) are often followed as was also evaluated in our IGF study.\(^{(71)}\) More recently serial measurements of SNIPs (sniff inspiratory pressures) have shown that a SNIP < 40 cm H20 is also a negative prognostic indicator in MND, this was not used in our IGF study as functional and nutritional indices were the main focus of the study.\(^{(72)}\) Cerebral degeneration as measured by proton magnetic resonance spectroscopy is predictive of reduced survival in MND although this is predominantly used as an experimental tool and is not widely available. All of these measures may be serially monitored in MND patients to assess disease progression. See later section on prognostic indicators.

2.5.2.4.12 Effect Modification

If a third factor magnifies the odds ratio (OR) or the relative risk (RR) in a study it is said to be an effect modifier of the association between the exposure and the outcome. These variables can affect the exposure-outcome association without confounding it. Effect modification may be recognized with stratification or subgroup analysis as was done in our exercise and MND study.\(^{(40)}\)
2.5.2.4.13 Unknown Familial Disease

A family history of disease has to start somewhere i.e. it is possible that a patient may present as a proband (first affected case in the family) and later siblings or children present with symptoms of MND. Familial and sporadic MND are indistinguishable clinically and some family cases only become apparent over time. For this reason a family history is a very important part of the initial assessment of any patient and our database allows is updated over time with regard to cases of apparently sporadic MND that later reveal themselves as familial disease. Less than 8% of the Irish MND population have familial MND (which correlates well with other countries) thus in general classic familial disease is considered unlikely to significantly alter the overall incidence and prevalence rates of sporadic MND.\(^ {10-11}\) An exception to this statement is disease caused by 'de novo' mutations i.e. deleterious genetic mutations causing MND that occur over the course of gametogenesis and reproduction. These rare mutations can result in MND cases erroneously assumed to be sporadic, but the genetic mutations that occur are potentially transmissible to the next generation. There is currently no way to exclude these patients from studies of sporadic MND until the next generation presents and familial MND is identified.\(^ {73}\)

2.5.2.4.14 Other Confounders

A confounder is a factor associated with both the exposure and the risk of developing the disease which can account for some of the association between exposure and the disease.\(^ {40, 41}\) To identify confounders in a study one may measure the magnitude of the exposure-outcome association before and after adjusting for all the potentially confounding variables. If the change in the estimate of effect is small (<10%) the variable may be considered not to be a confounder. In order to do this one must be able to identify all possible confounders. In the case of MND this may not be easy to do. Potential confounders once recognized should be controlled for at the start of a study and factored into the study design. One simple method of avoiding confounders in a study is to match controls and cases for the variables most likely to confound i.e. sex and age. We matched controls in our physical activity study for age, sex and geographical location, in our IGF
study we matched controls for age, sex and weight. Multivariate analysis looks at one variable at a time while controlling for the other confounding variables mathematically.

2.5.3 The Ideal MND Register
Swingler who founded the Scottish MND Register said the advantages of a population based register such as the Irish and Scottish registers were:

- Less ascertainment bias (everyone is included).
- More statistical power than case series/ clinic based studies.
- Reflected “the real world” situation with regard to available care etc.
- Encouraged collaboration.

The disadvantages were:
- Observer bias.
- Bureaucracy.
- Lack of Funding.
- Data needed to be sustained & maintained over time.
- Multiple referral sources making capture recapture difficult if not impossible.

A register needs to be easy to use but also protected for privacy. The structure may need to be updated over time to reflect changing thinking with regard to diagnosis and management of disease. There needs to be someone specifically assigned to review data as it becomes available and to update the register accordingly. The aims of the register should be realistic and recognize its’ limitations. Any study undertaken with the register should have defined end points. Part of the responsibility of those using the register should be to fundraise for its’ upkeep. \(^{(34)}\)

2.5.4 The Irish MND Register
Much information pertaining to the Irish MND Register has already been published in the methods section of this chapter. Established in 1993 the first published figures from the register appeared in 1999. \(^{(10)}\) Since then it has been the source of numerous Irish
publications on MND epidemiology, genetics, psychology, endocrinology and health care resources. Data produced from the register has been used to define Irish incidence and prevalence figures, to analyse subtypes of disease and survival times over the last decade. The data produced is available as an ongoing source of information from which to conduct smaller studies on risk factors for disease and prognostic indicators. With interventions such as RIG/PEG feeding (radiologically or percutaneously inserted gastrostomy tubes), cough insufflators, overnight oximetry and non invasive positive pressure ventilation (NIPPV) survival data can be compared over time to analyse their usefulness. The Register stores clinical and phenotype information on both sporadic and familial MND, recently we have also started collecting data on MND and frontotemporal dementia families for genetic studies. The data that is presented in this thesis predominantly pertains to sporadic MND unless otherwise stated, incidence and prevalence figures do include both sporadic & familial cases. The occurrence of familial disease is then expressed as a percentage of the total incidence and prevalence at any one time. The causes of sporadic MND are not known although some isolated genetic risk factors (new mutations) have been identified and environmental agents such as cigarette smoking may be relevant, though this is controversial. (74-76) Other potential risk factors will be discussed in more detail in the next chapter. In the majority of cases the disease is likely to be multifactorial with both genetic and environmental triggers required for onset of disease. The MND register can be extremely helpful in this regard identifying non random patterns of occurrence of the disease and searching for clues to aetiology. Using the results we aim to find causative agents or environmental triggers, identify risk and factors for the disease which may be avoided in those “at risk” or susceptible to the disease and evaluate new therapeutic strategies with regards to survival benefit.

2.5.5 Incidence, Prevalence Data

MND frequency is defined by either retrospective mortality studies with information gleaned from death certification data or by population studies. In the latter disease frequency is estimated in a prospective or retrospective manner by calculating the number of patients affected within a specific time period in a region. (6) MND is a suitable disease to estimate incidence rates in this way because we have defined criteria to recognize the
disease pre-mortem, there is a 100% fatality rate and it has a relatively short median survival time from onset.\(^{(77-80)}\) The incidence of MND worldwide excluding the areas in the Western Pacific region is traditionally described as fairly constant ranging from 0.6-3.3 per 100,000 per year in each country.\(^{(4, 6, 10, 12, 21-22, 63, 79-98)}\) Cronin et al recently standardized twenty two studies from all over the world to the 2000 US population using the 45–74 age band and found that reported incidence rates range from 0.8-8.2 per 100,000 person years depending on location.\(^{(6)}\) Available MND population data from the following locations will be discussed below:

- Northern and Southern Europe
- Ireland* 
- Central and Eastern Europe
- Russian
- North America
- Central and South America
- Western Pacific Regions
- Non Western Pacific Regions and Asia
- Middle East
- Africa

2.5.5.1 Northern and Southern Europe

Until 2009 the highest reported crude incidences within Caucasian populations were 2.6 and 2.5 per 100,000 person-years from the PARALS study in 2001 and middle-Finland in 1983, the lowest reported is 0.6 per 100,000 person-years in Italy in both Sardinia and Messina a decade apart.\(^{(97-98)}\) PARALS recently published new figures suggesting a crude incidence of 2.9 per 100,000 population which is similar to Irish figures.\(^{(12)}\) We have a wide variety of data on Northern European MND epidemiology. A recent study from Sweden reported age standardized incidence rates of 2.98 per 100,000 person-years in 2003-2005 which is again within the confidence intervals of Irish data.\(^{(82)}\) In Scotland 1226 people with MND were diagnosed between 1989-1998. Approximately half the
patients were diagnosed using modified WFN criteria for MND pre 1994, the other half were diagnosed using the EEDC from 1994 onwards. Only 44 of these patients underwent PM examinations of whom 100% had the clinical diagnosis of MND confirmed after death. Overall specificity varied between 91.7% and 98.5% for a consultant neurologist diagnosis of MND. When they examined the sensitivity of the EEDC they found that 1/10 patients by time of death still don’t reach definite or probable diagnosis by the time of death.\(^{(51, 61)}\) (Traynor et al reported similar findings in 2000 i.e. that approximately 10% of MND patients followed up over a 6 year period who were initially diagnosed as suspected or possible disease failed to satisfy criteria for definite or probable disease by the time of death).\(^{(59)}\) The Scottish MND Register has been able to estimate their ascertainment rate as 98% by comparing referral, discharge and mortality data on MND patients with their own registry data using the capture recapture method.\(^{(61)}\) Scottish incidence and prevalence rates are similar to Irish rates with a total incidence of 2.4/100,000 (male 2.73/100,000 and female 2.10/100,000) and prevalence of 4/100,000 which is lower than Irish prevalence figures. Most recently published Scottish incidence figures show that their figures are stable over a 10 year period similar to our findings in Ireland. There has been a trend towards increasing prevalence in Scotland over the past decade which may be related to increased early pick up, increasing survival, better care standards for patients and or Riluzole. Estimated number of cases missed over the period 1989-1998 in Scotland is 27 which indicates a very high ascertainment level using data based on two sources that were highly dependent.\(^{(8, 61)}\) Apart from 4 studies from Iceland, Denmark and UK most studies estimate incidence of MND in Northern Europe at 1.6-2.9 per 100,000 population. The 4 aberrant studies are all small or use single data sources.\(^{(100-103)}\)

Prevalence rates across Northern and Southern Europe vary from 1.6 to 7.1 per 100,000 population from Italy (Sardinia) in 1977 to Sweden in 1988. In the former study there appeared to be low ascertainment of patients after the 60-69 year age group and their population age demographics were biased towards a younger band which might explain artificially low figures. Age specific figures were not included and this study pre-dated the EEDC.\(^{(97)}\) In the latter study rates were based on a single year of study and have not been replicated since in the same area.\(^{(104)}\) In Northern Sweden Forsgren et al had found
an incidence rate of 1.7 and prevalence rate of 4.8 per 100,000 population in 1983 which would be lower than Irish rates. (105) More recently Fang et al have reported an increased age standardised incidence of ALS among Swedish natives from 2.32 per 100,000 person-years in 1991-1993 to 2.98 per 100,000 person-years in 2003-2005, representing an annual increase of approximately 2% during the 15 years. The age-specific incidence rates increased in all age groups except those younger than 50 years which might therefore indicate increased disease pick up in the typically affected age groups. (82) Norwegian rates were reportedly similar in 1991 to the Forsgrens’ study with a crude incidence of 1.6 and prevalence of 3.7 per 100,000 person years, although this data was sourced from a single referral centre which is not ideal for reasons previously discussed. (88) Excluding single data sources and the 4 problematic studies mentioned above most Northern European studies of prevalence agree on a prevalence rate of between 3.7 – 6.4.

Pertaining to Southern Europe we have 7 studies published from various regions in Italy estimating incidence and prevalence rates from 0.6-2.9 and 1.6-5 per 100,000 person years respectively. These low incidence and prevalence figures correlate well with the majority of other studies of the time based on a single data source and pre-dating EEDC. (7, 97-98,106-110) Only 1 early study from 1988 provides any data on MND in Spain, incidence and prevalence between 1974-1985 is reported as 1.0 and 3.5 per 100,000 person years respectively. (111)

A comprehensive review by Cronin et al reported a remarkably uniform frequency of MND across Northern and Southern Europe once figures were standardized to another population by the direct method. (6) Close correlation of figures was also demonstrated by the four prospective, population-based studies from Ireland, Scotland and Italy. (7,11-12,19,61,106) The initial results of the N.Ireland register have been mentioned above and correlate well with results from the Republic of Ireland MND register. (17,25) Hirtz et al recently performed a meta-analysis of studies from the USA and Europe and found that the median annual incidence rate of MND including all age groups in these studies was 1.6 per 100,000 (range 0.7-2.5) and from analyzing class I studies only, the figure became 2.1 per 100,000 (range 0.7-2.5). Most studies showed lower rates among women,
median rate ratio of men: women was 1.3:1. The distribution of age-specific rates showed
an acceleration in the upward trend for rates after the age of 40 years, this has also been
demonstrated in our age specific rates. (Figures 2-4) Estimated rates in the seventh
decade of life were broadly distributed around 5 per 100,000. Beyond this age group
studies differed greatly not allowing any generalisations to be made confidently. The
same study addressed USA disease prevalence. Results did not vary greatly between
counties. There was a median reported prevalence of 4 per 100,000 among all ages. MND
was weakly associated with male sex and strongly associated with older age in all the
studies. Most sources of data from the USA are clinic based, healthcare is predominantly
private insurance based and studies therefore contain a predominance of middle class
caucasians and figures may not be truly representative of the population as a whole. (112)

2.5.5.2 Ireland*
Using census values from the year 2002 provided by the Irish Central Statistics
Organisation (CSO) we calculated the crude incidence (CI) of MND on 31st December
2004 at midnight to be 2.0/100,000. When adjusted for age > 15 years the incidence
became 2.6/100,000 population. These figures would be similar to previous Irish figures
produced for the period 1995-1997 (CI = 2.1/100,000 and Age adjusted incidence
2.8/100,000). (10) Age specific rates were also calculated and showed a peak in the 65 to
80 years group during both periods, both the 1995-1997 and the 2002-2004 graphs are
almost identical.

Comparing age- and gender- specific incidence rates for bulbar- and limb- onset disease
the average annual age adjusted incidence of bulbar onset disease in Ireland in our study
was identical among men and women (1.1/100,000; CI 0.9-1.3) for all age groups except
the 80-84 year olds. In this group male incidence was four times that of female incidence.
The number of cases diagnosed in this age group however was small (11 men and 5
women) making it difficult to interpret these findings conclusively. In comparison the age
adjusted annual incidence of limb onset disease was 2.0/100,000 in males (95% CI 1.7-
2.3) compared to 1.1/100,000 in females. (95% CI 0.9-1.3) Female incidence rates for
bulbar onset and limb onset disease were similar at all ages whereas the incidence rates of
limb onset disease in males were consistently higher than those for bulbar onset disease for all age groups.

As a rule of thumb the crude prevalence rate may be expected to be approximately three times the incidence rate or close to 6 per 100,000 in most population studies reported in the literature. Age adjusted rates will be slightly higher.\(^{(43, 85, 88)}\) We estimated that crude prevalence on the 31\(^{st}\) December 2003 at midnight was 6.4/100,000 (95% CI 5.5-7.2) compared with 6.2/100,000 (95% CI 5.3-7.1) in 1996 and age adjusted was 8.7/100,000 compared with 4.7/100,000 and 6.2/100,000 respectively between 1995-1997.\(^{(10)}\) It is interesting that incidence and prevalence statistics in the Irish MND population have remained stable with only minor fluctuations over time. (Figure 15) Similar stable findings have been reported recently by the PARALS group in Italy.\(^{(12)}\) The Irish bulbar onset MND prevalence in 2003 was 2.0/100,000 (95% CI 1.5-2.5) accounting for less than 33% of all (prevalent) cases of MND alive at that time (63/200 patients = 31.3%). The incidence percentage of bulbar onset disease was greater at 42.3% (1.1 out of 2.6/100,000) of total incidence figures. This would be in keeping with the known worse prognosis and shorter survival time of bulbar onset disease.

![Graph showing incidence and prevalence rates over years](image)

**Figure 15:** Stable Incidence and Prevalence of MND in the Irish population over time. There have been only minor fluctuations in the Irish MND population incidence and prevalence over time, the figures are relatively stable.

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We might expect some fluctuations in prevalence figures in the future, there are several potential reasons for this:

- Reflecting rising incidence.
- Earlier diagnosis of the disease.
- Influx of cases of MND to country.
- Improving clinical management:
  - NIPPV.
  - Gastrostomy feeding.
  - Emphasis on nutrition & bulbar dysfunction management.
  - Widespread use of riluzole +/- other therapeutic agents.

2.5.5.2.1 Rising Incidence
Given the stable incidence rate of MND over the last decade a sudden increase in incidence is not expected in the near future. However, when the Irish baby boom population that are currently in the 20-40 year age group enter the 50-70 year age bracket a change in incidence and hence prevalence may occur.

2.5.5.2.2 Earlier Diagnosis
New Awaji criteria have been proposed to diagnose MND at an earlier stage but have not yet been widely accepted thus at present the EEDC are still the gold standard for diagnosing ALS. Until such time as the official diagnostic criteria change or a useful biomarker of disease is discovered MND cases are not expected to be diagnosed any earlier by neurologists than is currently the case. (31,51)

2.5.5.2.3 Influx of MND Cases
During the economic boom Ireland has become an increasingly multicultural society but this has not yet been reflected in our MND register. Out of a total of 1055 on the register before 31st December 2004, only 7 non Irish patients appear (0.56%). It is difficult to predict the effects of the influx of other nationalities into Ireland on future MND rates. There are reports that suggest that MND is less common in Hispanics and African
Americans, however this may reflect their lack of access to healthcare due to poorer socioeconomic status. In Cuba where mixed ethnicity including both afro-Caribbean, Hispanic and Caucasian heritage is common overall mortality from MND was found to be lower than in Northern Europeans. If this were the case future generations of mixed heritage might actually have a reduced risk of MND. as is reported in mixed ethnic groups in Cuba. This effect is not exclusive to African and Hispanic races, a recent Taiwanese study also found lower than expected rates of MND among their population which is a mixed 98% Han-Chinese and 2% Taiwanese Aboriginals. The majority of their population are currently between 16 and 64 years with a Life expectancy of 75 years for a male and 80 years for a female. Although the authors suggest that this race may have a reduced risk of MND they speculated that their rates of MND may increase as their population ages. They did not report age specific rates of disease. In a much earlier UK study by Elian et al rates of MND among Asian, African and Carribean immigrants were lower than expected but interestingly white immigrants from the Indian subcontinent had the expected MND mortality rates of their UK counterparts. Olivares et al in 1972 claimed that Mexicans had resistance to MND based on their figures. They reported an incidence and prevalence rate of 0.4 and 0.8 per 100,000 population in their study. This study has not been replicated. If anything these studies suggest that any significant influx of other nationalities to Ireland excluding Northern Europeans should serve to reduce Irish MND rates within several generations.

2.5.5.2.4 Improving Clinical Management

Increases in prevalence may reflect increased survival of patients due to better standards of care and improved clinical management as discussed by Traynor et al in 2003. This study clearly demonstrated that MND patients in Ireland who attended a specialised multidisciplinary MND clinic had increased survival compared to those who did not. The findings of this study were mirrored by Chio and colleagues in 2006 who demonstrated that their MND patients also did better attending an MND tertiary clinic, having less acute hospital admissions and improved survival. Effects of interventions such as NIPPV and gastrostomy will be discussed in more detail in the next chapter. Riluzole in MND lengthens life in patients typically by the order of months only. This effect is
most pronounced in rapidly progressive and bulbar onset MND. (Figure 4) Riluzole has been available on compassionate grounds in Ireland from 1994 and so benefits should already be seen in the 1995-1997 study. The lack of significant change in prevalence over the last decade also supports this theory. Unless a new agent is introduced with synergistic effects with riluzole, it is unlikely to further influence Irish patient figures.

Median survival among Irish MND patients was 16.4 months from the time of diagnosis. One might have expected that interventions such as improving nutritional management, increasing gastrostomy tube usage and non-invasive positive pressure ventilation (NIPPV) might have improved survival producing a secondary increase in prevalence. Although several studies have shown improvement in survival with the use of NIPPV, gastrostomy insertion probably influences quality of life more than survival length in most patients although the two interventions in MND are closely linked. For example poor respiratory function prior to gastrostomy insertion may in fact significantly shorten survival, although there is a window during which poor respiratory status (generally regarded as a SNIP < 40cm H2O) can be compensated for by using NIPPV prior to gastrostomy insertion. Our study did not demonstrate a significant difference in survival over the different time periods however it may have been too early to detect such changes as the use of the above interventions would only have become widespread in Ireland from approximately 2005 onwards. It will be interesting to see if future follow up studies show similar prevalence rates or demonstrate the survival benefit of these interventions.

Finally, many countries are actively collecting data and DNA samples on MND patients. These results will be analysed and ultimately compared amongst each other, this raises the question ‘are we all studying the same disease?’ Irish incidence, prevalence and survival rates (mean 16.4 months) remained relatively static over the time period studied which is similar to findings reported in Olmsted County between 1925 and 1998 and in Italy from 1995-2004 and Northern Ireland from 2004-2005. There were however significant differences noted in the demographics, clinical features and survival
times of the prevalent and incident cohorts of ALS patients in Ireland. (Table 2) The prevalent cohort had fewer bulbar-onset patients (37.0% Vs 42.3%) and fewer patients over the age of 65 years (37.0% Vs 52.5%) compared to the incident cohort. These differences are likely due to the shorter prognosis associated with bulbar-onset disease (414 days Vs 605 days among limb-onset cases) and with disease over 65 years of age (373 days Vs 649 days among patients younger than 65 years). The survival pattern of the 2003 Irish prevalent cases differs markedly from that of the Irish incident cohort (Figure 4: logrank = 127.4, p<0.0001). This has implications when collecting data genetic or otherwise on patients. As already alluded to earlier North American studies on MND, particularly genetic studies involve collections taken from the prevalent population. In this situation large numbers of patients with poor prognostic features such as bulbar onset disease or increasing age will be underrepresented. (136) The UK and Irish genetic banks however collect only new incident cases which is a slower process but may be more representative of the entire MND population spectrum. (131)

2.5.5.3 Central and Eastern Europe

Within central Europe we have 6 retrospective studies available from Poland, Serbia, Estonia and Greece. In Poland incidence but particularly prevalence figure vary by region and the majority of studies pre-date EEDC. From Lodz between 1980-1986 incidence and prevalence are estimated as 1.8 and 4.5 per 100,000 population. (132) Potemkowski (133) and Cendrowski (134) et al reported incidences to be closer to 0.9 and 0.8 respectively and prevalences 2.7 and 2.2 respectively per 100,000 population in Szczecin from 1986-1995, and Poznan, Western Poland in 1970. With the latter two studies ascertainment of patients was either limited to a single city or unclear so the first study from Lodz although old is likely to be more accurate and it is unsurprising then that these figures are closer to those of Ireland, Scotland, Northern Ireland and Italy. (8, 10, 11, 12, 17, 24) Serbian rates of MND were ascertained by studying three tertiary referral centres only. The figures for incidence and prevalence (0.5 and 1.1 per 100,000 population) not surprisingly therefore appear lower than expected. (135) In Estonia incidence is estimated at 1.3 per 100,000 using data from multiple sources from 1986-1995. The incidence rates among Russians and other nationalities living in Estonia was found to be 2.5 and 2.6 per 100,000 person years. The
reason for this discrepancy was unclear but could be related to ethnicity.\(^\text{136-137}\) Greek figures produced by Argyriou et al between 1990-2003 estimate incidence at 1.1 per 100,000 from a single data source only.\(^\text{138}\)

### 2.5.5.4 Russia

There is one quite old retrospective study available from Russia by Khondkarian et al, that reports a very low prevalence rate with quite a large range from 0.5-2.5 per 100,000 person years. This study is old and the methods used are unclear, the results are also questionable and have not been replicated to date.\(^\text{139}\)

### 2.5.5.5 North America

In North America reported incidence rates and prevalence rates have varied from 1.1-2.3 and 3-4.9 per 100,000 person years which are low compared with Irish figures.\(^2\text{1}, 78, 89, 113, 129, 140-142\) Interestingly non Caucasians consistently show lower rates of MND than their Caucasian counterparts in these studies. Ascertaintment in these groups may be low because African American, Hispanic and other minority populations traditionally have not had good access to the predominantly private healthcare services in the USA.\(^2\text{1}, 89, 113, 140-142\) Alternatively it may be due to a true protective effect of certain ethnicities, where mixed ethnicity appears to be a protective factor as in Cuba which has previously been discussed.\(^\text{143}\)

### 2.5.5.6 Central and South America

We have 6 studies that report frequency of MND in Central and South American populations. From Brazil Dietrich-Nato et al report an incidence and prevalence of 1.7 and 5.2 per 100,000 person years which are low compared with Ireland but may reflect the previously discussed ‘hispanic/ethnic protective factor’. Incidence was estimated from mortality data which should reflect incidence in a disease such as MND as has been discussed elsewhere.\(^\text{86}\) Their figures differ markedly from Moraes et al between 1991-1997 where incidence was estimated as an extremely low 0.3 per 100,000 population. This data was also based on mortality data which does not explain the discrepancy between the two groups’ results.\(^\text{85, 144}\) In Uruguay a prospective population based study
reported incidence and prevalence as 1.4 and 1.9 per 100,000 person years although ascertainment may be incomplete reflected by low prevalence figures, alternatively it may reflect low incidence in a hispanic population with additional poor access to health care.\(^{(83)}\) Chilean frequency rates are also quite low with incidence and prevalence reported as 0.4 and 0.8 per 100,000 person years respectively. This data is based only on a single referral centre however and is pre EEDC.\(^{(145)}\) A Mexican study from Olivares et al in 1972 claimed that Mexicans had resistance to MND based on their figures. They reported an incidence and prevalence rate of 0.4 and 0.8 per 100,000 population in their study. At the time of publishing their cases were taken from a public health program limited to government workers only and over three quarters of the target population were younger than 50 years of age. Their reported incidence rates were in fact based on 16 patients only. Their results have not been replicated as no further studies post EEDC have been done.\(^{(117)}\) It has been suggested that it also may be incorrect to label all people from Central and South America as Hispanic as many diverse cultures have settled in this area over the centuries thus most countries in this region share Spanish, Portugese, other European, Carthagian, Amaranidian and Arabic heritage and genes.\(^{(146)}\)

2.5.5.7 *Western Pacific Regions*

The western pacific form of MND was first described among the indigenous populations of Guam\(^{(147-148)}\), the Kii peninsula in Japan\(^{(149-150)}\) and the Auyu and Jakai people of Irian Jaya (Western New Guinea).\(^{(151)}\) It is commonly seen in association with a parkinsonism-dementia complex (PDC) and similar to the sporadic disease it is also progressively fatal. In contrast to the wealth of MND epidemiological publications from Europe and North America we have very little detail on rates of standard sporadic disease in the Western Pacific regions excluding the Western pacific Mariana islands, Western New Guinea, and the Kii peninsula where rates were very high and are now declining. These areas in the past had exceptionally high rates of MND, at its’maximum the annual incidence was approximately 50 times that of the USA in the 1950s. These aberrant figures were felt controversially to be due to specific dietary factors enjoyed by locals in these regions, although other environmental and genetic factors may also have contributed. Their rates of MND started to decline in the 1980s and in some areas are
reportedly approaching those of other regions in the western world although Kuzuhara recently reported that rates of ALS-parkinsonism-dementia, ALS alone and the parkinson-dementia complex are still high and the 3 diseases often occur among the same families arguing against an environmental factor and more for a genetic cause, the same sentiments were expressed by Steele et al in their 2008 paper. (6, 80, 94, 141, 152, 153)

2.5.5.8 Non Western Pacific Regions and Asia

From the Pacific area we have 3 studies from New Zealand and Hawaii respectively. The reported prevalence among New Zealand hospital inpatients of MND was 3.5 per 100,000 person years in 1981. Lower rates of disease were also reported among Maoris at the time. (154) Murphy et al have recently reported an increasing incidence of MND on the South island of New Zealand with estimated incidence of 3.3 per 100,000 person years in 2006 compared to 1.6 per 100,000 person years in 1985. There was a slight increase in the proportion of elderly patients from 1985-2006 (12.3% - 13.8% of total population were > 65 years respectively). There were only 2 Maoris who developed MND in their population, the study looked at the South island only however. New Zealand has two islands North and South. The total population is 4.3 million, 600,000 of the population are Maoris, the majority of whom live on the North island. (63) It will be interesting to see if this relatively high incidence is consistent throughout the entire New Zealand population and over time and whether there is a true difference between caucasian and Maori populations.

Capture recapture techniques in this study suggested that only 9 patients may have been missed during the study period hence the authors feel there is an increased risk of MND in New Zealand. The most recent incidence figures from this study would correlate with most international and European studies on incidence and prevalence. It is therefore possible that the earlier figures were an underascertainment. Also the population in New Zealand is quite skewed with the majority (3.3 million) living on the North island and only 1 million on the South island. The South island is also 91% Caucasian with very few indigenous people compared with only 78% Caucasians on the North island. Although this study is interesting it is difficult to make any extrapolations for the larger New
Zealand population on the basis of a study on the South island alone which does not reflect the whole island. (155) If for example as has been suggested in other minority groups (117), MND is less common in Maori people then the figures quoted for the South island would be reduced in the North island where there is a larger Maori population and a smaller Caucasian population. The expected age of survival in Maori populations traditionally has been lower than Caucasians because of inherent drug, alcohol and violence problems in these communities. In the past many would not survive to reach the age group where MND most commonly occurs. The current life expectancy for a Maori male is 70.4 years and for a female 75.1 years compared to 79.0 and 83.0 years respectively for Non-Maori New Zealanders. (155)

High incidence of MND has been reported on the Pacific island of Hawaii by Matsumoto et al. (93) In a retrospective study they estimated an incidence rate of 1.6 per 100,000 population among a mixed population of Filipinos, Japanese and Caucasians. When these figures were broken down by race the Filipinos had a striking incidence of 5.1 per 100,000 compared to their Japanese (1.1 per 100,000) and Caucasian (0.8 per 100,000) counterparts. There was little difference seen between the Caucasian and Japanese populations of Hawaii in comparison to low rates reported in Japan compared to Western and Northern European and North American disease rates. (93)

In Northernmost Japan Moriwaka reported an incidence rate of 0.7 per 100,000 person years and a prevalence of 2.3 per 100,000 person years over a 10 year period. When this result was standardized against the 2000 US population the incidence becomes 2.5 per 1000,000 in the 45-74 year age bracket. This is still quite low in comparison to Ireland and other European countries. (6,156) Fong et al reported an incidence of 0.3 and prevalence of 0.9 per 100,000 person years in the Hong Kong Chinese population attending hospitals with MND. These figures may be incomplete due to the Chinese traditions of attending local alternative therapists or Chinese medicine specialists before hospital specialists. When this figure was corrected to the 2000 US population age band 45-74 years the results were still low at 0.8 per 100,000 incidence. Alternatively there
may be a true lower rate of MND among some Asian populations which warrants further study. (6,157)

2.5.5.9 Middle East
Kahana et al in 1984 examined MND frequency in Israel and found an incidence rate of 0.8 per 100,000 population although all non-jewish people were excluded. When corrected to a standard population this figure became 2.3 per 100,000 population. (158) This demonstrated how a biased sample can skew the data making disease frequency appear artificially low or high when in fact it is similar to other countries.

2.5.5.10 Africa
Perhaps not surprisingly we have very little data about MND from Africa. There are 3 epidemiological studies from Northern and Eastern Africa. Radakhrishnan et al studied epidemiology of MND in Libya and reported incidence and prevalence figures of 0.9 and 3.5 per 100,000 years respectively among a young population base (81% of their population were under 40 years). (95) When this incidence rate was standardized to the 2000 US population by Cronin et al, the new incidence rate became 4.5 per 100,000 which did not differ markedly from the results of several other European countries. Libya has < 1% black Africans and the rest of the population are a mixture of Arabs, Europeans and non black or mixed race Africans. (6,95) A layperson study from Nigeria that included juvenile spinal muscular atrophy (SMA) under the umbrella diagnosis of motor neuron disease suggested a prevalence of 15.0 per 100,000 which is extremely high. No details on MND excluding juvenile SMA were given. (159) Finally the Ethiopian prevalence of MND including juvenile SMA was estimated at 5.0 per 100,000 population in 1990 by Tekle-Haimanot et al. (160) This was also a layperson study the results of which are difficult to reconcile with those of the Nigerian study.

2.5.6 Migration Studies
Migration studies examine the rates of disease among ethnic subgroups that have migrated into an area compared to local inhabitants. In London between 1979-1988 MND mortality rates were studied among migrant groups. MND mortality was lower among
Asians, East Africans, Carribeans and Western Africans than among native Londoners. White immigrants from India to London had the same mortality rates as native English. This study was flawed because ethnicity was assigned based on name rather than history and little detail on the size of the ethnic communities represented in the study in London at the time were not known.\(^{(116)}\) Israeli Caucasians showed a higher rate of MND than Afro-Asian immigrants who migrated into Israel in a similar study by Kahana et al in 1976. Cronin et al reviewed the frequency of MND across different ethnic groups and found that MND may be lower among African, Asian and Hispanic ethnicities while it remains fairly constant among Caucasians.\(^{(6)}\) It has been suggested that racial and ethnic backgrounds influence the expression of disease genotypes in neurodegenerative conditions.\(^{(161-163)}\) In fact there has been a slight increase in mortality due to MND in most western countries for a variety of reasons. Most notably due to improved healthcare standards and availability, peoples’ life expectancy has increased. As a result most patients are reaching the typical age range of MND onset but also people are surviving other medical conditions that were previously fatal but are now treatable and developing MND instead.

2.5.7 **Irish Population Age of Onset, Gender, Disease Subtype, FTD Data**

Over the past decade the annual incidence of MND has been consistently higher for Irish men than for women. During these six years (1995-1997 and 2002-2004 respectively) four hundred and sixty-five residents of Ireland were diagnosed with MND. Of these 29 (6.2%) had familial disease based on family history the remaining 93.8% are presumed sporadic disease. There was an overall preponderance of males with 268/465 (57.6%) of patients male gender. (Male: female ratio = 1.4: 1) In the period 1995-1997 the female and male incidence rates of MND in Ireland were 1.8 (1.4-2.1)/100,000 and 2.5 (2.0-2.9)/100,000 compared to 1.6 (1.3-2.0)/100,000 and 2.3 (1.9-2.7)/100,000 respectively. The median age at diagnosis of MND was 64.8 years for males (range 18-88.4) and 67.2 years (range 35.3-92.5) for females. 267/465 patients (57.4%) presented with spinal or limb onset disease and 164/465 (35.3%) presented with bulbar onset disease. 34/465 (7.3%) had generalized onset disease. Mean time from symptom onset to diagnosis was 13 months overall. Patients with bulbar onset disease had a shorter diagnostic delay than
spinal onset disease (9.4 Vs 15.7 months). Only 32 cases (6.9%) of patients had frank frontotemporal dementia by Lund and Manchester Criteria (164) which excludes patients who may have had more subtle neuropsychological defects. The mean survival time of Irish patients from the time of diagnosis was 16.4 months overall. Survival time in the 1995-1997 period was actually 1.1 months longer than that in the 2002-2004 period (17.2 Vs 16.1 months). It is probable that patients from both periods were on riluzole but one might have expected improved survival with close nutritional management, gastrostomy usage and NIPPV use in the later period studied. We feel that it might have been too early to see a response as neither were widespread at the time of study. Also it has become apparent that to see benefit with NIPPV it must be used for more than 4 hours at night, which may not have been fulfilled by many patients. There may be issues with the type of patients (spinal onset Vs bulbar onset) that tolerate it or the supports required to use it successfully. (72,165) Gastrostomy use initially appeared to enhance survival (166) More recent studies failed to demonstrate a significant survival advantage and a Scottish study suggested that patients undergoing PEG have reduced survival. (126) This may be related to poor respiratory status prior to PEG insertion. Other groups have shown that use of NIPPV prior to insertion in these patients often helps. (167) In most epidemiological studies to date the reported mean and median ages of onset of disease are 65 years and mean and median duration of disease from clinical onset to death are 3 years approximately. (10,168-170) In comparison figures from specialist referral centres and clinical trials often have a younger mean age of onset of disease, usually approximately 55 years. Survival figures in these centres are often longer than the usual mean because patients are younger and tend to survive longer but can vary. (64,171-172) There are several factors which have been associated with poorer prognosis and shorter survival time in MND: older age, bulbar onset disease, respiratory muscle involvement. (173-176) Clinical features at time of assessment that are associated with a poorer outcome include low forced vital capacity, low stiff inspiratory nasal pressure (SNIP < 40cm H2O), low body mass index, recent weight loss and low serum chloride. (71,72,177) Predominant upper motor neuron signs has been associated with a better outcome in one study, but has not been replicated. (178)
Overall the demographics and clinical features of MND in Ireland have not altered significantly over the six year period studied. In contrast Noonan and colleagues in 2005 reported wide variations in mortality in the USA between 1969-1998, a non population based study. (113) There had been a 50% increase in mortality in both males and females over this period. Mortality rate in older age groups has in fact doubled in those over 65 years. It is unclear whether these changes in mortality over time are due to improved data collection, increasing recognition of the disease or improving healthcare access for the population. Also the average lifespan of a person in the western world has greatly increased due to improvements in sanitation and nutrition and the discovery of treatments for many infectious agents. We as physicians are also able to recognize and treat many more medical conditions that may have previously proven fatal. Thus patients who in another time or place would have passed away from Tuberculosis for example are now surviving to reach an age where MND is far more common. The incidence of and mortality from many neurodegenerative diseases increases exponentially with increasing age. A decline in mortality from one disorder such as heart disease due to better medical management will increase mortality figures in another disorder. Noonan's figures also demonstrated that there was a geographical gradient from North to South across America. A similar gradient is seen in Ireland from North-west to the midlands which in Ireland’s case may represent two differing and homogenous gene pools. In contrast to the Scottish and Irish figures Noonan also found an increase in survival/ decrease in mortality in the USA MND population although his figures were based on data from death certification which may have varied in reliability. (11, 61, 114)
Risk factors for MND may be either endogenous which would include genetic susceptibility or exogenous which would include environmental exposures. Important putative RFs have included:

- Advanced age
- Male gender
- Genotype including family history of other neuro-degenerative disorders
- Race
- Smoking
- Exercise
- Trauma
- Hormonal and Growth factor deficiencies
- Heavy metal and Toxin exposure
- Occupations

In the past examination of clusters of MND cases was a popular study method but one which has failed to produce any sound aetiological data. Most studies failed to consider the occurrence of suspected clusters by chance alone and to compensate for this.

Armon suggests that the current lowest value for the ratio of observed to expected cases that is considered of epidemiological significance should be increased to save clinical and public health resources. Case ascertainment and field investigations should be reserved only for those clusters confirmed unlikely to be due to chance. Even allowing for this the likelihood of small clusters yielding any useful epidemiological information is low. To date only increasing age, sex and smoking have been supported as RFs for MND. Unfortunately the Irish MND Register has failed to actively collect data on risk factors for all patients with MND. From our data however we can conclude that MND has a peak occurrence in older generations and also that there is an increased male:female ratio of 1.4:1. An examination of heavy physical activity as a risk factor for MND is presented in chapter 3. What follows is a discussion of the literature on MND risk factors to date.
2.5.8.1  *Advanced Age*

The incidence of MND increases exponentially with increasing age from 40-75 years, the effect is more pronounced in males than females. Thereafter the incidence starts to fall but is still higher than those <40 years. We can see this illustrated earlier in this chapter in figures 2-4. Logroscino et al evaluated age specific rates of MND from four population based registries including Ireland and found an increased incidence of MND among patients 65 years and over in all the examined populations. The data from all four locations correlates well. (Figure 16) It is unclear why rates appear to decline by 75-80 years. This may be related to under ascertainment or under reporting of MND in the elderly. The disease may be missed due to frequent other co-morbidities or may be more aggressive with a rapid decline, precluding register contact. Interestingly Swingler evaluated those > 80 years diagnosed with MND over a ten year period in their Scottish MND register. He reported an increased incidence of bulbar onset disease in this age group and a tendency towards a shorter survival (6 months shorter than average) which may be related to reduced motor neuron pool, bulbar onset disease and/or other co-mobidities. It is clear from our data and other studies that MND risk increases with increasing age particularly between 65 -75 years approximately.

![Graph showing age specific incidence of MND](image)

*Figure 16: Age specific incidences of MND in males (left) and females (right), demonstrating similar peaks in MND incidence between Scottish, Irish and Italian populations. (Courtesy of Logroscino G, 2008)*
2.5.8.2 **Male Gender**

MND incidence is classically reported as being higher in males than females. Prior to the 1990s most publications quoted a male:female ratio of 2:1. These were predominantly non population based studies. Subsequent to this in population based studies the ratio fell to 1.6:1 male:female. In the early 2000s studies by Seljeseth and Sorenson in Norway and Rochester, USA respectively suggested that the sex ratio in MND was declining. These studies have not been followed up to date. This may have reflected improvement in ascertainment of females cases at the time, decrease in mortality of women from other causes or equalization of exposure to exogenous risk factors with increasing sex equality and female independence. A recent review by McGuire et al suggested that the MND sex ratio may in fact be trending towards 1 over time. The Irish Register data does not support this theory, clearly demonstrating a static male: female incidence ratio of approximately 1.4:1 over the last decade. Interestingly while bulbar onset disease incidence has a roughly 1:1 ratio, limb/spinal onset disease is responsible for most of the increased ratio between the sexes and analysed separately has a ratio of 2:1. The difference in incidence between the sexes in Ireland appears to roughly equalize after the peak incidence age period i.e. >65-75 years. This is supported by similar findings reported by the Scottish register and by the PARALS register in Italy. The static gender ratio among the Irish and Italian populations both studied over a decade each argues against a narrowing sex ratio over time but we will need to follow the trends for longer to know for sure.
Figure 17: Is the male:female ratio approaching 1 in MND? This point plot of male to female MND incidence ratios over time suggested that this is the case. Note that the points start to converge to the right of the plot. This is controversial. (Courtesy of McGuire V et al, 2006.)

Kennedy’s disease is an interesting, rare, x-linked trinucleotide repeat expansion disease resulting in abnormal androgen receptors that produces an exclusively male MND mimic syndrome. The clinical phenotype is a progressive lower motor neuron disease with prominent bulbar features and facial fasciculations. It does not appear to affect female carriers clinically. It is always considered in any male patients with predominantly lower motor neuron symptoms, facial fasciculations, gynaecomastia and a subclinical sensory neuropathy. Undiagnosed Kennedy’s disease in theory could account for an increased proportion of males with spinal onset disease but in reality it is a disease that neurologists specialising in MND are very aware of and frequently test for (in suspicious cases), in addition neurophysiological testing will reveal any element of sensory nerve abnormality that should raise a red flag for the disease. Kennedy’s disease patients/families once genetically confirmed are stored separately to the sporadic MND population on the Irish Register. There are currently less than 10 families with
Kennedy's disease on a separate part of the register with multiple family members affected.

2.5.8.3 *Genotype and Family History of Other Neurodegenerative Disorders*

There is evidence that a family history of other neurodegenerative states such as frontotemporal dementia, Alzheimer's disease and Parkinson's disease are risk factors for sporadic MND.\(^{(201)}\) Although the evidence is more robust in familial cases of MND i.e. those with known mutations such as those with TDP-43 or progranulin mutations, pathologically there appears to be significant overlap between neurodegenerative diseases.\(^{(202,208)}\) although not all are in agreement on this topic.\(^{(209)}\) Early studies suggested that the presence of ApoE-4 genotype correlated with an increased risk of MND, an earlier age of onset of disease and a shorter survival time.\(^{(174,210-213)}\) Using data from the Irish MND Register in a separate unrelated study Hardiman et al found increased reporting of Parkinson's disease and dementia in first degree relatives of patients with MND suggesting that these family members all might share a genetic susceptibility factor as yet unknown for neurodegenerative diseases. This is the topic of another MD thesis and will not be mentioned further.\(^{(201)}\) With advances in technology recent genome wide European studies on sporadic MND samples have focused attention onto chromosome 9p and 19p as a possible location for such factors.\(^{(214,215)}\) Abnormal survival motor neuron-1 (SMN1) gene copy number has also been associated with significantly increased risk of sporadic MND, deletion of this gene is associated with the development of spinal muscular atrophy, a motor neuron disease that generally occurs in children. Future publications from the Register will reveal further information on the phenotypes and genotypes of these families with multiple neurodegenerative diseases.\(^{(216)}\)

2.5.8.4 *Race*

Ireland is only beginning to see the effects of immigration and genetic admixing of different racial backgrounds. In the United States in from the early 1960s to the 1980s however there appeared to be lower rates of MND among non- Caucasians especially females of Hispanic background. Experts feel that this probably reflected reduced case
ascertainment and poorer health care among those of non-caucasian background (113, 132, 217) rather than a true racial difference. However, much of the data we have on MND comes from middle to upper class Caucasian populations where disease frequency appears to be relatively uniform. We still know relatively little about MND incidence in non caucasians. (6, 143, 172, 218) A recent study from Cuba suggests a reduced frequency of MND among ethnically diverse populations. Cuba’s three main ancestral groups classified by skin color are white (65%), mixed (24%), and (black 10%). (115) More details on genetic factors in MND are to be found in the introductory chapter, further discussion is beyond the scope of this thesis.

2.5.8.5 Smoking
Twenty-eight studies have evaluated the role of smoking in MND. This topic has been reviewed in depth by Armon. (219) 21 studies were excluded from his review as they failed to meet inclusion criteria. Only 2 of the remaining 7 studies provided class II-III evidence of an association. None of the 7 studies refuted the association therefore he concluded that smoking may be considered an established risk factor in sporadic MND. A later meta-analysis that included all studies on the subject of all classes of evidence failed to demonstrate a definite association except that smoking might be associated with a higher risk of MND in women. (220) The authors had noted this finding in their prospective UK study and followed it up with a meta-analysis on the subject. The inclusion of poor quality studies however may have hidden a true association among the male population. (221) The large European Prospective Investigation into Cancer and nutrition (EPIC) study also supports a role for cigarette smoking in MND aetiology. How smoking might predispose to MND is not known. Increased production of neurotoxic free radicals and alterations in lipid peroxidation have been proposed by the EPIC authors. (222) Unfortunately the MND Register has not collected data on smoking consistently therefore it is not possible to make any comment on smoking and MND in the Irish population.

2.5.8.6 Exercise
There are many postulated reasons why exercise might be associated with MND. This is the subject of scrutiny in the next chapter and will not be discussed further here.
2.5.8.7  Trauma

Trauma has long been proposed but never proven as a risk factor in MND. The difficulty is that trauma is common and it remains unclear what kind of trauma, which site of injury and what severity of injury should be considered significant. In addition trauma often coincides with exposure to other proposed risk factors such as exercise, occupation and toxin i.e. pesticides, electricity etc exposure. In a recent small retrospective case-control study on trauma and exercise in MND patients Beghi et al found no significant difference between cases and controls in their reporting of trauma. Armon has reviewed the evidence available for trauma and an association with MND on a number of occasions most recently 2007 and concluded that trauma is “more likely than not” not a risk factor for MND. An additional small case-control study (109 MND cases) and subsequent meta-analysis of studies available on the topic suggested that there may be an increased incidence of MND among people with repeated head injuries, this study has not been replicated. There are several case reports of a disease indistinguishable from motor neuron disease occurring after electrocution. Similar reports of patients developing a motor neuron syndrome after being struck by lightning exist. There are 2 reported motor neuron syndromes following electrical injury. The first is a progressive motor neuron like disease which can be typical for classic MND (both UMN and LMN signs), a pure UMN syndrome or a pure LMN syndrome. Onset is within approximately 2 years of the exposure. The second syndrome is a non-progressive motor neuron syndrome with features similar to classic MND whose onset is typically within the first week of injury. Site of onset for both syndromes correlates with the site of initial injury (entry point), usually a limb in the majority of cases with a relative paucity of bulbar onset disease. The more severe the electric shock, the more likely patients are to have a rapid onset non-progressive disease. Younger people may have a more delayed onset of symptoms after the initial injury (36 years in one case, though the injury may have been coincidental given such a long delay). MND is extremely rare after electrical trauma but a higher incidence of MND among electricians has also been reported. This topic was reviewed in 2007 by Abhinav et al who concluded that the evidence for an
association between electrical injury and a progressive MND syndrome is currently weak. The Irish MND Register does not currently hold data on histories of trauma preceeding MND.

2.5.8.8  *Hormonal and Growth Factor Deficiencies*

Much has been written about various growth factors and whether abnormal levels may contribute to MND. No consistent deficiency has been associated with the development of MND. This topic is reviewed in the introduction chapter and a later chapter on IGF in MND and will not be discussed further here. In 1991 Chio et al reported a possible role of sexual hormones in the aetiopathogenesis of MND. In their case-control study of risk factors in 512 MND patients they found that women with MND had a later menarche and an earlier menopause and therefore a significantly shorter reproductive period than controls. This observation has not been replicated. Del Aguila et al found that among those diagnosed with MND before the age of 40 years, 80% were male hinting at a potential hormonal factor in the disease.

2.5.8.9  *Heavy Metal and Toxin Exposure*

Heavy metals are a diverse group of elements some of which are vital to living organisms in small quantities for life (iron, cobalt, copper, managanese, zinc), excessive levels of the same elements can be toxic. Heavy metals such as mercury, plutonium and lead are always toxic and have no known vital or beneficial effect on organisms. Toxicity can be acute or slow with accumulation over time in the body. Derangements in serum, spinal cord and/or CSF copper, zinc, manganese, selenium, aluminium, iron and their metabolites in MND have been described. In addition MND like syndromes have been described after exposure to toxic levels of lead, cadmium, depleted uranium and mercury. The subject of metal exposure and MND risk has been recently reviewed by Sutedja et al. The authors reported a high number (47/50) of poor quality exposure assessment studies dealing with this topic and as a result no conclusions can be made about metals and MND risk. Morahan et al have suggested that some people may have an inherent genetic inability to correctly break down and deal with environmental toxin exposures. This is also of relevance in pesticide and chemical exposure. In their review Sutedja et al found increased
risk of MND with exposure to pesticides in a number of studies although the class of evidence was low.\textsuperscript{(236)} It has been suggested that this may explain the apparent causative link between football and MND.\textsuperscript{(238)} In addition there appears to be an increased risk of MND among Gulf War veterans that may be related to toxin exposures in Iraq.\textsuperscript{(239-240)} None of the studies currently available have a high class of evidence and are good quality exposure assessment studies. These will be needed to definitively decide if any of the above agents are responsible in some cases, though clearly not all cases of MND. The Irish MND Register does not currently document prior exposures to toxins.

2.5.8.10 \textit{Occupations}

As with other proposed risk factors much has been written on the topic of occupation and MND. Occupation may act as a surrogate for other exposures such as environmental toxins or physical activity. Sutedja et al also recently reviewed this topic for the ALS journal. Starting with close to 4000 potentially relevant studies the authors eventually found only 12 studies that included risk assessments for individual occupations. All studies reached only level IV class of evidence, which is poor. The authors concluded that their may be an increased risk of MND among veterinarians, health workers, athletes, hairdressers, power production plant staff, electrical and military workers however there is currently no good quality studies available on this topic.\textsuperscript{(241)} The Irish MND Register does not currently consistently record occupations therefore no comment can be made on this topic about the Irish population.

2.5.9 \textit{MND Prognosis and Survival Data}

Survival in sporadic MND is typically 3-5 years after symptom onset but can be extremely variable. Factors known to affect prognosis that are not controlled for in clinical trials can complicate the interpretation of the data later on. Proposed factors that can aid in predicting survival time in sporadic MND include:

- Age.
- Sex.
- Site of onset of disease.

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• Disease variants.
• EEDC.
• Rate of progression of disease.
• Cognitive decline.
• Mood disturbance.
• Nutritional status.
• Respiratory status.
• ALSFRSR.
• Riluzole.
• Specialist care clinics.
• Biological markers.

Prognostic factors are difficult to study individually in MND because they rarely occur independently of one another. This topic has recently been extensively reviewed by Chio et al, brief summaries of individual prognostic factors pertinent to MND are discussed below. (242)

2.5.9.1 Age
Many studies have demonstrated that older age, particularly if bulbar in onset and with respiratory muscle involvement is associated with a bad prognosis. (172-175,186) In addition patients with young onset disease (<40 years) survive longer than is typical and elderly patients (diagnosed at >80 years) have a shorter survival than average. (16) Examining MND deaths occurring in 2004 only (N=74), the shortest survival times seen in the Irish MND Register (0.13-0.7 months) were among 10 patients > 75 years. The longest survival times were amongst 2 patients in the 40-45 age bracket (7.9 months). Although the numbers of patients were small these findings would correlate with the previously mentioned studies.

2.5.9.2 Sex
Most epidemiological studies have failed to demonstrate a significant difference in overall MND survival between the sexes. Exceptions to these findings included 4 studies,
2 of which were population based suggesting a shorter survival amongst women with MND even when controlling for disease subtype. (235, 243) Bulbar disease occurred with equal frequency among males and females in the Irish Register.

2.5.9.3 Site of Disease Onset
It is well recognized that bulbar disease is associated with a worse prognosis as is respiratory onset MND. (173) Respiratory onset disease is rare but survival may now be improved with the use of NIPPV if it is tolerated for over four hours a day. (123, 244) It is unclear whether there is a significant difference in survival between patients with onset in the upper or lower extremities. (243) The Irish MND Register has consistently shown a worse survival among patients with bulbar onset compared to limb onset disease however onset in the upper and lower extremities has not been studied.

2.5.9.4 Disease Variants
The pure lower and upper motor neuron variants of the disease (progressive muscular atrophy and primary lateral sclerosis) have a better prognosis. (245-247)

2.5.9.5 EEDC
Survival time may be approximated by the level of certainty of the MND diagnosis by the revised EEDC. ‘Definite MND’ generally carries a worse prognosis than ‘Possible MND’. Exceptions occur, such as in predominantly respiratory onset disease which may not even fulfill criteria for Definite MND but has a very grave prognosis. The specificity of the diagnostic criteria for reliably predicting prognosis between all of the EEDC categories of disease is probably poor. (242)

2.5.9.6 Rate of Progression of Disease
The rate of progression of disease in MND is thought to be relatively stable, a shorter interval from symptom onset to diagnosis represents a more rapid decline in function and therefore predicts a shorter survival. (242) Objective measures of disease progression such as muscle strength testing, FVC testing or ALSFRSr scores (248, 249) are probably more reliable than physical symptoms to assess rate of progression. (120, 175, 178, 248, 250, 251, 257)

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Motor Unit Number Estimation (MUNE) can also be followed serially to assess disease progression but is laborious and resource consuming.\(^{(252)}\) The Irish MND Register has only recently started to collect data on serial FVC, SNIP, weight and ALSFRS\(_{r} \). 

2.5.9.7 **Cognitive Decline**

Patients with MND and evidence of co-existent FTD have a shorter survival than those with MND alone.\(^{(253)}\) Cerebral degeneration as measured by magnetic resonance spectroscopy, which occurs as part of the MND process regardless of the presence or absence of frank cognitive disturbance has also been correlated with survival in one study. The Irish MND Register has found an approximate rate of frank FTD in 6% of the incident and 7% of the prevalent population. Information has only been gathered on cognitive impairment in MND since the year 2000. It will be interesting to see if this rate remains stable over time.\(^{(254)}\)

2.5.9.8 **Mood Disturbance**

Whether low mood is due to rapidly progressive MND or vice versa is unclear. Approximately one third of patients with neurological disorders develop depression at some stage during their illness. Patients with MND who are psychologically distressed with have a twofold increased risk of dying sooner than those that are psychologically well.\(^{(254,255)}\) We do not have data on the Irish MND Register currently with regards to psychological and psychiatric comorbidities.

2.5.9.9 **Nutritional Status**

Loss of >10% of body weight has been associated with a poorer prognosis in MND.\(^{(177)}\) In addition a BMI of < 18.5 is an independent predictor of death.\(^{(71)}\) Although it is much debated as to whether gastrostomy feeding increases survival as well as quality of life, it was suggested in a single study that PEG may be an independent prognostic indicator.\(^{(173)}\) This finding has not yet been replicated. The issue of nutrition and MND is discussed in more detail in chapter 4.

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2.5.9.10  Respiratory Status

Respiratory function is universally accepted as the most important prognostic indicator in MND. The majority of studies in MND have concentrated on % FVC. Slope of decline of FVC correlates well with survival. A drop in FVC of > 50% is considered particularly significant, a difference of > 20% between sitting and supine FVC is also a useful marker of respiratory involvement.\(^{(257)}\) More recent studies have found Sniff Inspiratory Pressure estimation to be useful in the MND population. This test has the advantage of not requiring a good mouth seal and can be performed with a modified FVC/MIP/MEP respiratory meter but does require a trained respiratory technician to get accurate readings. SNIP readings tend to fall off gradually whereas FVC often remains normal until there is a sudden precipitous drop off. SNIP may be less accurate in bulbar than spinal onset MND. Once the SNIP falls below 40cm H\(_2\)O there is significant risk of death within the next 6 months unless non invasive ventilation is instituted.\(^{(72,258)}\) Sitting and lying FVC, MIP and MEP and nocturnal oximetry have also been shown to correlate with increased survival tracheostomy free.\(^{(259,260)}\) Once respiratory failure is identified in an MND patient ability to tolerate NIPPV will greatly influence survival thereafter. Tolerance is increased in spinal onset disease.\(^{(259,261)}\) NIPPV can add a median of 205 days to survival if tolerated by the patient.\(^{(262)}\) In addition early rather than late NIPPV introduction has a more profound effect on survival.\(^{(263)}\) There has been no significant change in MND survival over the past decade, we feel our study may be too early to show the effects of NIPPV on survival as it was not widely available in most centres in Ireland until 2005. It will be interesting to see future MND survival figures which should demonstrate the benefit of this intervention for patients.

2.5.9.11  ALSFRS\(\_\)r

This is the most commonly used functional rating scale in MND patients. It correlates well with outcome and the respiratory subscore in particular most closely predicts survival.\(^{(264)}\) Even in the ventilated patients the ALSFRS\(\_\)r continues to predict survival.\(^{(265)}\) Serial ALSFRS\(\_\)r values have only recently been added to the Irish MND Register data collection.
2.5.9.12  Riluzole
It is well established that Riluzole confers a modest survival benefit in MND in both Irish and international studies. (69, 118)

2.5.9.13  Specialist Care Clinics
Two population based studies have demonstrated that specialist MND clinics improve prognosis including a landmark Irish study using data from the Irish MND Register. (64, 68) A 3rd population based study did not replicate these findings. (266) It remains unclear and controversial whether this is a true prognostic indicator in MND.

2.5.9.14  Biological Markers
No one marker has consistently been found to be useful in predicting prognosis in MND. A low serum chloride and low serum creatinine have been associated with a worse prognosis in MND. (177, 267) High levels of LDL/HDL were associated with prolonged survival in another study perhaps reflecting improved nutritional status. (268) Chio et al failed to replicate these findings in their MND population but reported that low lipid levels did correlated with poor respiratory reserve. (269) Certain APOE Genotypes have inconsistently been associated with a poorer prognosis in MND and high serum APOE levels predicted a poorer prognosis in MND in 1 study. (270) The presence of a homozygous deletion of the SMN2 gene in MND confers a shorter survival time. (271) Most of these biomarkers have not been consistently shown to be useful in predicting prognosis and the biology behind some of them is poorly understood.

2.5.10  Irish Mortality and Morbidity in MND
Mortality rates in MND should track incidence rates closely as it is a terminal disease with a relatively short survival time. Unfortunately not all studies use the same definition for survival, some measure survival from onset of symptoms (i.e. Scottish MND Register) while others measure survival from time of diagnosis of disease. Apart from ongoing confusion in definitions, previously reported changes in MND morbidity and mortality rates since the 1980s were felt to be most likely due to improved case
ascertainment, lessening of mortality from other diseases and improved reporting on
death certificates. (170,272-276) Scottish figures on survival have fluctuated mildly over time
with a mean survival rate of 31 months from onset of symptoms in 2002 and 25 months
in 2004 which was reported as an unexpected decline in survival. (8) Only one other small
study from Rochester in 2002 reported similar findings. (129)

Although gastrostomy insertion is now the international standard of care in MND, there is
a cohort of patients in whom early post gastrostomy survival is reduced despite our best
efforts. These patients typically have rapidly progressive MND and are already quite
cachectic at the time of insertion and are at increased risk of peri-operative complications
in including a 'refeeding' phenomenon that is also seen in anorexic patients. (277) Some
studies not specific to MND have reported improved survival with close pre and post
procedural monitoring of the patients' nutritional status. (278) It is suggested that
radiologically inserted gastrostomy tubes may have a lower complication rate than
percutaneously inserted tubes. However either procedure can be successful in experienced
hands and both are acceptable according to AAN guidelines. (279-281) An important point to
note is that MND patients requiring gastrostomy placement may have undiagnosed
respiratory failure. Post procedure pain may cause the MND patient to splint their
diaphragms weakening an already compromised respiratory reserve. It is essential that
patients are checked for evidence of respiratory failure before gastrostomy insertion. A
number have studies have demonstrated that more patients with respiratory failure survive
gastrostomy placement if there is pre and peri placement non invasive ventilatory support.
(167, 282-285) Although it greatly improves quality of life in the majority of patients who
undergo gastrostomy insertion do not demonstrate consistently altered survival in MND.
(173) Future studies may indicate patients in whom gastrostomy insertion may be ill
advised. In our cohort there was no significant improvement in survival over the decade
studied. The median survival for Irish patients was 16.4 months and between the 1995-
1997 and 2002-2004 time periods prognosis did not alter significantly (logrank = 0.281, p
= 0.60). We feel however that neither gastrostomy placement nor (particularly) non
invasive ventilation became widely used until after our study period. We would expect
that the next follow up study should demonstrate improved survival in our MND patients particularly in relation to non-invasive ventilation.

As discussed in the section on prognostic factors in MND above Johnston et al (256) and Chio et al (173) showed that rate of disease progression is established early in the disease course and is relatively stable throughout the disease. A useful early indication of future outcome is time from first symptom to diagnosis. In general patients that are progressing rapidly will be seen quicker and diagnosis occurs shortly after onset of symptoms and prognosis in these patients is poor. Conversely patients who have their symptoms some time before they are diagnosed will continue at this rate of progression and so survival is better. (174-178) Future Irish studies should try to correlate this window between symptom onset and diagnosis with later survival. It might be a useful indicator of prognosis when the patient presents to clinic.

![Graph showing survival rates with and without Riluzole](image)

Figure 18: Survival of Irish MND patients with and without Riluzole 1996-2000. This graph demonstrates the marginal survival benefit of riluzole therapy in the Irish cohort. (Courtesy of Traynor BJ et al, 1999.) (69)

### 2.5.11 Death Certification

As part of our study death certificates were reviewed where available on all of our MND population identified from the Irish MND register during the period of study. The office of births and deaths registration at the time was unable to release to us a list of names of
patients with MND according to their death certificates. This was due to the fact that the office was in the process of changing from a paper to a computer database and also for confidentiality reasons. We were able to access figures of the number of cases of MND without names according to the central statistics office (CSO) for the same period and compare the figures. Since that time the Irish MND register has again applied for and received the data requested enabling a capture recapture study. The accuracy of this data has been discussed in an interesting recent review by Yeo et al. (286) Using capture recapture techniques and unanonymised data from the Irish MND resister, the CSO and the Irish MND association Yeo found that CSO death certification data likely overestimates the number of cases of MND in Ireland during a given period.

In our study we wished to assess whether GPs and other physicians were accurately recording MND on death certificates in patients who had MND. We searched individually for the death certificate of each patient recorded on the Irish MND register as having died with MND for the period studied. (True positives) It should be noted that we could not ascertain the number of patients who had an erroneous diagnosis of MND entered on their death certificate. (False positives) Only 77% of death certificates were available on patients registered as deceased by the Irish MND ring the period studied, this will be discussed below. Death certification data is said to have a moderate to high sensitivity and specificity for MND. Death certification accuracy studies in North America, Europe and Japan have reported a false negative rate of between 4-28%. Irish death certification however has been notoriously inaccurate in the past but had been improving. (287) An Italian study in 2004 found that the accuracy of death certification greatly depended upon the district the patient died in. (91, 94, 199, 288-290)

We compared the anonymised reported number of deaths by the CSO and the Irish MND association during the same periods there was a trend towards the Irish MND association reporting more deaths than the CSO except in 2002. This may be related to the fact that the Irish MND register is population based and from multiple sources. The CSO relies heavily on hospital based records and the information written on the death certificate. Although the sensitivity of death certification for documenting true MND is relatively
high (94%) in the records available, death certificates that most commonly omitted MND were completed in the hospital setting. Unfortunately we cannot comment on the accuracy of the missing 23% of death certificates in our population.

The reasons for the missing records may have included:

- delay in registering the death.
- delay in transferring paper based data to computerised database.
- inaccurate demographic details on the IMND Register i.e. familiar rather than christian/legal name or wrong date of birth.
- death abroad (known to have occurred in at least 1 case).

Yeo et al(286) was able to compare unanonymised Irish MND mortality data and found that approximately 80% of death certificates from patients on the MND register recorded MND as a primary or contributory cause of death. In that study approximately 80 patients were contained either on the CSO or IMND register but not on both. The IMNDA list was the least accurate of the three lists. Accuracy rates for death certificate reporting of MND cases vary widely. In 1977 two studies from Finland and Japan found that 4-8% of their MND population were classified non-MND and 6% of death certificates with the diagnosis of MND were not actually MND cases. (77,199,291) A 1987 UK study by O’Malley et al examined mortality statistics on known cases of motor neuron disease (MND) and multiple sclerosis (MS). 20-26% of respectively did not have the diagnosis mentioned on their death certificates. The authors speculated that either the certifying doctor was unaware of the diagnosis of MND or MS or failed to appreciate the importance of accurate death certification to national statistics. (292) This situation is not unique to the community setting or to the UK, Doyle et al also showed serious errors in completion of death certificates in 3 of the major teaching hospitals in Dublin. 45/100 death certificates that were reviewed before and after autopsy contained 55 errors. 19 certificates were ‘so inaccurate as to warrant change in the underlying cause of death’. (287)
Chio et al also examined MND patient death certificate accuracy in Northern Italy (Piedmont region) between 1966-1985. Certification was obtained in 96% of cases and MND was the primary cause of death listed in approximately 75% of cases. True positive rates did not vary over the 15 year period studied although the proportion of true positives showed a decreasing trend as age of patient at death increased. In 15% death was attributed to intercurrent illnesses such as myocardial infarction, respiratory failure, pneumonia and malignancy. In 10% cases erroneous diagnoses were observed such as MS, malignant tumours and muscular dystrophy. The authors concluded that death certificate diagnosis of MND in Italy was sufficiently accurate for use in descriptive and analytical epidemiology. (293)

Maudsley et al found that a significant percentage of house officers and general practitioners felt undertrained in completing death certificates accurately. (294) Chancellor et al demonstrated that 30% of patients coded with MND in hospital did not have the disease and that 23% of patients in hospital with MND were not coded as having the disease. The same group demonstrated a 10% false positive and a 6% false negative rate in death certificate records with regards to MND. (295) The authors comment that false positive cases might contribute to occasional reports of apparent increases in MND mortality. (94, 198, 199, 290, 292, 295) However in cancer mortality studies similar disease mortality fluctuations are usually attributed to ‘improved accuracy of death certificate reporting’ rather than increased false positive cases. (296)

From 1963-1990 Dean et al found that MND was recorded on the death certificates of 86% of UK MND cases, with no change in accuracy of recording over time. (297) A 1996 US study suggested that knowledge about the importance of accurate death certification was quite poor among hospital physicians of all levels and medical students making improved reporting an unlikely scenario unless there was a serious effort made to improve education on this matter. (298) A Northern Ireland study around the same time confirmed that this problem affects all health systems with an overall 34% level of inaccuracy among GPs and hospital doctors. Interestingly in that study hospital doctors were responsible for 62% of mistakes compared with 38% due to GPs, possibly reflecting
the inexperience of junior doctors in this setting.\textsuperscript{272} Myers et al designed an educational intervention on death certification for internal medicine residents at their institution. Major errors were found in 32.9% of death certificates prior to intervention, a rate comparable to other institutions and improved to 15.7% after the intervention, demonstrating that education can help improve death certification accuracy but probable needs to be ongoing and mandatory. A similar program could also be implemented among GPs in the community. A 2000 UK study showed an overall poor (9-27%) documentation of prosthetic heart valves on death certificates over a 12 year period. Recording was more likely in the young who died than in those aged > 65 years which concurs with Chio’s earlier study showing that documentation of details of death in older patients is less detailed and accurate.\textsuperscript{293, 299}

Ragonese et al reported very poor true positive rates (48% and 46%) in their study of MND and MS in Palermo, Italy.\textsuperscript{300} A multi-region follow up study in 2004 reported true positive rates of MND with 67% for Northern Italy and 52% for Southern Italy over a 26 year period.\textsuperscript{288} A Finnish study of death certification errors at an academic institution had pathologists complete a mock certificate in 50 cases after reviewing the patients’ charts and this was then compared with the actual death certificate. 64% of the actual death certificates respectively contained grade III-IV (moderate to severe) errors, 82% had multiple errors. The authors concluded that physicians need regular and continual training on the accurate completion of death certificates.\textsuperscript{301} Interestingly once unanonymised lists of deaths from MND recently became available to the Irish MND group both the CSO generated and the MND register generated lists had an estimated accuracy close to 80%. Both lists were missing approximately 80 patients using capture recapture but these figures may be inaccurate as the capture recapture suggested a crude incidence of 3.45/100,000 for MND in Ireland which is higher than expected and outside the CI for most MND registers in Europe.\textsuperscript{286} There are many reasons why death certification data might be inaccurate or incomplete and errors can occur at any stage in the completion of registration from the doctor at the bedside or general practitioner in the community to the HIPE technician in the hospital administration section or the registry office clerk who transfers the data to their computer system. When a person in Ireland
passes away at home in unsuspicious circumstances it is up to their general practitioner to assign a likely cause of death. This is thus a guesstimate if you will of what process the patient died from. The cause of death may not be due to failure of a specific organ or system ie respiratory failure but must be due to a specific pathological process ie pneumonia. The death certificate allows for the inclusion of up to three recent other significant illnesses or diseases that may have contributed directly to the persons death and three longstanding significant medical issues that the patient may have suffered from. The most common listed causes of death at home in the elderly in Ireland would be myocardial infarction, stroke and pneumonia. In the case of MND the majority of people in Ireland die at home from pneumonia and occasionally pulmonary embolus, but the death is usually expected and makes assignment of cause of death easier. Some practitioners will put MND as the direct cause of death although most will place it in the second category with for example pneumonia as the direct cause of death. Accuracy of the listed cause of death in MND is multifactorial but largely reliant on the physician who signs the death certificate, how well they knew the patient and experience with completing death certificates. One could for example merely put pneumonia on the death certificate and exclude MND and in Ireland this would be considered acceptable by the office of births, deaths and marriages. Once the relative of the deceased receives the death certificate they may bring this to the registration office and the handwritten form must be read and interpreted by the office employee and filed accordingly, there has been an effort to gradually computerize this system. In the past even looking up death certificates was by hand. This antiquated system was limited by the form design and the persons completing the form. Currently the data still needs to be read and transferred from the official handwritten death certificate to the computerized form and some offices are merely scanning original death certificates onto their systems which can be of varying quality.

The CSO in Dublin produces yearly figures of mortality due to different diseases using death certificates and hospital inpatient enquiry database (HIPE) services. These services run in all hospital and code all inpatients on discharge or death according to the diseases mentioned in their notes. The people trained to do this are not medically trained and can
frequently misinterpret potential differential diagnoses lists as diagnoses assigning
diseases to patients electronically when they are not proven. Computerized discharge
letters have been introduced in many but not all Irish hospitals which should improve the
accuracy as these are written by medical staff and can be used as a template for HIPE
staff. All of these issues greatly impact national statistics produced on motor neuron
disease incidence, prevalence and survival and also budgets for motor neuron disease
care, comparison between different services caring for MND and attempts to improve
support services for these patients. These are not problems isolated to MND but can be
applied to many other patients with other conditions also. In our population where MND
as the cause of death was completely omitted on the death certificate (6%) Pneumonia
and respiratory failure were the two most common causes of death. These would be
common direct mechanisms of death in MND patients. Similarly Yeo et al found
pneumonia, cardiovascular disease and pulmonary embolus were the most common cause
of death cited in those patients where the MND diagnosis was omitted. (286)

Although we only have death certification data on 77% of our cases, the majority of
MND patients from both periods were from either Leinster or Munster (Figure 8). This
would mirror the population spread of Ireland but also reflecting the location of the only
multidisciplinary specialized clinics for MND which are in Dublin (Leinster) and more
recently Cork (Munster). This places considerable financial burden on the involved
hospitals and their healthcare budgets. There was little change in the location of death of
patients over the two periods studied with approximately 50% of patients with MND
dying in acute hospitals. We expected or hoped to see a reduction in deaths located in the
acute hospital setting as the majority of patients would prefer to die at home or in a
hospice. However, the majority of patients died in acute hospitals which reflects the
significant burden that MND places on the acute hospitals. Approximately 1/3 of patients
died at home during both periods with a trend towards increased home deaths in 2002 and
2003. (Figure 10) These patients would all have had the hospice home care team involved
unless the service was refused or the death was unexpected. In cases where death is
expected and the course of disease is not extremely rapid it could be argued that the
hospice or home setting would be a more suitable setting for patients who are dying.
Acute hospitals are in general noisy, busy places with fast turnover of patients, staff and environment and rigid visiting hours. A more peaceful constant environment with regular staff, knowledgable in the care of the dying and flexible visiting hours are more suitable when a patient is dying. The hospice movement in Ireland has undergone considerable change in the last 20 years. In the past the emphasis has largely been on those patients dying with cancer though increasingly a wider variety of terminal conditions, including MND are being referred to and accepted by hospice teams in Ireland. In both periods approximately 12% of all MND patients died in the hospice setting although there was a trend towards increased numbers of patients dying at home in 2002 and 2003 with hospice home care involved in the majority of cases. Yeo et al also reported that 95% of the MND population died in hospital, at home or in the hospice in their 2010 study.\textsuperscript{(286)}

Marital status does appear to influence location of death. Although less married patients died in nursing homes or the hospice, surprisingly more married patients died in hospital than at home. Most widowed or single patients died in hospital but a significant proportion also died in the other locations. (Figure 11) Sex also influenced location of death. Males outnumbered females in all locations reflecting the ratio of males > females except in nursing home deaths where females outnumbered males. Less females died at home than males except during the period 1998-2001. The reasons for this are unclear. (Figure 12)

2.6 Conclusions

Irish MND incidence and prevalence figures have remained relatively static over time and are identical to figures in Northern Ireland. The rates of familial MND and MND with FTD in Ireland were similar. As we have only begun to document FTD among MND patients the latter figure may rise in the future. Time from onset of MND symptoms to diagnosis has also been static, thus our ability to recognise cases early or patients’ ability to recognise something is wrong and seek help has not particularly improved and unfortunately waiting times for consultant neurologists in Ireland can also be unacceptably long. The ratio of males to females among the Irish population has
remained at 1.4:1 over the last decade. Bulbar onset disease also remains less common than spinal onset disease but appears to influence survival times the most. The survival of Irish MND patients has not improved statistically despite the introduction of gastrostomy feeding and non-invasive ventilation over the past decade. One might expect to notice improved survival at the next time of analysis as use of NIV becomes more widespread. Gastrostomy feeding appears to influence quality rather than quantity of life in most patients in other studies to date.

Death certificates are a useful data source in MND but our study was hampered by almost 25% of documents being unavailable and the anonymity of the CSO data. The latter has since been rectified and has been reported by Yeo et al. (286) We found a high percentage of the certificates obtained documented MND accurately in definite cases. We were not able to comment on cases that were inaccurately called MND as we were working from a list of known cases. The biggest burden of disease was in Leinster and Munster and most cases died in hospital or at home so it is appropriate that the MND specialist clinics (an established clinic in Dublin (Leinster) and a new clinic in Cork (Munster)) are in these regions and are financially supported. Future funding for the terminally ill should be aimed at the hospice (to improve hospice-home care and in patient care) and the key hospitals involved in MND care.

Epidemiological studies in MND have advanced somewhat over the last 2 decades. Prospective population based studies are preferred to retrospective case studies or case series. The establishment of diagnostic criteria have removed many mimic syndromes from studies and EMG/NCS studies have advanced technically to allow definitive and reasonably early diagnosis in most cases. The search for other early disease biomarkers may allow future earlier inclusion of patients into studies. In addition establishing both environmental and genetic risk factors for sporadic MND is becoming increasingly important as we recognise the complex interactions between man and the environment in the development and progression of the disease. In diseases such as MND patient numbers are often limited due to the high turnover of cases which is an obstacle that can only be overcome either by longer term studies, meta-analysis or international co-
operation. Recently a European consortium of investigators with established national registries (EURALS) have joined forces to share knowledge and clinical/biological data on MND populations to advance knowledge on the disease and to evaluate potential therapies.

Future epidemiological studies in MND should focus on the identification of biomarkers for early disease, risk factors (both genetic and environmental) and new prognostic indicators. The optimum clinical pathways of diagnosis and treatment should also be studied on an ongoing basis. In our study significant clinical differences were noted in the incident and prevalent MND populations which may have a significant impact on MND related research worldwide. At the moment European and USA study groups have a preference for the former and the latter populations respectively which will make it difficult to compare the findings in studies on these MND populations. This is partially related to the health systems in these territories but it is something that should be addressed before these communities can truly share data on an equal footing. Circular MND epidemiology where similar ideas have been proposed and discussed in numerous articles with no useful decisions or conclusions being drawn by the end can and should be avoided in future epidemiological work. This may be achieved by aiming for the highest possible quality of MND studies and the highest possible classes of evidence while recognising the epidemiological limitations of a disease such as MND.
References


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Chapter Three
Exercise and MND

3.1 Introduction

Exercise and motor neuron disease is a controversial topic that has divided the experts since the time of Lou Gehrig. In the 1940s Wilson and Bruce stated ‘the view that strenuous occupations predispose (to ALS) must be received with caution; workmen, athletes and others engaged in heavy muscular performances are sometimes affected, but to a very large number more, the theory does not apply’.\(^\text{(1)}\) In 1962 Critchley wrote 'Nothing has been said about the possible role in aetiology of a previous habit of athleticism. I have the uncomfortable feeling that a past history of unnecessary muscular movement carried out for no obvious reason may be followed in later life by the development of motor neuron disease in a statistically significant number of cases.'\(^\text{(2)}\) To date accepted risk factors for MND include male gender, increasing age and family history. Smoking statistically also appears to increase risk of developing the disease though this is controversial.\(^\text{(3-9)}\) Identifying a potentially modifiable exogenous risk factor such as excessive physical activity could increase our knowledge of the disease process and could prevent or delay disease onset in susceptible individuals. To date the literature on this topic has been confusing and conflicting and fraught with confounding variables. What the exact mechanisms behind such an association might be and how we might identify those at risk remains unknown.

Italian epidemiological studies have shown an increased risk of MND among soccer players in Italy compared to the general population.\(^\text{(10)}\) Whether this is due to excessive exercise triggering some catastrophic event, chronic exposure to chemicals such as fertilizers used on the grounds or toxins from other sources i.e. illegal drugs or recurrent traumas during the games remains unclear. An increase in polymorphisms in the promoter region of the enzyme paraoxonase (PON1) which detoxifies organophosphate fertilizers has been described in sporadic MND but is controversial.\(^\text{(11)}\) Sceptics argue that the majority of elite athletes do not develop MND and that those that do stick out in
our memories because their sporting talent and athleticism makes the occurrence of MND all the more tragic and memorable. A number of high profile cases of MND in sportsmen worldwide have drawn attention to the issues involved: Lou Gehrig (baseball), Catfish Hunter (baseball), Ezzard Charles (boxing), Jimmy Johnstone (soccer), and Matt Hazeltine (American football). Clearly not all sportsmen get MND and in fact a recent Polish study has shown that compared with actors and clergymen, sportsmen who participated in the Olympics for Poland in the past had a longer mean survival using mortality data from 1946-2000. No figures are given for mortality from specific diseases.\(^{(12)}\) It is likely that a combination of genetic susceptibility factors and environmental exposures combine to produce the disease which may explain why all athletes are not affected. However, experiments on MND mouse models have failed to demonstrate acceleration of disease onset or course with sustained heavy exercise. In contrast some studies suggest that limited physical activity early on in the disease may delay progression particularly in male mice.\(^{(13-15)}\)

There are a number of theories as to why excessive exercise may be harmful. Excess physical activity can alter the balance between free radical formation and radical scavenger systems leading to oxidative stress.\(^{(16-17)}\) Physical activity in susceptible individuals can also cause overstimulation of motor neurons (excitotoxicity) thus enhancing motor neuron death.\(^{(18)}\) Quantifying physical activity or lifetime energy expenditure is a complicated process. Currently the accepted best method is a retrospective questionnaire of which there are many validated options. This is not an entirely optimal method for reasons that will be discussed in more detail below. As we cannot predict who will develop sporadic MND there are no alternative methods at present.\(^{(19)}\) In this article we will present and summarise the data available both for and against the association of exercise with MND, discuss the challenges associated with using questionnaires based on retrospective recall in an ageing population and present our own data on exercise in the Irish MND patient population compared with normal controls.
3.2 Aims

3.2.1 Study 1
To determine if the incidence of MND is higher among Irish individuals who completed the Dublin city marathon.

3.2.2 Study 2
To interview MND patients with regards to lifetime physical activity habits prior to diagnosis and calculate total metabolic energy expenditure in comparison to a normal population using a recognised and validated activity questionnaire.

3.3 Subjects and Methods

3.3.1 Study 1
Individuals completing the 1994 and 1996 Dublin city marathon (DCM*) were identified from publicly published lists. Names and ages were compared to names and ages of MND patients listed on the Irish MND register from 1993-2002. Crude standardised mortality ratios for all individuals who completed the DCM* in 1994 or 1996 were calculated using SPSS version 15.0.

3.3.2 Study 2
MND patients were randomly selected from the Irish MND Register or approached at the MND clinic in Beaumont hospital, Dublin and asked to participate in this study. All patients included fulfilled the EEDC for definite or probable sporadic MND. Patients were age, sex and weight matched to controls. Smoking status was also documented, if a subject had ever previously smoked on a regular basis they were documented as having ‘smoker’ status. Kriska’s physical activity questionnaire was employed which has been previously validated.\(^{20-23}\) The majority of interviews were in person, though some were conducted via telephone because of geographical distance. Patient controls were age, sex and geographically matched to within the same county to allow for urban/rural lifestyle
differences. SPSS version 15.0 was used for ANOVA and logistic regression statistical analysis.

3.4 Results

3.4.1 Study 1
5,069 individuals completed either the 1994 or the 1996 marathon. 1,069 runners were not from Ireland and were excluded. Data was incomplete for 68 runners who were excluded from the analysis. 396 individuals ran both the 1994 and the 1996 marathons. Their data was included only once. In total, name and age data was available for 3,536 marathon runners who were Irish or unknown nationality. 1,048 patients were diagnosed with MND in Ireland between 1993 and December 2004. Comparison between the 3,536 marathon runners and the 1,048 Irish MND patients revealed 1 patient who had run the DCM and then subsequently developed MND. The affected subject ran both the 1994 and 1996 marathons. He developed disease within 10 years of completing the marathon. Observed incidence of MND among the cohort of individuals who had run the 1994 or 1996 marathon was 1 per 3,536 runners over 9.2 years of follow up. Based on previously published age- and gender- specific incidence rates for MND in Ireland, the expected number of cases among 3,536 individuals observed for a 10 year period was 0.51. Crude standardised mortality ratio for MND was 1/0.51 = 1.96.

3.4.2 Study 2
One hundred and four MND patients agreed to participate in the study however 3 patients were judged unreliable historians and were excluded. None of these patients had formal neurocognitive assessments so this decision was made on a case per case basis by the interviewer. 1 patients had their diagnoses revised and was excluded. Data was analysed once 100 MND patients were included in the study. Patients were analyzed as a single MND group and also according to their MND subtypes (bulbar Vs spinal). The only significant demographic difference between the groups was seen in mean weight, where controls were heavier than MND patients (p < 0.0001). Weights were similar among subtypes of MND. (Table 1)
Table 1. Demographic characteristics of the sample

<table>
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<tr>
<th>Variable</th>
<th>Cases Bulbar N</th>
<th>Cases Spinal N</th>
<th>Cases All MND n</th>
<th>Controls n</th>
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</tr>
<tr>
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<td>47</td>
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<td>54</td>
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</tr>
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<td>Mean Age (yrs)</td>
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<td>58.7</td>
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<td>43</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>11</td>
<td>42</td>
<td>53</td>
<td>57</td>
</tr>
<tr>
<td>Mean Weight (kg)</td>
<td>68.6</td>
<td>71.2</td>
<td>69.9</td>
<td>77.6</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

There were more males than females in the control and spinal onset disease groups. This ratio was reversed in the bulbar onset group although the difference was not found to be significant ($p = 0.1563$). (Figure 1)

![Type of Disease by Gender](image)

Figure 1: Similar ratios of male:female were seen in the spinal onset and control groups. There was a non significant trend for females>males in the bulbar group

No significant differences were found in the ages of the respondents in any of the three groups ($p = 0.3252$). (Figure 2)
Figure 2: Mean age in all studied groups were similar

The control group showed a significantly higher mean weight than either bulbar onset or spinal onset subjects ($p < 0.0001$). However, spinal and bulbar onset cases showed no difference in weight between one another. (Figure 3)

Figure 3: Control group had a significantly higher mean weight (kg) than either the bulbar or spinal onset patient weights. ($p < 0.0001$) There was no difference in weight between the spinal and bulbar groups.
No differences were found between controls and MND disease subtypes and smoking rates \((p = 0.8462)\), the percentage of smokers stayed fairly constant between all groups. (Figure 4)

![Type of Disease by Smoking](image)

**Figure 4: Smoking rates among study groups were similar**

Total exercise differed between spinal and bulbar onset disease \((p = 0.0287)\), spinal onset patients had consistently higher reported rates of physical activity than controls and bulbar onset cases had consistently lower rates of exercise than controls, but the difference was not significant in either group. (Figure 5)
Figure 5: Total exercise (excluding work related activity) differed between spinal onset and bulbar onset ($p = 0.0287$). Neither group was found to be significantly different to the control group.

There was a significant difference between total exercise plus work among the spinal onset disease group and controls and also the spinal and bulbar onset disease groups with a higher reported rate of physical activity among the spinal onset group ($p < 0.0001$). The bulbar onset MND group and controls were not found to be significantly different to one another. (Figure 6)
Figure 6: Total exercise (including work related exercise) was significantly higher in the spinal onset group compared to both controls and bulbar onset disease ($p < 0.0001$)

Reported vigorous exercise differed between spinal onset MND patients and controls and also between spinal onset and bulbar onset subtypes of MND ($p = 0.0016$). Bulbar onset patients and controls were not found to be significantly different to one another. (Figure 7)

Figure 7: Vigorous exercise (> 6 MET) was significantly higher in the spinal onset group compared to both controls and bulbar onset patients. ($p = 0.0016$)

Due to the differences in exercise found between the two types of Motor Neuron Disease, three logistic models were evaluated to investigate fully the relationship between the
types of disease and a history of physical activity and other risk factors for MND: bulbar and spinal MND were examined individually and MND was then examined as a whole. Total exercise including work was the figure used for evaluation as as potentially increasing risk of MND. risk factor for MND. As the three main measures of exercise activity were related to one another in their calculation, only one of the three measures was chosen for evaluation. Since total exercise (recreational plus Work (MET-hrs per week)) was the most significant measure in predicting control versus Motor Neuron Disease, this variable was chosen. Gender, age and smoking were not found to be significant predictors of spinal onset MND, however, exercise and weight were. (Table 2)

Table 2: Spinal onset MND Logistic Model Calculations

<table>
<thead>
<tr>
<th>Effect</th>
<th>Point Estimate</th>
<th>95% Wald Chi-Square Confidence Limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender M Vs F</td>
<td>1.428</td>
<td>0.666</td>
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<tr>
<td>Age (Years)</td>
<td>1.018</td>
<td>0.988</td>
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<tr>
<td>Smoking No Vs Yes</td>
<td>1.153</td>
<td>0.588</td>
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<tr>
<td>Total Exercise plus Work MET hr</td>
<td>1.004</td>
<td>1.002</td>
</tr>
<tr>
<td>Weight (KG)</td>
<td>0.941</td>
<td>0.909</td>
</tr>
</tbody>
</table>

Weight only was found to be a significant predictor of bulbar onset MND. Gender, age, smoking and exercise were not. (Table 3)

Table 3: Bulbar onset MND Logistic Model Calculations

<table>
<thead>
<tr>
<th>Effect</th>
<th>Point Estimate</th>
<th>95% Wald Chi-Square Confidence Limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender M Vs F</td>
<td>0.815</td>
<td>0.277</td>
</tr>
<tr>
<td>Age (Years)</td>
<td>1.009</td>
<td>0.962</td>
</tr>
<tr>
<td>Smoking No Vs Yes</td>
<td>0.897</td>
<td>0.332</td>
</tr>
<tr>
<td>Total Exercise plus Work MET hr</td>
<td>1.000</td>
<td>0.997</td>
</tr>
<tr>
<td>Weight (KG)</td>
<td>0.934</td>
<td>0.891</td>
</tr>
</tbody>
</table>

Exercise and weight but not gender, age or smoking were found to be significant predictors of all types of MND. (Table 4) For every hour of exercise, MND risk increases slightly but the Odd Ratio is very close to 1 i.e. for every extra hour of
exercise the respondent is just slightly over one times more likely to suffer MND. For weight, increased weight indicates a lower risk of MND. In this instance, for every extra kg the subject weighed, they were 0.94 times as likely to have MND.

Table 4: Logistic Model Calculations = ‘All MND’

<table>
<thead>
<tr>
<th>Effect</th>
<th>Point Estimate</th>
<th>95% Wald Chi-Square Confidence Limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender M vs F</td>
<td>1.25</td>
<td>0.63</td>
</tr>
<tr>
<td>Age (Years)</td>
<td>1.02</td>
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<tr>
<td>Smoking No vs Yes</td>
<td>1.07</td>
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<tr>
<td>Total Exercise plus Work</td>
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<tr>
<td>MET hr</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Weight (KG)</td>
<td>0.94</td>
<td>0.91</td>
</tr>
</tbody>
</table>

3.5 Discussion

Only 1 case of MND was identified among the marathon runners studied, statistically however this was significant because only 0.5 cases were expected. It is difficult to know how to interpret this data given that you cannot in real life have half a person affected. Anecdotally the Irish MND register during that period did contain at least 3 other serious marathon runners who for various reasons did not run the DCIM in the years studied. This study would need to be replicated to establish whether the results are truly meaningful. There are no similar studies in the literature with regards to long distance running and MND, however the reported cases of Italian football players in Italy were picked up using similar records kept by the Italian football Association and will be discussed in more detail later.

The modifiable or historical leisure activity questionnaire used in study 2 was originally developed by Kriska et al to assess lifetime and recent physical activity in Pima Indians.\(^{(20)}\) It has been shown to be useful, valid and reliable in a variety of populations of all age groups and ethnic backgrounds.\(^{(21-23)}\) Lifetime physical activity can be calculated indirectly by averaging the total amount of time spent doing each activity throughout a lifetime and multiplying each activity by its’ metabolic equivalent (MET) or the energy
cost of that activity.\textsuperscript{(24)} Total energy expenditure for each activity can then be calculated using the following equation: kilocalories = MET x hours of activity x body weight in kilograms. 1 MET (which is equal to 1 kcal/hr/kg body weight) is considered to be the resting metabolic rate while sitting quietly.\textsuperscript{(19)} Individual METs for 605 specific activities at different levels of physical activity from mild to vigorous are described in the published Compendium of Physical Activity. An activity that has an energy cost of > 6 MET is considered to be vigorous energy expenditure.\textsuperscript{(24)}

A number of assumptions are made using this method firstly, that the person being interviewed will be able to accurately and honestly recall activity from decades before. Secondly, that the level of energy expenditure is maintained throughout the period of exercise and also maintained over many decades of performing the same activity. Thirdly, that all the activities described are present in the compendium. Fourthly, that the estimated energy expenditure is based on assumed resting metabolic rate that may vary amongst individuals, the compendium of activities is only a guide. The resultant calculation is therefore an estimate only of previous physical activity with some accepted inherent flaws. Such estimates from physical activity questionnaires are valuable because they can be used to rank persons or groups of persons within a population from the least to the most active.\textsuperscript{(22, 25-26)}

Studies comparing actual metabolic energy expenditure as measured using the doubly labelled water technique with recalled short term energy expenditure.\textsuperscript{(27)} have shown good correlation in short term testing indicating that most people do recall physical activity accurately and honestly using a physical activity questionnaire with regards to recent exercise.\textsuperscript{(21, 27)} However, it is also recognised that quantitative recall questionnaires are subject to some recall bias with time spent in vigorous-intensity activities overestimated and habitual daily activities such as walking difficult to recall and underestimated. Responses may be biased based on demographics such as age, sex and education of the subjects.\textsuperscript{(19)}
There are a number of features of the MND population that are somewhat unique and potentially troublesome when assessing physical activity. The majority of the MND population is > 60 years of age and recall may be impaired, although notably Kriska et al have used the same questionnaire in an elderly population without difficulty.\(^{(22)}\) It is now accepted that 5-10\% of ALS patients have a frontotemporal dementia while other patients demonstrate more subtle cognitive changes.\(^{(28)}\) The frontal variant of FTD is most common which can affect verbal fluency, executive functioning and attention but not specifically recall.\(^{(28-29)}\) Alzheimer’s disease has only rarely been described with MND.\(^{(30)}\) In our population the average age in all groups was similar between cases and controls (mean: 56-59yrs). (Figure 2) In 3 cases the patients either appeared disinhibited, were unable to recall details from their past clearly or gave answers that were inconsistent and this data was not used.

In MND the male: female ratio is reported as 1.5:1 and males tend to exercise more than their female counterparts. It should be noted however that this ratio male: female appears to approach 1:1 as patients become older, particularly after the age of 60 years.\(^{(31-33)}\) For this reason we also matched patients and controls by gender. We found that there were more males than females in the control and spinal onset disease groups but that this ratio reversed in the bulbar onset group although the difference was not found to be significant and overall our cases and controls were well matched. (Figure 1) In our study neither gender nor age significantly predicted the development of MND regardless of subtype of MND.

Lifetime physical activity is calculated as a function of weight in kilograms. Most MND patients become underweight throughout the course of their disease. Despite our best efforts to match patients and controls with regards to weight there was still a significant overall difference between cases and controls in our study. (p<0.0001) The mean weights of MND patients were approximately 7-9kg lower than controls, with bulbar onset patients having the lowest weights. (Figure 3) In practical terms a higher weight should increase your overall MET-hours per week calculation which would mean that we might expect overall energy expenditure to be higher in those with higher weights i.e. the
controls, however this was not the case. (Figures 5 & 6) Interestingly risk of MND increased as weight declined. Scarmeas et al also reported a trend towards increased risk of MND in those with a 'lean body habitus', for slimness the OR was 2.1 in that study (95% CI: 1.40 to 3.47).\(^{(34)}\) In our study for every extra kg the subject weighed, they were 0.94 times as likely to have MND (conversely for every kg lighter the respondents were they were 1/0.94 = 1.06 times more likely to have MND). It is important to note that the reduced body mass in MND may also be a result of the disease process rather than contributory to development of the disease itself.

Smoking has been reported as a risk factor in MND in a number of studies.\(^{(8-9,35-36)}\) We did not control specifically for smoking when assigning controls to MND cases in our study however despite this, numbers of smokers and non-smokers were fairly evenly distributed between the groups. (Figure 4) In addition smoking did not increase risk of MND according to our study. It is possible that because there was an excess of physically active cases in our study that these people were less likely to smoke.\(^{(8-9,35-36)}\) This topic is reviewed in more detail in the section risk factors for MND in the chapter on MND epidemiology.
The literature up until 1998 is littered with articles both supporting and refuting the association of physical activity and MND, mostly small retrospective case control studies and case series. The standard of these studies however is variable. The worldwide diagnosis of MND was not standardised until a meeting at El Escorial in 1994. These criteria were further refined in 1998 at Arlie House. Earlier studies may have erroneously included large numbers of MND mimic syndromes and so cannot be fully trusted. Methodology of these studies was frequently flawed with inadequate numbers, poorly matched controls and inadequate consideration of confounders/other risk factors. A paper in 1991 by Longstreth et al deserves mention because it discusses some of the potential mechanisms by which exercise in excess may cause harm to the nervous system. Vigorous physical activity might increase exposure to environmental toxins, facilitate the transport of toxins across the blood-brain-barrier, increase absorption of a toxin by the lower motor neurons, or increase susceptibility of motor neurons supplying fast twitch fibres by stressing activity. It has been shown for example that polio virus affects preferentially limbs that have recently been exercised around the time of infection. Armon has published guidelines on studying exogenous risk factors such as physical activity in MND, however few studies to date have produced high levels of evidence on this topic. Veldink et al produced a useful table showing the studies to date (as of 2005) and their level of evidence using Armon’s methods. Additional studies since 2005 have been added to the original table in the figure below. (Figure 8) According to Armon only class III or higher evidence can inform conclusions.

In 1996 Strickland et al published a retrospective case control study examining the association between MND and physical activity and trauma. A positive association was noted between MND and sweating during work, or physical activity, or having been involved in competitive school or college activities. (OR: 1.6 (95% CI 1.1-2.4); OR 1.6 (95% CI: 1.1-2.5); OR 3.1 (95% CI: 1.04-9.3)) respectively. Criticism of the study included the small sample size, use of prevalent rather than incident cases and selection as the patients all came from a specialist MND clinic. The questionnaire used was not described in detail with regards to validity or methods. Logistic regression was used to analyze the data because of multiple hypotheses testing.
Class of evidence for physical activity as a RF for MND studies

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<td>25</td>
<td>55</td>
<td>88</td>
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Figure 8: Adapted from Veldink et al, 2005 with new information from Armon, 2007. Figure demonstrates the classes of evidence available for studies on physical activity and MND. (16, 49-50)

Longstreth et al examined the risk of amyotrophic lateral sclerosis and physical activity at work and leisure in a 1998 population based case control study. (52) His group found overall no difference in physical activity between cases and controls but that MND cases were more likely than controls to have participated in organised school sports. (OR 1.52, 95% CI: 1.03-2.25) There was also a non significant trend of an association between ALS and vigorous physical activity in and out of the work place at a young age. (OR not significant) Physical activity at home was also recorded but not discussed in the paper. 174 incident cases were recruited from three Washington counties over a period of 4 years with 97% ascertainment. 174 cases and 348 controls were interviewed. Details of sporting activities and work related physical activity were documented until 10 years prior to MND. 10 years was chosen to exclude the potential effects of subclinical disease before the reference date of the patients’ diagnosis. Strengths of this study include evaluation of work and leisure time activity, adequate population size and two controls per patient interviewed. The authors calculated hours of vigorous physical activity from 15 years up until 10 years prior to diagnosis of MND. Weaknesses include inclusion of a number of familial cases and use of 2 different methods for obtaining controls due to inadequate response rates with the initial method, the second method used Medicare eligibility lists which may have skewed the control population. Also, 20 patients were deceased before they could be interviewed and surrogate information was introduced. (52)
Scarmeas et al examined premorbid weight, body mass and varsity athletics in MND in their paper of 2002. In this retrospective case control study of MND cases from 1992-2000 patients were more likely to have participated in varsity athletics and to have had a slim body habitus than controls. (OR 1.89 (95% CI: 1.05-3.4)) and OR 2.1 (95% CI: 1.08-4.07)). A ‘yes’ and ‘no’ style questionnaire was used which was not previously validated. BMI was documented on all cases. Although 279 cases of MND were included, standardised EEDC were not used. There were only 152 controls who were not a ‘normal population’ but who had various other neurologic diseases recruited from a single specialist neuromuscular clinic over an eight year period. Obviously any neurological disease has the potential to seriously impact ones’ exercise capabilities, thus the control and may have resulted in inaccurate results. The adjusted ORs were only significant for slim habitus and participation in varsity athletics. This study had the potential to be simple and work well having a decent sample size but the lack of validation of the questionnaire used and the use of a smaller number of neurologically impaired patients as controls makes the findings difficult to interpret.

Vanacore et al postulated that branched chained amino acid supplements should be considered as a potential toxin in MND associated with exercise due to their potential glutaminergic effects in the brain during catabolism. Interestingly skeletal muscle and the brain contain the highest quantities of enzymes involved in the catabolism of branched chain Amino Acids (BCAAs) such as branched chain amino transferase (BCAT) and branched chain alfa ketoacid dehydrogenase (BCKD). Two mammalian BCAT exist, mitochondrial (BCATm) and cytosolic (BCATc) localised to both astroglia and neurons. BCATc is particularly prevalent in pyramidal neurons.

Valenti et al published a multicentre retrospective case-control study in 2005 on amyotrophic lateral sclerosis and sports. This study failed to demonstrate an association between sports or sport associated trauma and MND. 300 new consecutive cases of probable or definite MND by EEDC were recruited from 10 MND speciality clinics in Italy over a 17 month period. (9 familial cases were also included) Cases
included 193 males and 107 females. Controls were age, sex and geographically matched but details of ascertainment are not given. A ‘standardized questionnaire’ on sports participation and related trauma was used, validity was not discussed. Although this study has good case ascertainment, the lack of validity discussion on the questionnaire used and the scant details on how controls were obtained raises doubt about the credibility of their findings. There is also no data included on how many ‘trained operators’ there were or about inter- and intra-rater variability if this is a newly designed questionnaire.\(^{(57)}\)

Also in 2005 Veldink et al investigated lifetime physical activity and MND in a population based case-control study. No significant association between MND and lifetime physical activity was seen, however patients with earlier onset of disease had more cumulative physical activity before the age of 25 than other cases.\(^{(16)}\) (HR 1.6 (95% CI: 1.2-2.2)) The study involved 219 incident cases, diagnosed by EEDC with definite, probable or possible disease, excluding known familial cases. All cases attended one of two national MND referral centres; the population encountered had a sex distribution as described in other population but a younger mean age of onset of disease and a relative predominance of spinal onset disease suggesting referral bias. 254 controls were volunteered by the patients themselves and so were likely to have similar lifestyle histories potentially hiding any difference between cases and a truly normal population. The planned case: control ratio was 1:2 but this was not achieved. A postal questionnaire was used which may have led to response bias and incorrect interpretation of questions. Temporal relationship between timing of physical activity and onset of disease was documented as physical activity in early, late or total lifetime. Analysis of physical activity was adjusted for age, sex, BMI, education, alcohol and smoking. The authors found that smoking and alcohol were both independently associated with MND by logistics regression analysis. Higher cumulative leisure times were associated with an earlier age of onset of MND (early physical activity: HR = 1.7, 95% CI = 1.3-2.4, p < 0.001 and late physical activity: HR = 1.6, 95% CI = 1.2-2.2, p < 0.001).\(^{(16)}\) Kaplan Meier curves showed that in patients with higher leisure time activities before the age of 25, onset of disease was 7 years earlier (logrank test: p = 0.001) and that higher leisure time activities during the 10 years before the reference date resulted in earlier onset of disease.
by 3 years (logrank test: p = 0.02)\(^{(16)}\) In a review of the literature on physical activity and MND, applying the guidelines suggested by Armon in 2003 the authors concluded that there was less evidence of a link between MND and physical activity at higher evidence levels.\(^{(16,49)}\)

There has been increasing interest in a possible association between MND and soccer for the last decade. A study of all cause mortality in Italian professional soccer players reported a higher than expected MND occurrence in this group.\(^{(58-61)}\) Following a complaint by a football coach that illegal drug use was widespread amongst football players, Mr Raffaele Guarinelli had ordered an enquiry in 2003 into the causes of death in a cohort of approximately 24,000 professional soccer players who played in the Italian professional leagues A, B and C between 1960 and 1996. 375 deaths were reported, 8 of whom were documented as having died from ALS. Italian mortality statistics predicted 0.69 expected ALS related deaths for the same period. The corresponding SMR was 11.6 (95% CI: 6.7-20.0).\(^{(61)}\) These results prompted a number of MND Research groups to perform further studies to confirm the initial results.

Annual reports of active soccer players during the above time periods were the main source of data mentioned in the text. Vital status of former and current players were ascertained through the national tax office. The last year of tax payment was checked and those who failed to register for tax contributions in the following years were considered potentially deceased. The municipality of birth was then contacted for those potentially deceased players to ascertain if a death had been registered. All registered deaths were ICD coded except for 25 players where cause of death was unknown. Case files were not examined nor family or physicians contacted to confirm robustness of the diagnosis of MND.\(^{(61)}\) Because of insufficient data on age at onset of sporting activity, age at entrance into professional activity, age at change of team or league and age at change of tactical role in field the authors were unable to estimate amount of exposure to professional soccer playing or person-years at risk accurately and so used Standardised Proportionate Mortality Ratios (SPMRs) as opposed to standardised mortality ratios. SPMR was estimated as 1158 (95% CI: 672-1998). This corresponds to an underlying 10 fold
increased mortality rate for this disease among soccer players. Proportional mortality rates were measured which is only a relative measure of risk. There have been criticisms that the calculated ‘cause specific expected number of deaths’ was underestimated and the matched reference group was inappropriate but this is controversial. Interestingly the age of disease onset in these soccer players was earlier than usual. (3/8 cases occurred < 39 years and 6/8 < 59 years.) (47,60)

In the same year another Italian group looked at a cohort of 7325 male Italian soccer players playing in the Italian professional leagues A and B from 1970-2001. (10) Two sources were used to identify the study cohort, football pension archives and the Panini football card company. Non Italian players had been excluded due to difficulty with follow up after repatriation. There were 137,078 person-years of follow up in total. The authors confirmed the diagnosis of MND with review of clinical data and by interviewing the patient, his family/carers or his physician. Only cases with definite or probable MND were included. Detailed family and playing histories were obtained. A total of 18 cases of MND were identified initially. 3 players were non Italians and 10 players were excluded because they played before 1970. 5 Italian players who played after 1970 and subsequently developed MND were identified where only 0.77 cases would be expected. 3/5 cases were bulbar onset disease (p = 0.003) and 2/5 were spinal onset, 1 upper and 1 lower limb onset disease. Mean age of onset of disease was 43.4 years. Overall a standardised morbidity ratio of 6.5 (95% CI: 2.1-15.1) was observed. This is a significantly higher SMR than would be expected for MND onset, SMR before 49 years was even higher at 7.5 (95% CI: 2.0-19.2) using the PARALS as a reference. (62-63) A dose-response relationship between duration of professional football playing career and risk of MND was also reported (playing > 5 years, 15.2 (95% CI: 3.1-44.4); playing <= 5 years, 3.5 (95% CI: 0.4-12.7)). By the end of 2003 4/5 cases were deceased. (10) Some of the cases included in this study were also part of the 2005 Belli study. (61) None of the cases had a family history of MND or a history of significant trauma. Playing a midfield position increased SMR to 12.2. Delay from end of football career and onset of MND varied from 4-19 years although one case had onset while still playing. Mean survival
from diagnosis was 32.4 months in the 4 deceased cases, 1 case was still living at 42 months. This is lower than would be typically expected in young onset MND.\(^{(10, 63-65)}\) Other case reports during the same period supported this association.\(^{(66)}\) Soccer players may be exposed to high levels of physical activity, repeated microtrauma, pesticides on the pitch and the use of illicit or performance enhancing drugs or high dose health food supplements thus the relationship is by no means clear-cut. Similarly an increased risk of MND has been reported among American military particularly Gulf war veterans. The reasons for this apparent association are multifold including increased physical activity, exposure to both chemicals and trauma.\(^{(49, 67-71)}\) However, a later study by Horner et al in 2008 would suggest that the increased risk seen in Gulf war veterans was limited to the decade following the war suggesting that a toxic environmental exposure during the war may have been the overriding cause in that cohort of patients.\(^{(72)}\)

In 2006 Vanacore et al described a further case of early onset bulbar MND in an Italian professional soccer player, definite by EEDC.\(^{(59)}\) This case was not included in the 2005 Belli or Chio studies but was similar to many of the cases they described being young onset (45 years) and bulbar in onset. The subjects all played midfield for longer than 5 years, both of which have been proposed as potentially increasing your risk of MND in sub analysis by Chio et al.\(^{(10)}\) The patient played professional football at high level for 17 years. He had a family and personal history of autoimmune disorder and consumed prescribed drugs and dietary supplements to accelerate recovery from injury and enhance performance.\(^{(59)}\)

A 2007 follow up mortality study on Italian soccer players from 1975-2003 was commissioned by the Italian ministry of health.\(^{(61)}\) A total of 5389 players, aged 14-35 years at enrolment (in 1975) were identified from public sources and followed up until 2003. All players played in the Italian professional A and B leagues. A total of 204,125 subject-years of follow up were involved. Overall and cause-specific mortality rates were calculated and compared to age cause-specific mortality rates for men during the same calendar period. Ratios between observed and expected deaths gave the standardized mortality ratios. During this period 63 players died, 4 of who had MND. The expected
number of deaths from MND during this period in this age group was estimated to be 0.2. A significantly higher than expected number of deaths was noted for car accidents as well as for MND. Deaths from diseases of the circulatory system, cancer and immune deficiency was less than expected. Vital statistics were incomplete for 180 players. Mean age of death was 40.6 years. Standardised Mortality Rate/Ratio (SMR) was calculated as 18.18 (95% CI 5.00-46.55). Italian age and gender specific MND mortality rates were not available for the period in question so results were compared with US figures at the time. Using death certificates as a source of data is always controversial however the reported accuracy of death certification in MND is reportedly better than in other diseases, one of the referenced studies having been done in Italy where most of the soccer and MND data has been published. This study demonstrated a near 20 fold risk increase for MND in young professional soccer players. Limitations include incomplete data on 3% subjects, use of US mortality data as a comparative population, lack of information on concomitant risk factors and use of death certificates whose accuracy is controversial.

In 2007 Wicks described a cluster of three soccer playing friends in the UK who all developed MND simultaneously between the ages of 40 and 54 years. All patients grew up and lived in the same rural district in the UK and participated in the same amateur soccer league from their teenage years into their thirties and forties. The last to give up playing football discontinued playing at 42 years, one year after symptom onset. In the other two cases there was a 28-30 year gap between stopping playing and onset of symptoms. Two of the players had suffered from multiple minor fractures and traumas over their playing careers. The patients worked as a builder/carpenter, engineer and an electrician respectively and had all received multiple electric shocks at 240V without losing consciousness. The age and sex adjusted incidence of MND in that region of the UK was 1.66/100,000 person years, 2.04 for males and the population was 489,346 (241,142 male). Although 5 cases might be expected on this basis, the close connection between the cases is unexpected. None of the cases had a family history of MND or had used performance enhancing drugs. The authors discuss possible contributions from pesticides and chemicals used on the pitches or recurrent microtrauma.
but one of the difficulties in establishing putative causes in these cases are that these players had so much in common. It has been suggested that the apparent increase in MND seen in football players is soccer specific,\(^{(65)}\) this would be supported by apparent lack of increased MND risk in professional cyclists.\(^{(78-79)}\)

Our studies demonstrate that there may be an excess of MND among those who engage in heavy physical activity. Our marathon study suggests this finding may not be soccer specific as has been suggested in the Italian studies. However, our marathon study would require replication given the low ‘affected’ figures involved but would also be easy to replicate in other sporting events. In our second study controls and cases were well matched (sex and age) except with regards to weight. MND patients were lighter by a mean of 7-9kgs \((p < 0.0001)\). Because energy consumption calculations are dependent on weight, if this were to erroneously effect calculations MND cases should have had less total energy consumption than controls which did not occur. In fact, the opposite was found; our MND patients had higher levels of energy consumption than controls. Excess physical activity was only demonstrated among spinal onset disease when work activity was also included\((p < 0.0001)\) or when in particular vigorous activity was examined\((p = 0.0016)\). Exercise and low weight were found to be significant predictors of the development of MND. Interestingly exercise increased risk of spinal onset disease but not particularly bulbar disease in contrast to the excess of bulbar disease reported among Italian football players.

### 3.6 Conclusion

Our studies show that a history of heavy physical activity may increase the likelihood of developing MND, in particular spinal onset disease. A number of interesting points have also come to light. The effect may not be soccer specific as has been previously suggested. It is important to include analysis of work activity in calculations in addition to leisure time physical activity or the effect may be missed which may explain previous negative studies. In addition analysis of vigorous activity has not previously been done but also demonstrates significant differences between cases (spinal) and controls. The
idea that certain levels of activity might be more toxic than others to the nervous system is an interesting idea that probably merits further investigation.
References


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Chapter Four
Insulin like Growth Factors in Motor Neuron Disease

Figure 1: Ribbon like structure of IGF-1. (Courtesy of www.Wikipedia.com from the Protein Data Bank (PDB), 2009.)

4.1 Introduction

Aberrant levels of Insulin like growth factors (IGFs) have been reported in motor neuron disease and supplementation with extrinsic IGF-1 has been examined as a putative treatment for MND. It is likely that this was an overly simplistic view of the IGF system and its’ interplay with diseases such as MND. (5, 7) IGFs are polypeptides which share a high sequence homology with insulin and are part of ‘the IGF axis’. (Figures 1 & 2) The axis contains the ligands IGF-1 and IGF-2, two cell surface receptors IGF-IR and IGF-IIR and high affinity IGF-binding proteins (IGFBPs) numbered 1-6. There are also 8-10 low affinity binding proteins termed IGF-binding protein related proteins (IGFBP-rPs). (Figure 3) Although much is known individually about the various components of the IGF system,
the exact roles of IGF-1 and IGF-2 in nerve cell growth promotion remains unclear. In particular the complex nature in which the IGFs interact with other mitogens and the contribution of each of the BPs in inhibition, augmentation and distribution of IGF throughout the body are not yet fully understood. \(^{(1-5)}\)

Figure 2: Comparison of the pre-peptides for Insulin, IGF-1 and IGF-2. (Courtesy of google images, publisher unknown, 2009) \(^{(6)}\)
Figure 3: Insulin and IGF receptors and their ligands. Three binding sites for the insulin like growth factors (IGFs) are expressed on the surface of target cells; the insulin receptor, the IGF-1 receptor and the IGF-2 mannose 6 phosphate receptor (IGFM6PR). Insulin and IGF are structurally homologous, both are tyrosine kinases and interact with various intracellular mediators. The IGFM6PR differs from these receptors in structure and has no known signalling action. Insulin binds both the insulin and the IGF1 receptor. The relative affinities for each of the ligands for the receptors is indicated by the width of the arrows in the upper part of the diagram. (Courtesy of LeRoith D, 1997) (5)

There are many mechanisms by which IGF-1 may influence the nervous system, some of these are shown in figure 4 and are also well described in the review article by Sakowski et al. (15). In contrast very little is known about the function of IGF-2 or its’ role in MND. Studies suggest that serum and spinal cord IGF-1 levels are deficient or low-normal in Post polio syndrome (PPS) (16-17) and MND patients, (18-20) Most of these studies evaluated IGF-1 levels at a single point in time and MND is a very heterogenous disease. No longitudinal study of serum IGF-1 levels in MND patients throughout the course of their disease has been previously been performed. This is of interest because factors that can influence serum IGF-1 levels such as age, nutritional status and exercise are of all of relevance in MND, a progressively debilitating disease of older people which often
involves dysphagia and secondary malnutrition. A study indicating whether serial IGF-1 levels are consistently low in MND or whether they fluctuate would be useful to provide a positive evidence base for further investigation of alternative modes of IGF-1 supplementation in this group of people.

**Figure 4:** Sites and mechanism of IGF-1 action in MND. IGF-1 can impact various aspects of motor neuron (MN) degeneration and survival in MND. Its' effects are mediated in MN, muscle and glia via activation of the IGF type 1 Receptor (IGF-1R), subsequent activation of IRS and downstream activation of major survival related pathways including PI3K/Akt and p44/42 MAPK signalling pathways (as represented in the MN cell body). These pathways may act in an autocrine or paracrine fashion by either directly promoting MN survival themselves or by their effects on support cells and muscle cells (by mediating the release of toxic or inflammatory molecules or disrupting neuromuscular junction and motor neuron maintenance mechanisms. Pharmaceutical influence at any of these sites by IGF-1 could be important in MND therapy. ) (Courtesy of Sakowski SA et al, 2009.) IRS
Insulin receptor substrate; PI3K/Akt and p44/42 MAPK are important cell signalling pathways.\(^{(15)}\)

Animal studies have shown motor neuron rescue and protection in models of MND treated with IGF-1.\(^{(7-8)}\) At the time of this project there had been two contradictory double blind therapeutic trials of subcutaneous recombinant IGF-1 therapy in MND.\(^{(9,10)}\) A subsequent Cochrane review meta-analysis found that rhIGF-1 was safe and well tolerated in MND and that high dose IGF was of significant benefit in the primary outcome (Appel ALS functional scale) but showed only a trend towards benefit in secondary outcomes such as survival and quality of life.\(^{(11)}\) A third trial on subcutaneous IGF-1 has since been completed failing to show a consistent functional, prognostic or quality of life benefit among MND sufferers.\(^{(12,13)}\) However, an interesting small Japanese study in 2005 examined the effects of intrathecal IGF-1 supplementation in 9 MND patients. Motor deterioration slowed with this route of administration but bulbar dysfunction was unaffected. This small study has not been replicated since but does beg the question are we completely finished with IGF-1 in MND, is it possible supplementation by an alternate route or combined with other neurotrophic agents might still be beneficial even if not curative?\(^{(14)}\) Any further time dedicated to evaluating this agent might however be a waste of valuable resources. For this reason we felt it was necessary to return to the beginning of the story again, to establish whether IGF-1 levels were truly aberrant in MND, whether this was a consistent finding among all MND cases and over the course of the illness and to attempt to rationalise why aberrations might occur.

### 4.2 Aims

The aims of our study were:

- To identify aberrations in the peripheral IGF system in MND patients.
- To compare IGF systems in patients with MND, post polio syndrome (PPS), Multiple Sclerosis (MS) and normal controls.
- To define the timing of IGF-1 aberrations with respect to MND stage.
• To correlate IGF levels with nutritional status and functional capacity.
• To identify whether IGF-1 level abnormalities correlate with MND disease subtype (spinal or bulbar onset).

4.3 Methods

Fifty-two sequential MND clinic patients at Beaumont hospital were recruited. They were age (+/-3 years), sex and body mass index (BMI +/- 2.5) matched to controls. In addition 22 PPS and MS patients were also recruited. All MND patients fulfilled criteria for definite or probable MND by El Escorial diagnostic criteria (EEDC)(21) and all MS patients were diagnosed using the MacDonald criteria.(22) Post polio diagnostic criteria used were those recommended by the European neuromuscular consortium.(23) Controls included healthy spouses and friends of patients and public volunteers from Beaumont Hospital, Royal College of Surgeons of Ireland, University College Dublin and the University of Limerick who did not have a personal or direct family history of MND, polio or MS or any other neurological disorder. Informed consent was obtained from all patients and controls and the study had full approval of the Beaumont Hospital Ethics Committee. Serial fasting IGF-1 samples were collected at three monthly intervals. Participants also attended nutritional services regularly and had weight (kg), body mass index (BMI), 24 hour dietary recall and other nutritional parameters documented. Serial functional assessment using the modified ALS-functional rating scale (ALS-FRSr)(24) and Kurtze expanded disability scale scores (EDSS)(25) were calculated at each visit for MND and MS patients respectively. Any MND patients who became dysphagic, malnourished or hypermetabolic had percutaneous gastrostomy (PEG) tubes sited and received nutritional supplementation by this method and continued to participate in the study. None of the other disease groups or controls required this intervention. All patients with MND were taking riluzole.

There were two arms to the study, a cross sectional analysis of IGF, in particular IGF-1 and its’ related proteins at the start of the study in comparison with controls and two other neurological populations (MS and PPS). The longitudinal arm involved serially following the same parameters as evaluated in the cross sectional study over a period of two years or
until patients dropped out or died. Because of the expected high dropout rate we collected over twice as many MND patients initially as other patient groups. The number of MND patients needed to power the longitudinal arm of the study was calculated as 20-25. The heterogeneous nature of MND disease and survival is well recognized. Two interesting subgroups of MND were identified from the longitudinal group, 11 patients had a blood sample drawn and ALSFRS-r done <\= 30 days prior to death. These patients were labelled ‘pre-agonal’ (PA) MND. The other 10 patients were labelled ‘long term’ (LT) patients because they had all had a diagnosis of MND for > three years by the study end.

Blood & immunoassay samples were collected after an overnight fast from 10.00pm. 5 mls of blood was drawn by venepuncture from the antecubital vein. Samples were collected in Vacutainer® SST™ tubes (Becton Dickinson Diagnostics, UK Cat. #367977) inverted 5 times to mix clot activator with blood and allowed to clot for 20 minutes. Samples were then spun at 3000 rpm and at 4 degrees centigrade for 10 minutes. Serum was extracted, aliquotted and stored at -80 degrees centigrade until samples were assayed. To reduce analytical variance samples were batched using the same assay lot. The concentration (ng/ml) of serum IGF-1, IGF-2, IGF-BP-1, IGFBP-2 and IGFBP-3 was determined by immunoradiometric assay (IRMA: Diagnostic System Laboratories DSL, Webster, TX) according to the manufacturer’s protocol. Measurement of acid labile subunit (ALS*) was by enzyme linked immunosorbent assay (ELISA; Diagnostic System Laboratories, Webster, TX). Frozen sera with varying concentrations of IGFs/IGFBPs and a pooled serum sample were used as quality control samples to assess intra- and inter-assay precision. Intra- and inter-assay CVs were as follows; IGF-1 3.3%, IGF-2 1.0% and 2.6%, IGFBP-1 1.0% and 8.1%, IGFBP-2 10.9% and 10.1%, IGFBP-3 0.7% and 2.0% and acid labile subunit 9.2% and 6.6%. Serial blood samples were taken in Beaumont Hospital or in the patients’ homes from month zero for the cross sectional study and every three months thereafter for the longitudinal study until 24 months. Levels of IGF-1, -2, ALS, glucose, insulin and IGFBPs were measured in patients with MND, PPS and MS and corresponding healthy age (+/- 3 years), BMI (+/- 2.5kg.m⁻²) and sex matched controls. Statistical analysis was done using SPSS and Excel. Data for all analytes in patient groups
and controls were normally distributed. Analysis of patients versus matched controls was by paired Student t-test (2-tailed). Results are reported as the mean (SD).

### 4.4 Results

#### 4.4.1 Cross sectional study

For the cross sectional study the control group included 72 healthy volunteers, 26 male and 46 female. Mean age was 51.0 (14.6) years with a range of 25.8 – 80.9. Mean BMI was 25.4 (3.3) kg/m2, with a range of 17.9 – 36.7 kg/m2. 52 controls were required to match the MND population in the cross sectional study. The final MND population under study included 52 (30 male & 22 female). Patients were subcategorised into spinal onset and bulbar onset disease. Mean age was 60.2 (11.9) years with a range of 24.6 – 86.5 years. Mean BMI at onset was 23.9 (3.9) kg/m^2, with a range of 14.0 – 37.7 kg/m^2. 26 PPS patients (13 male and 13 female) were recruited: Mean age was 55.7 (9.7) years with a range of 44.5 – 80.9 years. Mean BMI was 29.1 (6.0) kg/m^2, with a range of 22.7 – 43.7 kg/m^2. The MS population consisted of 23 patients (8 male & 15 female) with a mean age of 49.8 (11.1) years, range 28.6 – 73.1. Mean BMI was 24.8 (3.3) kg/m^2, with a range of 20.2 – 32.0 kg/m^2.

In the cross sectional study the MND patient population had higher baseline levels of IGF-1 and IGF-2 by 16% and 11% respectively compared to their controls. The increase in both peptides resulted in a greater IGF \text{TOTAL} (IGF-1 + IGF-2) by approximately 11% compared with the control population. (p = 0.042) All binding proteins examined were lower in the MND population than in controls, IGFBP-1 (− 34%), IGFBP-2 (− 10%) and IGFBP-3 (− 8%) but only the decrease in IGFBP-1 reached statistical significance (p = 0.05). Serum ALS* [Acid Labile Subunit] did not differ from matched controls. (Table 2) PPS patients had similar IGF-1 and IGF-2 levels to the control population. IGF\text{TOTAL} was therefore also similar to controls. All binding proteins were lower in the PPS group compared to their controls IGFBP-1 (−12%), IGFBP-2 (− 22%) and IGFBP-3 (− 11%), none of these figures reached statistical significance. Similarly there were no aberrations in the ALS* in PPS patients. An increased incidence of previously undiagnosed impaired
glucose tolerance was discovered in the post polio population. This was independent of BMI, age and sex. (Table 2)

Table 1: Summary Baseline Data on Patients at Onset of Cross Sectional Study

<table>
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<tr>
<th>Characteristic</th>
<th>Controls</th>
<th>MND</th>
<th>PPS</th>
<th>MS</th>
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<td>No. subjects</td>
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<td>52</td>
<td>26</td>
<td>23</td>
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<tr>
<td>Female</td>
<td>46</td>
<td>22</td>
<td>13</td>
<td>15</td>
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<tr>
<td>Male</td>
<td>26</td>
<td>30</td>
<td>13</td>
<td>8</td>
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<tr>
<td>Mean Age (SD)</td>
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<td>60.2(11.9)</td>
<td>55.7(9.7)</td>
<td>49.8(11.1)</td>
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<td>Age Range (years)</td>
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<td>24.6-86.5</td>
<td>44.5-80.9</td>
<td>28.6-73.1</td>
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<tr>
<td>BMI (SD) (kg/m²)</td>
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<td>23.9(3.9)</td>
<td>29.1(6.0)</td>
<td>24.8(3.3)</td>
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<td>14.0-37.7</td>
<td>22.7-43.7</td>
<td>20.2-32.0</td>
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<td>Mean duration of Symptoms (yrs)</td>
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<td></td>
</tr>
<tr>
<td>Mean EDSS Score (SD)</td>
<td></td>
<td>13.8(10.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EDSS Range</td>
<td></td>
<td>1.8-31.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ALSFRS-r score (SD)</td>
<td></td>
<td>3.4(1.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.5-5.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In the MS population the mean concentration of serum IGF-1 was 38% (p = 0.018) higher than the control population. Similarly concentrations of the ALS* and IGFBP-2 were also higher in the disease population by 17% (p = 0.044) and 43% (p = 0.035) respectively. When stratified by disease subtype a significant increase in serum IGF-1 (41%; p = 0.028), ALS* (22%; p = 0.044) and IGFBP-2 (56%; p = 0.071) were seen only in patients with relapsing remitting MS (n = 11) as opposed to those with primary progressive or secondary progressive disease. Serum IGF-2 did not differ between the MS population and controls (p = 0.75). The dominant influence of IGF-2 resulted in no significant change in IGF\text{TOTAL} between MS patients and their controls. (Table 2)
Table 2: Summary of results of cross sectional IGF study. Data are reported as Mean (SD) and expressed in ng/ml except acid labile subunit expressed as ug/ml. Significant results labelled in red.

<table>
<thead>
<tr>
<th></th>
<th>MND</th>
<th>Controls</th>
<th>PPS</th>
<th>Controls</th>
<th>MS</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGF-1</td>
<td>184(84)</td>
<td>158(67)</td>
<td>149(69)</td>
<td>158 (77)</td>
<td>230(123)</td>
<td>167(66)</td>
</tr>
<tr>
<td>P value</td>
<td>0.179</td>
<td></td>
<td>0.714</td>
<td></td>
<td>0.018</td>
<td></td>
</tr>
<tr>
<td>IGF-2</td>
<td>1072(275)</td>
<td>969(193)</td>
<td>933(214)</td>
<td>954(187)</td>
<td>980(188)</td>
<td>961(148)</td>
</tr>
<tr>
<td>P value</td>
<td>0.089</td>
<td></td>
<td>0.774</td>
<td></td>
<td>0.747</td>
<td></td>
</tr>
<tr>
<td>IGF TOTAL</td>
<td>1126(225)</td>
<td>1126(225)</td>
<td>1082(218)</td>
<td>1112(213)</td>
<td>1210(265)</td>
<td>1128(175)</td>
</tr>
<tr>
<td>P value</td>
<td>0.042</td>
<td></td>
<td>0.710</td>
<td></td>
<td>0.268</td>
<td></td>
</tr>
<tr>
<td>IGFBP-3</td>
<td>4038(807)</td>
<td>4378(903)</td>
<td>3900(685)</td>
<td>4361(823)</td>
<td>4638(998)</td>
<td>4473(651)</td>
</tr>
<tr>
<td>P value</td>
<td>0.137</td>
<td></td>
<td>0.092</td>
<td></td>
<td>0.542</td>
<td></td>
</tr>
<tr>
<td>IGFBP-2</td>
<td>318(176)</td>
<td>352(216)</td>
<td>253(172)</td>
<td>324(198)</td>
<td>475(367)</td>
<td>332(204)</td>
</tr>
<tr>
<td>P value</td>
<td>0.545</td>
<td></td>
<td>0.281</td>
<td></td>
<td>0.035</td>
<td></td>
</tr>
<tr>
<td>IGFBP-1</td>
<td>30.7(23.8)</td>
<td>46.6(29.3)</td>
<td>33.4(18.2)</td>
<td>37.9(23.1)</td>
<td>37.1(19.2)</td>
<td>39.8(24.6)</td>
</tr>
<tr>
<td>P value</td>
<td>0.05</td>
<td></td>
<td>0.529</td>
<td></td>
<td>0.705</td>
<td></td>
</tr>
<tr>
<td>ALS*</td>
<td>13.9(4.1)</td>
<td>14.7(4.2)</td>
<td>16.2(3.5)</td>
<td>16.2(3.2)</td>
<td>17.7(5.1)</td>
<td>15.1(3.0)</td>
</tr>
<tr>
<td>P value</td>
<td>0.480</td>
<td></td>
<td>0.994</td>
<td></td>
<td>0.044</td>
<td></td>
</tr>
</tbody>
</table>

Of the initial 52 MND patients entered in the cross sectional study, only 21 (42%) of MND patients survived 16-18 months and took part in the longitudinal study. Baseline patient characteristics are shown in table 3. Serial evaluation of PPS patients was abandoned after 1 year because no alterations in the IGF axis were noted. Some aberrations were noted in the MS population specifically increases in IGF-1 (38%; p = 0.018/0), IGFBP-2 (43%; p = 0.035) and the ALS* (17%; p = 0.044). Further analysis demonstrated that alterations were probably associated with interferon-β use. These findings are not discussed in more detail here as they are part of another PhD thesis that is ongoing. The results of the longitudinal analysis are shown in table 4.
Table 3: Baseline characteristics of MND subgroup patients & controls in longitudinal study. (LT= long term patients; PA= pre-agonal)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>LT</th>
<th>Controls</th>
<th>PA</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. subjects</td>
<td>10</td>
<td>10</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Male</td>
<td>8</td>
<td>8</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Female</td>
<td>2</td>
<td>2</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Mean Age(SD)</td>
<td>48.9 (12.6)</td>
<td>48.9 (12.5)</td>
<td>68.4 (9.0)</td>
<td>65.0 (7.8)</td>
</tr>
<tr>
<td>Range (yrs)</td>
<td>25.8-63.9</td>
<td>24.6-64.0</td>
<td>55.9-86.5</td>
<td>52.4-80.9</td>
</tr>
<tr>
<td>Mean BMI (kg/m^2) (SD)</td>
<td>24.7 (3.3)</td>
<td>24.9 (5.1)</td>
<td>22.8 (4.6)</td>
<td>24.7 (2.2)</td>
</tr>
<tr>
<td>Range (kg/m^2)</td>
<td>20.3-32.5</td>
<td>19.7-37.7</td>
<td>17.6-30.6</td>
<td>22.0-29.3</td>
</tr>
<tr>
<td>Spinal onset</td>
<td>10</td>
<td></td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Bulbar onset</td>
<td>0</td>
<td></td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

Table 4: Summary results of longitudinal study. (Data are reported as Mean (SD) and expressed in ng/ml. Data for PA patients is last sample taken <= 30 days prior to death. Data for LT patients is month 0 sample representing furthest time from death.)

<table>
<thead>
<tr>
<th>IGF-I</th>
<th>LT</th>
<th>Control</th>
<th>PA</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>% change</td>
<td>169(82)</td>
<td>124(60)</td>
<td>143(86)</td>
<td>145(78)</td>
</tr>
<tr>
<td>P value</td>
<td>36%</td>
<td>0.032</td>
<td>-1%</td>
<td>0.975</td>
</tr>
<tr>
<td>IGF-II</td>
<td>1159(259)</td>
<td>1010(195)</td>
<td>832(216)</td>
<td>962(257)</td>
</tr>
<tr>
<td>% change</td>
<td>15%</td>
<td>0.187</td>
<td>-14%</td>
<td>0.212</td>
</tr>
<tr>
<td>P value</td>
<td>17%</td>
<td>1134(229)</td>
<td>976(283)</td>
<td>1106(288)</td>
</tr>
<tr>
<td>IGF_TOTAL</td>
<td>1328(308)</td>
<td>1134(229)</td>
<td>976(283)</td>
<td>1106(288)</td>
</tr>
<tr>
<td>% change</td>
<td>17%</td>
<td>0.115</td>
<td>-12%</td>
<td>0.282</td>
</tr>
<tr>
<td>P value</td>
<td>0.115</td>
<td></td>
<td>0.282</td>
<td></td>
</tr>
<tr>
<td>IGFBP-3</td>
<td>4247(975)</td>
<td>4449(848)</td>
<td>336(1059)</td>
<td>4178(1150)</td>
</tr>
<tr>
<td>% change</td>
<td>-5%</td>
<td>0.666</td>
<td>-19%</td>
<td>0.097</td>
</tr>
<tr>
<td>P value</td>
<td>-5%</td>
<td>0.666</td>
<td>0.097</td>
<td></td>
</tr>
<tr>
<td>IGFBP-2</td>
<td>228(147)</td>
<td>290(175)</td>
<td>713(546)</td>
<td>436(259)</td>
</tr>
<tr>
<td>% change</td>
<td>-21%</td>
<td>0.414</td>
<td>64%</td>
<td>0.127</td>
</tr>
<tr>
<td>P value</td>
<td>-21%</td>
<td>0.414</td>
<td>0.127</td>
<td></td>
</tr>
<tr>
<td>IGFBP-1</td>
<td>22.3(13.3)</td>
<td>53.5(33.2)</td>
<td>43.0(46.7)</td>
<td>37.3(21.5)</td>
</tr>
<tr>
<td>% change</td>
<td>-58%</td>
<td>0.02</td>
<td>15%</td>
<td>0.729</td>
</tr>
<tr>
<td>P value</td>
<td>-58%</td>
<td>0.02</td>
<td>0.729</td>
<td></td>
</tr>
</tbody>
</table>

An overall downward trend in IGF-1, IGF-2, IGF_TOTAL and IGFBP-3 was noted over time in MND cases compared to controls. (Figures 5-7) PPS patients also appeared to maintain stable IGF-1 and related protein levels over the first year studied. (Figure 8)
Figure 5: Longitudinal IGF-1 and IGF-2 in MND patients.

Figure 6: Longitudinal analysis of total IGF and IGFBP-3 in MND patients
Figure 7: Longitudinal total IGF levels in control population

Figure 8: Longitudinal total IGF levels in PPS population

Decline in IGF-1 in MND patients was mirrored by a gradual decline in functional ability although none of the IGF-1 parameters directly correlated with ALSFRS-r scores. (Figures 9 & 10) Our patients had a wide range of ALSFRS-r scores before death (6-36)
Figure 9: Longitudinal decline in functional ability in MND patients.
Figure 10: Lack of association between % change in IGF-1 and ALSFRS-r

Figure 11: BMI stabilization in patients with gastrostomy insertion & close nutritional management
10 patients were designated LT and 11 patients were PA. All 10 LT patients were spinal onset disease. Mean disease duration (SD) in this subgroup was 6.5 (3.1) years. This subgroup also demonstrated a 36% (p = 0.032) increase in IGF-1 and a 58% (p = 0.02) reduction in IGFBP-1 over time. Increases of 15% and 17% in serum IGF-2 and \( \text{IGF}_\text{TOTAL} \) and a decrease in IGFBP-2 (-21%) were also found but these values did not reach statistical significance. Concentrations of IGFBP-3 were similar in both LT patients and controls. Although there was an increase in serum IGF-1, because IGFBP-3 levels remained steady, a mean IGF-1 binding ratio (SD) of 0.87(0.16) was calculated i.e. insufficient IGFBP-3 to bind even 13% of IGF-1. This reduction in binding capacity was significant compared with controls (p = 0.009). (Table 4) In PA MND patients’ serum IGF-1, IGF-2 and \( \text{IGF}_\text{TOTAL} \) were similar to controls. IGFBP-1 and IGFBP-2 were increased by 15% and 64% respectively compared with controls (non significant trend only). IGFBP-3 was decreased by 19% in PA patients (p=0.097). (Table 4)

4.5 Discussion

4.5.1 IGF and Related Proteins Background

Background information on IGFs and associated proteins will be provided in this section followed by discussion and interpretation of the study results. In 1976, two peptides were isolated from human serum both having insulin-like-structure and function, they were named IGF-1 and IGF-2. Produced predominantly in the liver, nerves and muscle are also capable of local production. IGF-1 is under the control of and mediates the effects of growth hormone (GH) but the major functions of IGF-2 remain elusive.\(^{28-29}\) Both peptides have similar molecular structures: a single chain of peptides, approximately 70 residues cross linked by three disulphide bridges and having molecular weights of approximately 7.6 kilodaltons (kDa). The peptide regions are homologous to insulin A and B chains.\(^ {30} \)

Several unique ligands of human IGF-1 have also been described and deserve mention. IGF-1Ec, also called mechano growth factor (MGF) is upregulated in response to mechanical stimulus and found particularly in muscle tissue.\(^ {30-31} \) Des-IGF-1, a truncated
form of IGF-1 is found predominantly in fetal and adult human brains. With a reduced affinity for IGFBPs it displays a 10-fold greater potency than standard IGF-1.\(^{32}\) GPE are the first three N-terminal amino acids (Gly-Pro-Glu) which are cleaved from IGF-1 to form Des-IGF-1, these amino acids have potent biological activity within the brain.\(^{33-35}\) Three variants of IGF-2 have also been described to date although little is known of their function.\(^{36-37}\) IGF ligands are of interest as alternative therapeutic options for MND in the future.

The liver is the principal source of IGF-1 but the highest concentration is found in the blood.\(^{38-40}\) IGF-1 is more common than IGF-2 in the adult, the latter may be a predominantly fetal promoter of cell growth, survival and differentiation. IGF-1 circulates in the body at much higher concentrations than insulin and is largely bound to one of the six major IGFBPs that modulate its’ activity. The binding proteins, also synthesized primarily in the liver and less frequently in bone and muscle, may act in a paracrine, autocrine or endocrine fashion.\(^{41-42}\)

IGFs are anabolic promoting proliferation and differentiation of cells and inhibiting apoptosis. Metabolically they increase cellular amino acid production, glucose uptake and protein synthesis and decreases protein degradation and glycolysis. Growth hormone (GH) is the main regulator of hepatic IGF-1 production.\(^5,43-44\) IGF-1 levels follow a normal distribution curve throughout life with peak levels occurring in both sexes in the 12 to 15 year age range when there is a growth spurt and declining thereafter. (Figure 12)
75-90% of peripheral IGFs (both IGF-1 and -2) circulate bound to IGFBP-3 and the ALS* as a ternary complex. Only a small proportion of IGFs are bound to IGFBP-1 and -2. Binary but not ternary complexes can leave the circulation and target specific tissues.\(^2\) IGF-1 and IGF-2 may bind to both the insulin receptor and the IGF-type-I or type-II receptors to exert their effects. Activation of either the insulin or the IGF-I receptor will produce similar initial responses within the cell. However, since insulin regulates metabolic function and IGFs regulate growth and differentiation, the final pathways these hormones activate within the cell are separate and distinct. Only IGFs (not insulin) can interact with IGFBPs. Table 6 summarises the features of these proteins. Activation of the IGF-type-II receptor is not well characterized.\(^{43-45}\)
Table 6: Comparison between serum IGF-1, IGF-2 and insulin in humans. (Adapted from Thissen JP et al, 1994) (41)

(INS=Insulin; a=Daltons; +++=strong; +=weak; 0=nil;)

<table>
<thead>
<tr>
<th></th>
<th>IGF-1</th>
<th>IGF-2</th>
<th>Insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular mass</td>
<td>7649Da</td>
<td>7471Da</td>
<td>5734Da</td>
</tr>
<tr>
<td>Structure</td>
<td>1 chain, E-peptide</td>
<td>1 chain, E-peptide</td>
<td></td>
</tr>
<tr>
<td>C-peptide</td>
<td>Cleaved off</td>
<td>Cleaved off</td>
<td></td>
</tr>
<tr>
<td>Origin</td>
<td>Mainly liver</td>
<td>Mainly liver</td>
<td>Beta cells of islets</td>
</tr>
<tr>
<td>Secretion/Production Rate</td>
<td>Widespread</td>
<td>Widespread</td>
<td>Pancreas</td>
</tr>
<tr>
<td>Rate</td>
<td>Constant slow</td>
<td>Constant slow</td>
<td>Pulsatile</td>
</tr>
<tr>
<td></td>
<td>10mg/day</td>
<td>15mg/day</td>
<td>2mg/day</td>
</tr>
<tr>
<td>Circulating forms</td>
<td>Mostly bound</td>
<td>Mostly bound</td>
<td>Free</td>
</tr>
<tr>
<td>Binding proteins</td>
<td>6 distinct forms</td>
<td>6 distinct forms</td>
<td>None</td>
</tr>
<tr>
<td>Adult concentrations</td>
<td>200ng/ml</td>
<td>700ng/ml</td>
<td>0.5-5ng/ml</td>
</tr>
<tr>
<td>Daily variations</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Half-life</td>
<td>Little or none</td>
<td>Little or none</td>
<td>Yes</td>
</tr>
<tr>
<td>Receptor affinity</td>
<td>12-15 h</td>
<td>15h</td>
<td>10 min</td>
</tr>
<tr>
<td>Action</td>
<td>IGF-1-&gt;IGF-2-&gt;INS</td>
<td>IGF-2-&gt;IGF-1-&gt;INS</td>
<td>INS=IGF-1</td>
</tr>
<tr>
<td>GH dependence</td>
<td>E, P, A</td>
<td>E, P, A</td>
<td>E</td>
</tr>
</tbody>
</table>

E = endocrine; P = paracrine; A = autocrine

4.5.2 Insulin like Growth Factor Action

IGFs exert their effects by acting upon:

- cell cycle progression
- cell proliferation
- cell differentiation
- cell function
- cell death
4.5.2.1 Effects of IGF on Cell Cycle Progression
IGF-1 is a progression factor in the cell cycle, inducing quiescent cells to enter G₁ from G₀ and to continue through this phase and on to other parts of the cycle. (45-47)

4.5.2.2 Effects on Cell Proliferation
A wide variety of cells have a mitogenic response to IGF-1 stimulation including neuronal and skeletal muscle cells which is relevant in MND. IGF effects may be synergistic with other growth factors such as epidermal growth factor (EGF). High circulating levels of IGF-1 and low levels of IGFBP-3 have been shown to be associated with many types of cancer. (48-54)

4.5.2.3 Effects on Cell Differentiation
IGFs potently induce neural cell and myoblast terminal differentiation. Myoblasts also secrete IGF-2 themselves, creating a positive feedback mechanism. However, high concentrations of IGF-1 or ‘over-expression’ can also paradoxically inhibit cell differentiation. (55)

4.5.2.4 Effects on Cell Function
IGF-1 inhibits glutamate-stimulated release of GABA from Purkinje cells and may function as an in vivo neuromodulator for these cells. IGF-1 in skeletal muscle stimulates glucose uptake, glycolysis and glycogen synthesis. The anabolic effects of IGF-1 are particularly active on protein synthesis during health and disease. (55-57)

4.5.2.5 Effects on Cell Death
IGF-1 may inhibit cell death in certain cell lines i.e. haematopoietic cells. IGF-2 does not appear to be essential for cell survival. (55)

4.5.3 Factors that Influence Insulin like Growth Factors
IGFs are required for normal growth and development. GH stimulates IGF-1 gene expression, transcription and production. Serum IGF levels tend to parallel 24-hour mean
GH concentrations. In addition as well as anabolic effects IGF-1 feeds back negatively to the pituitary gland, inhibiting further GH secretion.\(^{46,55}\) IGF-binding proteins limit access of the IGFs to specific tissues and their receptors. IGFBP-3 binds most of the circulating IGF-1. The ternary complex it forms with IGF and ALS* has a half-life of several hours and is the only form in which these proteins can prepare to leave the circulation to enter a target tissue. GH increases the serum ALS* and IGFBP-3 in tandem with increasing IGF-1 expression and transcription.\(^{(2)}\)

Many variables may affect serum IGF concentrations including GH levels, age, sex (figure 12), illness, pregnancy, menopause and nutritional status.\(^{(56)}\) Fasting or starving results in complete GH resistance and hence high levels of GH. Restriction of protein or calories or both causes lesser degrees of GH resistance resulting in high GH levels though IGF-1 levels are low. GH receptor signalling may also become impaired, reducing instead of increasing IGF-1 and a state of catabolism ensues. Low serum IGF-1 has been associated with catabolic states such as severe trauma and sepsis.\(^{(57)}\) Nutrition is also one of the major regulatory factors in IGFBP levels which can also significantly impact functional levels of IGF-1. These factors are all of relevance in MND where significant nutritional failure is common.

The IGFs in the circulation and throughout the extracellular space almost entirely bound to members of the high affinity IGF binding protein family. IGFBPs prolong the plasma T\(_{1/2}\) of IGF-1 and IGF-2, control the rate of IGF exit from the circulation and regulate interaction between IGF-1 in particular and the IGF-type-I receptors on cell surfaces. IGFBPs have both stimulatory and inhibitory effects, thus controlling IGF bioavailability. Nutrient intake is the major regulator of plasma concentration of IGFBPs.\(^{(41)}\)

### 4.5.4 Insulin like Growth Factor binding Proteins

Six distinct IGFBPs have been characterized to date, four of which (IGFBPs-1-4) are found in the serum in significant quantities. They each have three distinct domains characterized by highly conserved cysteine rich NH\(_2\)- and COOH- termini. The N and C termini are high affinity binding sites for IGFs. The middle or linker domain defines the
structural differences between the IGFBPs though they share < 1/3 primary sequence homology. The general characteristics of IGFBPs are shown in table 7. All BPs share structural homology and specifically bind IGF not insulin. IGFBPs regulate IGF biological activity in four ways.

1. Plasma IGF transport proteins, control efflux of IGFs from circulation.
2. Prolong the T½ of the IGFs, regulate their metabolic clearance.
3. Allow tissue and cell specific IGF localization.
4. Modulate IGFs and receptors interaction.

Most IGFs circulate as part of a 150,000 Dalton ternary complex containing IGF-1, IGF-2, IGFBP-3 and the ALS*. ALS* is an 88 kDa glycoprotein that facilitate protein-protein interactions for example it increases IGFBP-3 to IGF-1 binding capacity x 3. The IGF-1-IGFBP-3-ALS* ternary complex is more stable than individual unbound circulating proteins thus preventing large amounts of IGF-1 leaving the vascular compartment at once, prolonging the IGF-1 T½ from <10 minutes to 12-15 hour. Most binding proteins have a T½ of 30-90 minutes unbound. The main properties of individual IGFBPs are shown in table 8. Key additional features of individual IGFBPs will be discussed below. It would be important to factor IGFBPs into analysis of IGF-1 levels in MND and also potentially when giving exogenous IGF-1 as a therapeutic agent in MND.
Table 7: Characteristics of IGFBPs. (Data courtesy of Rosenzweig SA et al, 2004; Baxter RC, 2000; Rechler MM, 1993 and LeRoith D, 1992.) (55, 58-60)

(RGD (Arg-Gly-Asp) sequence: integrin recognition site; Glycosylation: N-linked and O-linked; CSF: Cerebrospinal fluid; AF: Amniotic fluid; Mini= minimal)

<table>
<thead>
<tr>
<th>IGFBP-No</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular weight (kDa)</td>
<td>30</td>
<td>34</td>
<td>38-41</td>
<td>24</td>
<td>29</td>
<td>28-30</td>
</tr>
<tr>
<td>IGF Affinity</td>
<td>1&gt;2</td>
<td>2&gt;1</td>
<td>1&gt;=2</td>
<td>1&gt;=2</td>
<td>2&gt;1</td>
<td>2&gt;1</td>
</tr>
<tr>
<td>Phosphorylation</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glycosylation</td>
<td>-</td>
<td>-</td>
<td>N</td>
<td>N</td>
<td>O</td>
<td>O</td>
</tr>
<tr>
<td>Proteolysis</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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4.5.4.1 IGFBP-1

This low molecular weight protein associates with IGF-1 in small complexes (30-40 kDa) that can cross capillary endothelium unlike IGFBP-3. Its’ T½ is 90 minutes. Depending on the target tissue and the environment IGFBP-1 can block or enhance IGF-1 binding to its’ receptors. For positive action on IGF-1 it requires the presence of an as yet unidentified ‘other factor’ from CSF and plasma. The presence of the RGD (Arg-Gly-Asp) integrin recognition sequence in IGFBP-1 suggests that it acts by binding to the cell surface integrin receptor alpha-5-beta-1. IGFBP-1 isoforms that inhibit IGF action are phosphorylated and those that stimulate are non-phosphorylated. The phosphorylated (inhibitory) IGFBP-1 has a 4-6 fold higher affinity for IGF-1 than the non-phosphorylated (stimulatory) IGFBP-1. Thus IGFBP-1 function is mostly inhibitory.

4.5.4.2 IGFBP-2

This low molecular weight protein associates with IGF-1 in small complexes (30-40kDa) similar to IGFBP-1. It may be inhibitory or stimulatory to IGF-1 depending on the environment. As an IGFBP-2-IGF-1 complex it can cross vascular endothelium, delivering
IGF-1 to the tissues. It binds IGF-2 predominantly in an inhibitory fashion.\textsuperscript{(74-76)} T½ is 90 minutes unbound. Compared to IGFBP-1 relatively little is still known about IGFBP-2.

4.5.4.3  IGFBP-3
This is the larger, most abundant of the serum IGFBPs. (46-53 kDa) The ternary complex it forms with IGF-1 and ALS* cannot cross capillary endothelium, prolonging T½ of all the components. The high affinity binding site for IGF is the hydrophobic domain at the N terminus.\textsuperscript{(77)} It is the main IGF carrier in the serum (90% of IGF-1).\textsuperscript{(2)} It inhibits IGF-1 stimulated glucose incorporation into fat cells and IGF-1 stimulated DNA synthesis, by preventing IGF-1 binding to the IGF-type-1 receptors.\textsuperscript{(69,78)} IGFBP-3 has a higher affinity for both IGF-1 and IGF-2 than the IGF-1-receptor, however once in close contact with the cell surface its' affinity for IGF-1 and –2 diminishes and hence IGFs may become more available to the IGF receptors under the right conditions.\textsuperscript{(79-81)} This BP thus serves as a major storage pool for circulating IGF-1 and is the main regulator of IGF-1 function in humans.

4.5.4.4  IGFBP-4
Little is known about this low molecular weight binding protein. Its' T½ is undetermined. It can cross endothelial barriers. IGF-1 or –2 binding enhances serum and tissue protease mediated IGFBP-4 cleavage unless IGFBP-5 is present. It may have a role in cancer therapy as it appears to have pro-apoptotic properties.\textsuperscript{(82-83)}

4.5.4.5  IGFBP-5
In addition to IGFBP-3 this protein may also form a ternary complex with IGF and ALS*. Alternatively it can also form binary complexes with ALS* in the absence of IGF, allowing migration from the serum. IGFBP-5 and -3 share a highly homologous amino acid region which is involved in the formation of these complexes. Most IGF-1 preferentially binds IGFBP-3 over IGFBP-5 when available. Like IGFBP-3, ternary complexes form more readily in the presence of IGF-1 rather than IGF-2. IGFBP-5 can inhibit or stimulate IGF-1 action and can also inhibit the proteolysis of IGFBP-4 under different conditions.\textsuperscript{(83)}
4.5.4.6  IGFBP-6
This peptide is structurally similar to but much less potent than either IGFBP-3 or IGFBP-5. It cannot bind ALS* or form ternary complexes.\(^{(84)}\)

4.5.5  IGF Receptors
There are two principle types of IGF receptors. The type-1 receptor resembles the insulin receptor and the type-2 receptor has similar structure to the mammose-6-phosphate receptor. The type-1 receptor binds both IGF-1 and -2 and insulin with differing affinities whereas type-II receptor binds IGF-2 \(>\) IGF-1 but not insulin. Divergence of the receptor mediated actions of insulin and IGFs is likely related to the receptor relative affinities for different ligands. IGF-1 and -2 mediate most of their actions via the type-1 IGF receptors.\(^{(85)}\) IGF-1 binds to the cysteine-rich region of the \(\alpha\)-subunit, the binding site for IGF-2 has not been elucidated. Binding of the ligand to the \(\alpha\)-subunit causes autophosphorylation of the \(\beta\)-subunit which leads to the phosphorylation of downstream substrates, setting in motion either MEK or PI3K pathways.\(^{(86)}\) The action of different peptides such as IGF-1 and IGF-2 may be determined by the distribution of the different receptors on these organs. (Figure 13)

4.5.5.1  The Type-I IGF Receptor
Type-1 receptor consists of heterotetrameric (\(\alpha_2\beta_2\)) subunits like those in the insulin receptor. It possesses an intracellular tyrosine-kinase domain which undergoes autophosphorylation as the initial step in signal transduction. This type of signalling system is shared by receptors for a variety of growth factors including BDNF and PDGF.\(^{(84)}\) This receptor exhibits a hierarchy of binding affinities which favours IGF-1 \(>\) IGF-2 \(>>\) Insulin.
Figure 13: Structural characteristics of the receptors for insulin, IGF-1 and of the insulin/IGF-1 hybrid receptors. All three receptors consist of two extracellular α-subunits and two transmembrane β-subunits. The α-subunits confer ligand binding specificity. Locations are shown for the cysteine rich domains, which are required for ligand binding and the adjacent regions responsible for determining insulin-binding specificity. The intracellular tyrosine kinase catalytic domain of each β-subunits transphosphorylates sites on the opposite β-subunit. Tyrosine phosphorylations of the juxtamembrane and regulatory phosphorylation sites shown are required for most, if not all, signalling from these receptors. The function of C-terminal phosphorylation sites is undetermined. (Courtesy of Jones JI et al, 1995) (2)

These receptors are found on motor neuron perikarya and processes, throughout the spinal cord grey matter and in the median hypothalamus. Genetic mutations in the IGF type-1 receptor coding region have been associated with exceptional longevity and preservation of cognitive function in the elderly. A rare human hybrid IGF/Insulin receptor has also been identified having both one-alpha and one-beta subunit from each. Their relevance to waht is currently known about the mechanisms of the IGF system is unclear.
4.5.5.2 *The Type-II IGF Receptor*

Much remains unknown about the function of this receptor. Although it binds most of the circulating IGF-2 with high affinity it does not appear to mediate many of its biological effects. It appears to scavenge potentially biologically active IGF-2 out of circulation.\(^2\) This receptor also recognizes and binds IGF-1 but not insulin. It is identical to but distinct from the mannose-6-phosphate (M6P)-receptor. These receptors greatly outnumber type-1 IGF receptors in the brain but do not appear to carry out much of the IGF signalling which is performed by the type-1 receptors. Type-2 receptors are highly concentrated in the olfactory bulb, the hippocampus and the primary olfactory cortex.\(^{85-86, 92}\)

4.5.6 *IGF in Healthy Subjects*

The IGFs, IGF-1 in particular have many interesting effects on normal human metabolism and growth in addition to effects that occur in disease states. Intravenous IGF-1 in normal human volunteers causes rapid symptomatic hypoglycaemia (like insulin) but has less effect on free fatty acids (FFAs) and its' action is short-lived.\(^{93-98}\) IGF-1 suppresses insulin, glucagon and GH secretion, up regulates insulin receptors and suppresses GH mediated insulin resistance. IGF-I has anabolic and growth effects similar to growth hormone and increases noradrenergic and adrenergic responses to hypoglycaemia. Normal metabolic responses to IGF-1,-2 and insulin under different conditions are compared in table 9.\(^{93-98}\) Both IGF-1 and –2 are normally expressed in the central nervous system (CNS) and cross the blood brain barrier (BBB) poorly. Neuronal cell populations have abundant IGF-type-1 receptors therefore most IGF activity in the CNS is likely to be autocrine or paracrine. IGF-1 may act as a neurotransmitter and neuromodulator as well as a growth factor in the CNS.\(^{93}\)
Table 8: Dietary Regulation of the GH-IGF Axis in Humans. (Courtesy of Thissen JP et al, 1994) (41)

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▲ indicates increase
▼ indicates decrease
= indicates no effect
() indicates controversial effect
? indicates absence of data

There are a number of features of MND that may alter IGF-1 levels which is of relevance when we are serially measuring them. MND is a relative catabolic state particularly as the disease progresses and patients experience weight loss, increasing energy demands and respiratory failure. MND patients become chronically malnourished independent of the presence of bulbar symptoms. Poor nutritional status is well known to be an independent risk factor for reduced survival time in MND (99) and may be reflected in alterations in IGF-1 especially in the pre-agonal state. There have been a number of artificial experiments in healthy humans to evaluate how each of these factors interferes with IGF-1 and its’ proteins, what effect these factors combined produces on MND patients is probably extremely complex. In measuring IGF-1 levels serially in such a population it would be of relevance to know whether the patient was malnourished (low BMI) and also what stage of disease the patient was in i.e. early disease or pre-agonal.

4.5.7 IGF-1 in catabolic states

Clemmons et al gave a continuous IV infusion of IGF-1 to calorically restricted (starved) normal subjects. This resulted in an improved nitrogen balance comparable to that
achieved by administration of GH, except that serum glucose and insulin levels decreased rather than increased. These effects were less dramatic if GH was given concurrently.\(^{(96)}\) IGFBP-1 and -2 concentrations increased twofold during IGF-1 administration alone while IGFBP-3 decreased in some subjects. Combined IGF-1/GH resulted in a small decrease in IGFBP-1, no change in IGFBP-2 and a significant increase in IGFBP-3.\(^{(98)}\) The ALS* was increased by the combination therapy but decreased by IGF-1 alone. When GH and IGF-1 were administered together greater anabolic effects were seen this might be of relevance in the previous MND trials with IGF-1 where it was given alone.\(^{(9-12,96)}\)

### 4.5.8 Chronic under nutrition and IGF-1

Low IGF-1 and -2 bioactivity has been demonstrated in both marasmus and kwashikor.\(^{(100-102)}\) Low serum IGF-1 levels can be normalized by adequate nutritional rehabilitation though how long this should take is a matter of debate.\(^{(103)}\) Accompanying low IGF-1 levels typically are high GH levels and low/normal insulin levels. This suggests a metabolic adaptive mechanism making FFAs available to peripheral tissues as an alternative energy source.\(^{(102-103)}\) Kirschner in 1986 showed that IGF-1 concentrations in malnutrition increase in correlation with increased energy intake until normal growth is resumed.\(^{(104)}\) Similar findings have been shown in malnutrition states secondary to anorexia nervosa, inflammatory bowel disease, coeliac disease and HIV.\(^{(103-106)}\) Donahue et al\(^{(107)}\) demonstrated a decline in IGF-1 in their hospitalized malnourished patients which was most severe in those patients with protein or protein and calorie malnutrition, rather than calorie under nutrition alone, supporting the pivotal role of dietary protein intake in IGF-1 regulation. Hence nutrient intake is also the major regulator of IGFBPs concentrations in the plasma, which in turn controls bioavailability of IGFs to the tissues. Serum IGFBP-3 concentrations in healthy humans are relatively constant throughout the day and are largely responsible for the stability of serum IGF-1 levels. IGFBP-1 in contrast is rapidly and markedly suppressed by increased nutrient intake and secondary increases in serum insulin and glucose concentrations. IGFBP-2 levels are steadier and not subject to post-prandial change. Manipulation of diet alters serum IGFBP levels, as a general rule dietary restriction increases serum IGFBP-1 and -2 while decreasing IGFBP-3.\(^{(108)}\) During chronic malnutrition serum IGFBP-3 declines. In severe catabolic states

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serum IGFBP-3 undergoes proteolytic alteration so it has decreased affinity for IGF-1. This temporarily increases IGF-1 bioavailability for all the tissues However if IGF-1 binds instead to IGFBP-1 or -2, IGF-1 may be inadvertently excreted from the body. Some MND patients fall into the chronically malnourished category which is again of relevance to our study.

4.5.9 Exercise and IGF-1
Decline in IGF-1 seen with sustained increased exercise is caused by an insufficient supply of nutrients to meet energy expenditure. In MND where patients are not that active but probably require more energy to complete daily tasks this may be of relevance. Several studies on IGF-1 and IGFBP-3 levels in the normal population and relationship to physical fitness have not revealed any clear consistent pattern. Interestingly Kaspar et al has shown that a combination treatment regime of insulin-like growth factor via gene delivery and exercise in a mouse model of MND improved survival and function, but was not a cure.

4.5.10 IGF-1 and Age
IGF-1 levels decline with age which may partially be related to a decline in physical fitness but also reduced growth requirements with age. This is of relevance in MND where the average population age is 60-65 years. VO2 max, leisure time physical activity and weekly percent protein intake in diet directly correlate with IGF-I levels in men and women in studies of the IGF system in the elderly. Daily energy intake, oestradiol, age, BMI, menopausal state and fasting GH concentrations were not predictive of serum IGF-1 in an elderly population.

In our study we attempted to assess the entire IGF system in MND while controlling for several important variables that have been shown to influence IGF-1 concentration in the circulating blood such as age, sex and nutritional status. Torres-Aleman et al had previously reported low levels of serum IGF-1 in MND patients in a cross sectional study but there was little information on nutritional parameters provided. We predicted that over time nutrition would become difficult to control in our MND group and this decline
in weight and other nutritional indices would correlate with any decline in IGF-1 levels. We hypothesized that alterations in peripheral IGF-1 previously reported might be secondary to other events in the course of MND predominantly declining nutritional status. If this were the case then IGF-1 levels might be a useful early biomarker for nutritional decline in MND. Unterman et al had shown that in the normal population IGF-1 levels were more informative than anthropometric measurements and classical laboratory tests as indices of nutritional status.\(^{(115)}\) Similarly Minuto et al demonstrated that serum IGF-1 more accurately reflected changes in body composition than other serum markers such as albumin and transferrin.\(^{(116)}\)

There are several problems with using serum IGF-1 as a marker of malnutrition. Firstly, many disease processes may themselves alter blood IGF-1 levels and acceptable levels have a wide confidence interval in normal subjects. Secondly, there are several available commercial assays for IGF-1 available each with differing normal ranges. Thirdly, there is no universal agreement as to what level of IGF-1 would constitute 'malnourishment'. It is likely that serum IGF-1 and nutritional status do not have a direct linear relationship, instead when nutrient intake declines below a critical threshold, IGF-1 levels become altered. Above this threshold IGF-1 serum levels remain normal over a broad range of dietary intakes. Thisen et al suggested that this critical limit was when a patient was \(> 10\%\) below their predicted body weight.\(^{(117)}\) The use of IGF-1 to monitor response to increased nutritional support in malnourished patients has been used in other diseases.\(^{(118-120)}\) Fourthly, it is unclear what nutritional parameters most closely follow IGF-1 levels i.e. BMI Vs anthropometric measures. BMI is probably most widely used and easy to perform. Teramukai et al\(^{(111)}\) examined the IGF system in middle aged Japanese men and found that BMI strongly correlated with both IGF-1 and IGFBP-3 levels. In the same study smoking was inversely associated with serum IGF-1 and IGFBP-3 and alcohol correlated inversely with IGF-1 but directly with IGFBP-3. Thus the relationship between IGF-1, IGFBPs and nutrition is complex and also may be under the influence of a number of other factors that are not yet fully understood. In addition, because MND is a neurological disease it is not clear whether serum IGF-1 levels correlates to CNS levels or muscle/nerve levels of IGF-1. Two studies have examined both serum and CSF levels of
IGF-1 in MND, one study has examined CSF IGF-1 and binding proteins alone. Interestingly all of the studies reported normal serum levels of IGF-1 and IGFBP-3. In one study CSF free IGF-1 was up regulated.\textsuperscript{(121-123)} It should be reiterated at this stage that the above studies were all cross sectional rather than longitudinal.

Wilczak et al showed enhanced spinal motor neuron immunoreactivity for IGFBP-2, -5 and -6 in spinal cord segments from patients with MND.\textsuperscript{(124)} The same specimens showed upregulation of IGF-1 receptors compared to controls. IGFBP-2 inhibits IGF-1 action and IGFBP-5 can either inhibit or stimulate the actions of IGF-1, it can compete with IGFBP-3 for IGF-1 binding but is not as efficient as the former.\textsuperscript{(2,125-127)} IGFBP-6 also appears to be mainly inhibitory to the actions of IGF-1.\textsuperscript{(125-128)} The Wilczak study also demonstrated reduced free IGF-1 in the ventral horns of the spinal cord, which may have been secondary to the high concentrations of IGFBP-2, -5 and -6 in those areas.\textsuperscript{(124)} IGF receptors were upregulated perhaps to attract more free IGF-1. It is unclear whether these findings are a primary or secondary part of MND disease. Experimental lesions to lumbar motor neurons in rats produces a similar pattern of increased concentrations in binding proteins -2, -5 and -6 suggesting that the findings are a secondary phenomenon.\textsuperscript{(128)} Application of IGF-1 locally in the rats did not improve survival of their motor neurons because the exogenous IGF-1 was still unable to bind to IGF-1 receptors to exert an effect.\textsuperscript{(129)} This may be why trials with recombinant IGF-1 in MND failed, simply replenishing neurotrophic factors that are low is too simplistic an approach to a much more complicated problem than it first appears.

In our cross-sectional analysis of IGF-1 the mean BMI in MND patients was within the normal range (18.5-25) and patients were well matched for age and sex. Mean ALSFRSr score was 34/42 indicating only mild functional disability or early disease. (Table 1) Serum IGF-1 and IGF\textsubscript{TOTAL} were higher in MND and MS patients than in controls. PPS patients had normal values. This suggests that despite normal BMIs, patients with CNS disease were producing higher levels of IGF-1 perhaps as a compensatory strategy early in the disease. (Table 2) Notably in MND IGFBP-1 levels were reduced which is of uncertain significance as IGFBP-1 may inhibit or promote IGF-1 function.\textsuperscript{(72-73)}
In the longitudinal arm of the study only 21/52 (42%) of the MND patients survived to donate serial samples. PPS patients IGF indices remained stable throughout the first year and so further serial analysis was abandoned thereafter. MS patients showed some IGF system aberrations which correlated with interferon use. This is the subject of another PhD thesis and will not be discussed further here, details can be found in the referenced published article. Among the MND study patients 2 distinct subgroups emerged: ‘long-term’ (LT) survivors and ‘pre-agonal’ (PA) patients. ‘Pre-agonal’ patients were older (mean age 69 years) and behaved like classic MND, that is survival was approximately 2 years from diagnosis. Serum IGF-1 samples were collected in these patients within 30 days of death. ‘Long-term’ patients were younger (mean age 49 years) and survived beyond 2 years. There were more males in the LT group (8:2) and more females (7:4) in the PA group. The LT group consisted entirely of spinal onset disease and the PA group also had more spinal than bulbar (8:3) onset disease. (Table 3) An overall non significant downward trend in IGF-1, IGF-2, IGF_{TOTAL} and IGFBP-3 was noted over time in MND cases compared to controls. (Table 4) This mirrored but did not correlate directly with a decline in functional status. (Figures 6, 9 & 10) Suprisingly BMIs were relatively stable during the study. We believe this was due to close nutritional supervision and early PEG placement with supplemental feeding in our MND patients. We further suggest that the close nutritional supervision was responsible for the non significant downward trend in serum IGF-1, IGF-2, IGF_{TOTAL} and IGFBP-3 rather than a the more dramatic decline that might have been predicted. (Figure 11) It is likely that these findings would have been more significant in the setting of severe untreated nutritional failure associated with MND.

With regards to the MND subgroups, LT patients developed significantly higher IGF-1 levels as the disease progressed compared to controls. We presume this was to try and partially compensate for ongoing loss of neurons, because IGFBP-3 levels remained stable however, the additional IGF-1 was an ineffective strategy long term. In PA patients although IGF-1 levels remained similar to controls throughout the study there was a non significant trend towards reduction in IGFBP-3 which would render any existing IGF-1
less effective in the setting of neuronal destruction.\(^{130}\) Whether these effects are a intrinsic step in the pathogenesis of or a secondary event in MND is unclear.

Three studies have now failed to demonstrate consistent meaningful benefit in the treatment of MND patients with synthetic IGF-1. All of these studies gave IGF-1 via the subcutaneous route without any measurements of serum or CSF IGF and its’ associated proteins.\(^{9-10,12}\) The Cochrane review on rhIGF-1 in MND was performed prior to the last trial and had suggested there might be some benefit to IGF-1 treatment.\(^{11}\) Intravenous administration of rhIGF-1 is not an option as it can be harmful to the cardiovascular system.\(^{5}\) We have little idea of the amount of IGF-1 that can cross into the CNS from either of these routes. Given that IGF-1 is produced in the CNS it is likely that significant proportions of systemically produced IGF may not cross the BBB into the CNS. Nagano et al demonstrated a modest functional benefit after IGF-1 administration intrathecally in a small cohort of MND patients. This is a novel approach to treatment but has not been replicated to date.\(^{14}\)

### 4.6 Conclusion

Our data confirms the finding of perturbations in the IGF system in MND. An overall non significant downward trend in IGF-1 and IGFBP-3 was noted in our MND population over time. In addition two distinct subgroups emerged among the MND patients: ‘Long term’ MND patients show innate resistance to drop off in their IGF-1 levels early in their disease suggesting that these patients mount a compensatory overproduction of IGF-1 early on in the disease. In contrast ‘Pre-agonal’ MND patients have normal IGF-1 levels but their IGFBP-3 levels decline close to death. This may be a useful biomarker to identify patients who are reaching a stage where more frequent clinical monitoring is required. Close nutritional surveillance and early intervention stabilized BMI and IGF-1 levels for a time in MND patients studied however, more than the expected 10% of our longitudinal MND population had a longer than usual survival and so we must interpret this data with caution. ‘Long term’ patients may have had extended survival because of their intensive nutritional management (thereby increasing bioavailability of IGF-1 for
longer), attendance at a specialist MND clinic or other unidentified factors. It is not clear however why the ‘Pre agonal’ MND patients failed to respond to the same nutritional management program. Both groups of patients had similar demographics at the start of the study.

MND is a heterogeneous disease and some of the outcomes observed may have occurred regardless of our interventions. Although synthetic growth factors are an attractive treatment option in diseases such as MND it is clear from this study that growth factor systems are very complex. It is unlikely that replacement of one growth factor alone will treat such a complex disease. Future studies on growth factors in MND should pay particular attention to identifying factors that may alter the bioavailability of the ligand such as age, sex, and nutritional status, the presence of stimulatory and inhibitory binding proteins in the circulation and potential modes of transport of growth factors into the CNS. If growth factors do represent a potential treatment strategy for MND then it is far more likely this will take the form of a cocktail of multiple growth factors and their binding proteins rather than one growth factor alone.
References


Chapter Five
Final Thoughts

This thesis has covered a wide variety of topics relating to MND. It would not have been possible without input and co-operation from the multiple disciplines involved in the care and research of motor neuron disease in Ireland today. The Irish MND register is an ongoing unique and invaluable resource when conducting studies on MND in Ireland. Our epidemiological study confirmed a stable level of MND over a ten year period and similar levels of MND in Northern Ireland. This is both reassuring and disappointing.

It is reassuring because the data is almost identical in the two populations therefore it is likely that the data is accurate and representative of the Irish population. Because of the difficulties with highly inter-relating patient lists identified during this study, we were unable to perform capture-recapture analysis. We have since taken steps to separate the Irish MND register list from the Irish MND Association list and we have applied for access to unanonymised death certification data on MND cases from the CSO in the future. This will allow us to estimate our case ascertainment levels statistically in the future.

It is disappointing because the Dublin MND multi-disciplinary management clinic is now well established and yet our overall survival figures remain similar. This is despite the use of gastrostomy feeding and non invasive ventilation. (1) However, our figures represent the whole of the Republic of Ireland, not just the specialist clinic in Dublin. Not all patients choose to or are able to attend the Dublin clinic and not all hospitals in Ireland have immediate access to the above interventions. Specialist centres appear to have a survival benefit in MND, though this is somewhat controversial. (2-4) In addition it is suggested that gastrostomy may impact quality of life rather than survival in many cases of MND, though this is also debated and depends closely on the patients’ respiratory status. (5-7) Non invasive ventilation only officially became a standard of care relatively recently. (8) Future educational sessions for physicians and patients around Ireland involved in the care of MND are ongoing and aim to alert both groups to the available

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treatment options. We expect that in time the Irish survival figures will show the benefits of early non invasive ventilation that have been seen in other populations. (9)

The establishment of the sister register in Northern Ireland has certainly been a worthwhile and interesting project. This register is also being updated constantly and will produce independent data as well as occasional ‘all Ireland’ data, the first of which has recently been published. (10)

The Irish register also continues to be a useful data resource for studies on familial MND, MND with FTD and other interesting motor neuron diseases such as Kennedy’s disease. Our figures on familial MND and FTD associated MND are in line with other populations. (1,11) A large DNA databank has been collected from the Irish MND population in tandem with clinical data, including familial and FTD cases. This allows co-operative risk factor and genetic studies with other international MND communities. The clinical data collected and entered into the MND register is invaluable in conducting phenotype-genotype correlations.

The differences noted in our study between the ‘prevalent’ and ‘incident’ cohorts in the Irish population is topical given the climate of international co-operation among MND researchers. In the USA the ‘prevalent’ population, which includes younger patients, less males, less bulbar onset disease, longer delay to diagnosis is most commonly used in studies. These factors select out a population who will be expected to survive longer. In contrast, European populations such as the Irish MND population are studied typically as an ‘incident’ population who inherently include many patients with poorer prognostic markers. If the same drug or intervention were studied in both populations, a comparison between the results would be flawed. This is an important point that has not been raised before and probably needs discussion between different investigators before embarking on any future large clinical trial.
Both the epidemiological study and death certification information gleaned from our study could be used as important data when lobbying for future healthcare resources in MND. This is particularly true of hospital based facilities and the hospice foundation. Clearly the majority of MND patients in Ireland are centred around Dublin (Leinster) and Cork (Munster). This is where future resources should be targeted perhaps with a satellite clinic also in the West. Hospice services are currently underutilized with regards to MND palliative care. Investment in palliative care resources, in particular for non-cancer patients such as MND sufferers could result in large economic savings by the avoidance of inappropriate hospitalization of terminally ill MND patients. Future study of unanonymised MND death certification data will be useful in this regard.

In our search for risk factors in MND other than the previously identified age, sex and smoking, (12-15) we re-examined the topic of exercise and MND. Using a novel approach we evaluated lifetime physical activity mathematically in MND patients and controls. We found that those with a history of higher physical activity, in particular ‘vigorous activity’ were at increased risk of MND, especially spinal onset disease. Unlike previous studies the physical activity was not activity specific and included work related activity. The concept that excessive exercise might be a risk factor, perhaps in pre-disposed populations is an interesting one and deserves further evaluation in a larger population.

IGF deficiency had been proposed as a risk factor for MND, but previous studies failed to examine or control for the other factors such as binding proteins or nutritional status that also influence IGF levels. Our study confirmed abnormalities in IGF levels in MND patients. Initially total IGF levels (IGF-1 and IGF-2) were actually increased or up regulated in our MND patients. Later there was an overall downward trend in IGF-1 and a compensatory increase in IGFBP-3 as the disease progressed in pre-agonal patients. was stable. This pre-agonal may be useful in indicating those patients with critically advanced disease requiring urgent evaluation.
Future studies in the Irish MND population should aim to:

- Confirm the positive benefits of NIV epidemiologically.
- Expand the exercise and MND data.
- Correlate genetic and environmental risk factors (‘epigenetics’).
- Correlate genetic abnormalities with pathophysiological mechanisms of disease.
- Search for disease biomarkers and prognostic indicators.
- Evaluate further the Irish FTD-MND population.
- Encourage co-operation between different MND communities.
References


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Appendices

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D  Information Sheet for Exercise Study
E  Consent for Exercise Study
F  Information Sheet for IGF Study
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# Appendix A

**Sample Data sheet from MND Register**

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</tr>
<tr>
<td>Referral Source:</td>
</tr>
<tr>
<td>Research Source:</td>
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<tr>
<td></td>
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<tr>
<td>Risk Factors:</td>
</tr>
<tr>
<td>Notes:</td>
</tr>
</tbody>
</table>

Print All

| Clinical Evaluation | Clinical Details | Hospitals | G.P./P.H.N. | Death Details | Coordinator Details |
Appendix B

Information and Consent for Epidemiological Study

Patient name and Medical record number:

Bennaston Hospital
Medical consent research form, page 1

Title of Project: Epidemiological and genetic studies of familial amyotrophic lateral sclerosis (SOD1) and other forms of familial and sporadic motor neuron disease.

Principal investigator: Orin Hardman.

PURPOSE OF RESEARCH

I understand that I have been asked to participate in a research study of Motor Neuron Disease (MND). The overall objective of the study is to locate gene defects that might cause this disorder. Thus far, one gene has been identified as a cause of familial MND, which is the SOD1 gene on chromosome 21. Inclusion of those with specific forms of MND will allow determination in those cases of any genetic origin. The identification of other genes will improve diagnosis and may lead to improved therapy. The study is being conducted in collaboration with the University of Dundee, Benston Hospital and Harvard medical school.

PROCEDURE

If I agree to participate in this study, I will be asked questions about my medical and family history and I may have a medical examination in some cases. A venous blood sample will be obtained (approximately 20ml). Hospitalisation will not be necessary.

RISKS AND DISCOMFORTS

The blood sample is usually taken from a vein in the arm. The risks are minor. Occasionally there can be minimal blood loss, swelling or tenderness at the insertion site of the needle.

BENEFITS

There is no immediate benefit to me or my family in participating from this study. I understand that the results of the genetic tests will not be made available to me.

USE OF SPECIMENS

I understand that cells, tissue, blood or other specimens removed from me during the course of the study may be valuable for scientific, research or teaching purposes. I authorize Benston hospital and members of its professional staff to use my cells, tissues, blood or other specimens for these purposes.

CONFIDENTIALITY

The investigators will request information about my condition from my General Practitioner and the physician, usually the neurologist, who made the diagnosis. This information will then be converted to an anonymized form where patients cannot be identified by information held on the database. The information will be stored in the investigator's research file in accordance with the Data Protection Act. I can obtain a copy of the notes if I wish. Furthermore the blood samples will be anonymised so that they cannot be linked back to me.

If the data are used for publication in the medical literature or for teaching purposes, no names or other identifiers will be used. The Benston Hospital Ethical committee which has responsibility for scrutinising all proposals for medical research on humans in Benston hospital has examined the proposal and has raised no objections from the point of view of medical ethics.

REQUEST FOR FURTHER INFORMATION

I may ask more questions about the study at any time. Dr O'Tools or Dr Hardman are available at Benston hospital (telephone number = 013093000) to answer my questions or concerns.
Patient name and Medical record number

Beaumont Hospital
Medical consent research form, page 2

Title of Project: Epidemiological and genetic studies of superoxide dismutase 1 (SOD1) and other genes in familial and sporadic motor neuron disease.

Principal Investigators: Orla Hardiman.

A copy of this consent form will be given to me to keep for careful recording.

REFUSAL OR WITHDRAWAL OF PARTICIPATION

I understand that my participation is voluntary and that I may refuse to participate or withdraw consent and discontinue participation in the study at any time without affecting any present or future care at Beaumont Hospital. I also understand that Dr Orla Hardiman may terminate my participation in this study at any time after she has explained the reasons for doing so and has helped arrange for continued care by my own physician, if this is appropriate.

I have explained to ______________________________________________________________ the purpose of the research, the procedures required and the possible risks and benefits to the best of my ability.

Investigator's signature ___________________________ Date __________

I confirm that _________________________________________________________________ has explained to me the purpose of the research, the study procedures that I will undergo and the possible risks and discomforts as well as benefits that I may experience. I have read and understood this consent form. Therefore I agree to give my consent to participate as a subject in this research project.

Patient's name ___________________________ Date __________

Patient's name in capital letters ___________________________ Date __________

Witness to Signature ___________________________ Date __________

Witness to Signature in capitals ___________________________ Date __________
## Exercise Questionnaire for ALS

### Leisure Activity(s) and code

### Time / Age Period

<table>
<thead>
<tr>
<th>Leisure Activity(s) and code</th>
<th>12-18 yr (7 yr total)</th>
<th>19-34 yrs (16 yr total)</th>
<th>35-49 (15 yr total)</th>
<th>≥ 50 yr (____ yr total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. yr</td>
<td>Mon/yr</td>
<td>h/wk</td>
<td>No. yr</td>
<td>Mon/yr</td>
</tr>
</tbody>
</table>

01 Jogging (outdoor, treadmill)  
02 Swimming (laps, aqua-therapy)  
03 Bicycling (indoor, outdoor)  
04 Softball/BBall/Rounders  
05 Volleyball  
06 Bowling  
07 Basketball  
08 Skating (roll, ice blading)  
09 Martial Arts (karate, judo)  
10 Tai Chi  
11 Calisthenics/Fitness Exercises  
12 Rock Climbing  
13 Water/cold healing  
14 Walking for exercise (outdoor, indoor)  
15 Football/Soccer  
16 Racquetball/Tennis/Bowling  
17 Horseback Riding  
18 Hunting  
19 Fishing  
20 Aerobic Dance/Step Aerobic  
21 Water Aerobics  
22 Dancing (Square, Line, Ballroom)  
23 Gardening or Yardwork  
24 Badminton  
25 Strength/Weight Training  
26 Rock Climbing  
27 Scuba Diving  
28 Stall Riding  
29 Fencing  
30 Hiking  
31 Tennis  
32 Golf  
33 Cross-country/Skiing  
34 Water skiing  
35 Jumping Rope  
36 Snow Skiing (X-country/Nordic)  
37 Snow Skiing (Downhill)  
38 Snowboarding  
39 Yoga  
40 Other  
41 Rugby  
42 Cricket  
43 Hockey  
44 Sailing  
45 Bowling  
46 GAetetic Football  
47 Hunting
### Activity Survey – occupational activities

<table>
<thead>
<tr>
<th>Occupational activity - historical</th>
<th>Age at Start and Finish of Job</th>
<th>Job Schedule (Average Schedule)</th>
<th>Job activities</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>At work, is most of your time spent sitting or performing light activities? If not, how is it usually spent?</td>
</tr>
<tr>
<td>Job Titles</td>
<td>Start</td>
<td>Finish</td>
<td>Molyr</td>
</tr>
<tr>
<td>List all other jobs held during lifetime for more than one year</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

± If more than 4 jobs, list the jobs held the longest.

**Light activities** (including standing, slow walking, and all sitting activities): sitting, standing, light cleaning (ironing, cooking, washing, dusting), driving a tractor or harvester, slow leisure walking.

**Moderate activities** (includes most indoor activity): carrying light loads (5-10 lb), continuous walking, heavy cleaning (mopping, sweeping, scrubbing, scraping), gardening (planting or weeding), painting/plastering, plumbing/welding, electrical work.

**Hard Activities** (includes heavy industrial work, most outdoor construction, heavy farming): carrying moderate to heavy loads, shoveling, heavy construction, farming (bowing, digging, mowing), digging ditches, chopping (ax), sawing.
Appendix D

Information Sheet for Exercise Study

Title of Project: A population-based case-controlled study of risk factors for the development of motor neurone disease

Principal investigators: Orla Hardiman, Orna O'Toole, Paul Brennan.

PURPOSE OF RESEARCH

I understand that I have been asked to participate in a research study of Motor Neuron Disease (MND). The overall aim of the study is to determine whether exercise increases the risk for developing MND. If we find factors relating to exercise are important, we will look for gene defects that might be in part responsible for the increased risk. The identification of new genes will improve diagnosis and may lead to improved therapy.

PROCEDURE

(A) If I agree to participate in this study, I will be asked questions about my exercise habits. I may have a medical examination in some cases.

(B) I will be asked to donate a venous blood sample will be obtained (approximately 20mLs, or 4 tablespoons). Hospitalisation will not be necessary. The blood sample will be stored for genetic studies after the completion of the questionnaire study.

RISKS AND DISCOMFORTS

The blood sample is usually taken from a vein in the arm. The risks are minor. Occasionally there can be minimal blood loss, swelling or tenderness at the insertion site of the needle.

BENEFITS

There is no immediate benefit to me or my family in participating from this study.

USE OF DATA

I understand that my personal details will be made anonymous, and will be entered into a secure file of a computer. I understand that this data will be used to establish whether exercise, or sex hormone levels are related to the development of MND.

USE OF SPECIMENS
I understand that blood removed from me during the course of the study may be valuable for scientific, research or teaching purposes. I authorise Beaumont hospital and members of its professional staff to use my blood or other specimens for these purposes. I understand that the results of the genetic tests will not be made available to me, or to any party involved in my medical care, other than the research team.

I understand that it will not be possible to link the results of the genetic tests directly back to me.

CONFIDENTIALITY
The investigators may request information about my condition from my General Practitioner and the physician, usually the neurologist, who made the diagnosis. This information will then be converted to a form where patients cannot be identified by information held on the database.

The information will be stored in the research file at Beaumont Hospital, in accordance with the Data Protection Act. I understand that I can obtain a copy of this entry if I wish.

If the data are used for publication in the medical literature or for teaching purposes, no names or other identifiers will be used.

The Beaumont Hospital Ethical committee which has responsibility for scrutinising all proposals for medical research on humans in Beaumont hospital has examined the proposal and has raised no objections from the point of view of medical ethics.

REQUEST FOR FURTHER INFORMATION
I may ask more questions about the study at any time. Both Paul Brennan and Dr Hardiman are available at Beaumont hospital. Paul’s telephone number is 01 8093874 and is available to answer my questions or concerns.
Appendix E
Consent for Exercise Study

Title of Project: A population-based case-controlled study of risk factors for the development of motor neurone disease

Principle investigators: Orla Hardiman, Orna O'Toole, Colm Sheehan

The patient should complete this form himself/herself

Have you read the information sheet?
Yes/No

Have you had an opportunity to ask questions and discuss this study?
Yes/No

Have you received satisfactory answers to all of your questions?
Yes/No

Have you received enough information about the study?
Yes/No

Whom have you spoken to?
Dr/Mrs..........................

Do you understand that participation is entirely voluntary?
Yes/No

Do you understand that you are free to withdraw from the study:
• at any time?
  Yes/No
• without having to give a reason for withdrawing?
  Yes/No
• without affecting my future medical care?
  Yes/No

Do you agree to take part in this study?
Yes/No

Patient's/Next of
kin/Signature..........................Date..........................

Patient's name in capitals........................................
Appendix F
Information Sheet for IGF Study

Subject Information Sheet: Motor Neuron Disease

A Study of insulin like growth factor metabolism and neuroendocrine status in 3 chronic neurologic conditions:

Motor Neuron Disease

Multiple Sclerosis

Post Polio Syndrome

What is required from you:

- A fasting blood sample (drawn every 3 months).
- A nutritional assessment. This will involve taking your weight, height, diet history, and upper arm measurements (skin fold thickness and arm strength will be included).
- A psychological assessment. This involves answering 5 questionnaires.
- A neuroendocrine challenge. This involves the injection of growth hormone, after which blood samples will be drawn (to be carried out every 6 months).
- A muscle biopsy will be performed.

This research project will be conducted during a routine admission to hospital for a general assessment of your condition. This study will take place over a 3-5 day period including weekends.

Any personal requirements will also be dealt with during your stay.

Our findings will not have any direct impact on your treatment, however your participation allows us to gain further knowledge of your condition and may help explore and develop new treatment options.
Appendix G
Consent for IGF Study

Beaumont Hospital
Medical consent research form, page 1

Title of Project: A study of the relationship between altered neuroendocrinology, insulin-like growth factor metabolism and nutritional status in patients with Amyotrophic Lateral Sclerosis.

Principal investigators: Orla Hardiman Veronika O'Keane, Kay Nolan, Francesca Brett

REFUSAL OR WITHDRAWAL OF PARTICIPATION
I understand that my participation is voluntary and that I may refuse to participate or withdraw consent and discontinue participation in the study at any time without affecting my present or future care at Beaumont Hospital. I also understand that Dr. Orla Hardiman or Dr. Veronika O'Keane may terminate my participation in this study at any time after she has explained the reasons for doing so and has helped arrange for continued care by my own physician, if this is appropriate.

I have explained to ___________________________ the purpose of the research, the procedures required and the possible risks and benefits to the best of my ability.

________________________________________________________________________

Investigator’s signature Date

I confirm that ___________________________ has explained to me the purpose of the research, the study procedures that I will undergo and the possible risks and discomforts as well as benefits that I may experience. I have read and understand this consent form. Therefore I agree to give my consent to participate as a subject in this research project.

CONSENT

I agree to participate in this study

Yes

No

________________________________________________________________________

Patient’s name Date

________________________________________________________________________

Patient’s name in capitals Date
Title of Project: A study of the relationship between altered neuroendocrinology, insulin-like growth factor metabolism and nutritional status in patients with Amyotrophic Lateral Sclerosis.

Principal investigator: Orla Harvillman Veronica O’Keane, Kay Nolan, Francesca Brett

The patient should complete this form himself/herself

Have you read the information sheet? Yes/No

Have you had an opportunity to ask questions and discuss this study? Yes/No

Have you received satisfactory answers to all of your questions? Yes/No

Have you received enough information about the study? Yes/No

Whom have you spoken to?

Dr.

Do you understand that participation is entirely voluntary? Yes/No

Do you understand that you are free to withdraw from the study:
- at any time? Yes/No
- without having to give a reason for withdrawing? Yes/No
- without affecting future medical care? Yes/No

Do you agree to take part in this study? Yes/No

Patient’s signature................................Date..........................

Patient’s name in capitals.......................................................

Telephone number where patient can be contacted:

.................................(home).................................(work)
Appendix H
ALSFRS-r Sample

ALSFRS-r

1. **Speech**
   - 0. Loss of useful speech
   - 1. Speech combined with nonvocal communication
   - 2. Intelligible with repeating
   - 3. Detectable speech disturbance
   - 4. Normal speech processes

2. **Salivation**
   - 0. Marked drooling; requires constant tissue or handkerchief
   - 1. Marked excess of saliva with some drooling
   - 2. Moderately excessive saliva; may have minimal drooling
   - 3. Slight but definite excess of saliva in mouth; may have nighttime drooling
   - 4. Normal

3. **Swallowing**
   - 0. NPO (exclusively parenteral or enteral feeding)
   - 1. Needs supplemental tube feeding
   - 2. Dietary consistency changes
   - 3. Early eating problems — occasional choking
   - 4. Normal eating habits

4. **Handwriting**
   - 0. Unable to grip pen
   - 1. Able to grip pen but unable to write
   - 2. Not all words are legible
   - 3. Slow or sloppy; all words are legible
   - 4. Normal

5a. **Cutting food and handling utensils (patients without gastrostomy)**?
   - 0. Needs to be fed
   - 1. Food must be cut by someone, but can still feed slowly
   - 2. Can cut most foods, although clumsy and slow; some help needed
   - 3. Somewhat slow and clumsy, but no help needed
   - 4. Normal

5b. **Cutting food and handling utensils (alternate scale for patients with gastrostomy)**?
   - 0. Provides minimal assistance to caregiver
   - 1. Some help needed with clomuses and fasteners
   - 2. Chummy but able to perform all manipulations independently
   - 3. Unable to perform any aspect of task
   - 4. Normal

6. **Dressing and hygiene**
   - 0. Total dependence
   - 1. Needs attendant for self-care
   - 2. Intermittent assistance or substitute methods
   - 3. Independent and complete self-care with effort or decreased efficiency
   - 4. Normal function
7. **Turning in bed and adjusting bed clothes**

   4  Normal
   3  Somewhat slow and clumsy, but no help needed
   2  Can turn alone or adjust sheets, but with great difficulty
   1  Can initiate, but not turn or adjust sheets alone
   0  Helpless

8. **Walking**

   4  Normal
   3  Early ambulation difficulties
   2  Walks with assistance
   1  Nonaulatory functional movement
   0  No purposeful leg movement

9. **Climbing stairs**

   4  Normal
   3  Slow
   2  Mild unsteadiness or fatigue
   1  Needs assistance
   0  Cannot do

10. **Dyspnea (new)**

    4  None
    3  Occurs when walking
    2  Occurs with one or more of the following: eating, bathing, dressing (ADL)
    1  Occurs at rest, difficulty breathing when either sitting or lying
    0  Significant difficulty, considering using mechanical respiratory support

11. **Orthopnea (new)**

    4  None
    3  Some difficulty sleeping at night due to shortness of breath, does not routinely use more than two pillows
    2  Needs extra pillows in order to sleep (more than two)
    1  Can only sleep sitting up
    0  Unable to sleep

12. **Respiratory insufficiency (new)**

    4  None
    3  Intermittent use of BiPAP
    2  Continuous use of BiPAP during the night
    1  Continuous use of BiPAP during the night and day
    0  Invasive mechanical ventilation by intubation or tracheostomy

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Appendix I
Published Data

Epidemiology and clinical features of amyotrophic lateral sclerosis in Ireland between 1995 and 2004
O O'Toole,1 B J Traynor,2 P Brennan,3 C Sheahan,1 F Frost,2 B Corr,1 O Hardiman1

ABSTRACT
Background: We conducted a prospective, population-based study to examine trends in incidence and prevalence of amyotrophic lateral sclerosis (ALS) in Ireland from 1995 to 2003.
Methods: The Irish ALS Register was used to identify Irish residents diagnosed with ALS between the 3-year period from 1 January 1995 to 31 December 1997 and the 3-year period from 1 January 2002 to 31 December 2004.
Results: 465 Irish residents were diagnosed with ALS during the study periods. The annual incidence rate of ALS in Ireland remained stable over this time (2.5 cases per 100 000 person-years, 95% CI 1.8, 2.7). Median survival of Irish ALS patients was 18.4 months and did not change during this study period. Demographic and clinical features of the incident and prevalent Irish ALS cohorts were markedly different.

Although the development of a population-based register has acurately measured the epidemiology of amyotrophic lateral sclerosis (ALS) in European and North American populations,7 it is not known whether the incidence of this disease has changed over time (see page 4). It is also unclear if the clinical course of ALS has changed over the past decade as a result of improvements in supportive care, such as non-invasive ventilation and early gastrostomy tube placement, and the introduction of riluzole. Changes in the prognosis of ALS would significantly impact on clinical trial design and healthcare delivery.

The purpose of this study was to examine the temporal pattern of ALS epidemiology within Ireland over the 10-year period from 1 January 1995 to 31 December 2004.

METHODS
Details of the Irish ALS Register have been published previously.8 Briefly, the Irish ALS Register was used to identify Irish residents diagnosed with suspected, possible, probable or definite ALS according to the El Escorial criteria during the 3-year period from 1 January 1995 to 31 December 1997 and during the 3-year period from 1 January 2002 to 31 December 2004. Overall incidence rates were calculated as observed number of new cases during the 6 years of study (1995-1997 and 2002-2004) divided by the total Irish population during the same time period.9 There has been significant immigration to Ireland over the past decade (220 100 in the past 7 years, Irish population in 2002 was 3.917 288). To analyze the temporal trend of ALS in the face of shifting demographics, incidence rates for the 2002-2004 cohort were age and gender adjusted to the 1996-Irish population using the direct method.

The denominator for the calculation of prevalence rates was the Irish population on 31 December 1996 and 31 December 2000.5 Survival was estimated using the Kaplan-Meier method using time from date of diagnosis to death, tracheostomy (1995-97, n = 2; 1998-2004, n = 2) or last follow-up.

RESULTS
During the 6 years of study (1995-1997 and 2002-2004), 465 Irish residents were diagnosed with suspected, possible, probable or definite ALS. Of these, 29 (6.2%) had familial ALS based on a detailed family history, and 32 cases (6.9%) had clearly documented frontotemporal dementia (according to the Manchester-Lund criteria).

Demographic and clinical features of Irish patients with ALS did not change significantly between the 1995-97 and 2002-2004 cohorts (table 1).

Based on 465 newly diagnosed cases, the average annual incidence rate of ALS in Ireland was 2.5 cases per 100,000 person-years (95% CI 1.8, 2.7), see supplementary table 1 and supplementary fig 1. The average annual incidence rate for the population older than 15 years was 2.1 per 100,000 (95% CI 1.3, 2.9), see supplementary table 1 and supplementary fig 1. The 95% CI 1.3, 2.9). The incidence rate was 2.1 per 100,000 (95% CI 1.3, 2.9) for the 2002-2004 cohort after age and gender adjustment to the 1996 Irish population.

The incidence of bulbar onset ALS was identical among men and women (2.8% per 100,000 person-years, 95% CI 0.7, 1.4, see supplementary fig 2). In contrast, the incidence of limb onset ALS was almost twice as high among women compared with women (1.5 per 100,000 person-years, 95% CI 1.3, 1.7) vs 0.8 (95% CI 0.7, 0.9). Women had similar incidence rates for limb onset and bulbar onset disease at nearly all ages whereas the male incidence rate of bulbar onset disease was consistently higher than bulbar onset disease for all age groups in men.

The crude prevalence rate of ALS at 31 December 1996 was 0.2 per 100,000 population over the age of 15 years (95% CI 0.1, 0.3). The prevalence rate on 31 December 2005 was almost identical (0.4 per 100,000, n = 200, 95% CI 0.5, 0.7, see supplementary table 1). The clinical features of the prevalent and incident cohorts were markedly different (see table 2). For example, bulbar onset disease accounted for less than one-third of...
improvements in case ascertainment and diagnostic methods rather than a genuine increase in incidence.

Survival of Irish patients with ALS was similar to that reported in other population based studies and did not change over the 10 year study period, 1995-2004. This is an unexpected finding as there have been at least minor advances in treatment during the past decade. Several factors may explain this observation. Riluzole was available to a small number of Irish patients on compassionate grounds from 1994 and, thus, the therapeutic effect of this medication, albeit mild, may have influenced prognosis in both cohorts. Internal nutrition became widely utilized in Ireland from the late 1990s onwards, and was expected to improve survival of the 2002-2004 cohort. However, although early evidence suggested that percutaneous endoscopic gastrostomy assistance and external nutrition improved life expectancy, more recent studies failed to demonstrate a significant survival advantage and a study in the Scottish ALS population suggested that patients undergoing percutaneous endoscopic gastrostomy have reduced survival. The most significant beneficial intervention in the management of ALS over the past 10 years has been the use of non-invasive ventilation. Non-invasive ventilation was not widely used in Ireland until after 2004, meaning that the positive survival benefits of this intervention would not have been apparent in the 2002-2004 cohort.

There were significant differences in the demographics, clinical features and survival times of the prevalent and incident cohorts of Irish ALS cases (Table 3). The prevalent cohort had fewer bulbar onset patients (31.3% vs 42.3%) and fewer patients over the age of 65 years (57.9% vs 52.3%) compared with the incidence cohort. These differences are most likely a result of the shorter prognosis associated with bulbar onset disease (241 days vs 602 days among incident cases) and with disease onset over 65 years of age (267 days vs 649 days among patients younger than 65 years). Indeed, the survival pattern of the 2003 prevalent cohort differs markedly from that of the Irish incident cohort (fig 1; log rank  127.4, p <0.0001). This has important implications for the current North American and UK efforts to collect large numbers of ALS DNA samples. The North American initiative focuses on collecting prevalent cases and it is likely that cases with a poor prognosis, such as bulbar onset

### Table 1 Demographics and clinical features of Irish patients diagnosed with amyotrophic lateral sclerosis in the 1995-1997 and 2002-2004 cohorts

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>85.7 (19.7)</td>
<td>84.5 (18.2)</td>
<td>85.0 (19.4)</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>129/110</td>
<td>110/134</td>
<td>229/244</td>
</tr>
<tr>
<td>Limbic/limbic plus onset</td>
<td>125/60/43</td>
<td>120/64/44</td>
<td>245/124/89</td>
</tr>
<tr>
<td>Family history of ALS</td>
<td>9</td>
<td>8</td>
<td>17</td>
</tr>
<tr>
<td>Prevalence with FTD</td>
<td>41</td>
<td>39</td>
<td>80</td>
</tr>
<tr>
<td>Mean disease delay (months)</td>
<td>10.5</td>
<td>11.6</td>
<td>11.0</td>
</tr>
<tr>
<td>Median survival (months)</td>
<td>17.2</td>
<td>16.1</td>
<td>16.4</td>
</tr>
<tr>
<td>Incidence per 100,000 py</td>
<td>2.5 (1.8, 2.7)</td>
<td>1.9 (1.8, 2.9)</td>
<td>2.3 (1.9, 2.6)</td>
</tr>
<tr>
<td>Incidence rate per 100,000 py 15 y</td>
<td>2.8 (2.4, 3.1)</td>
<td>2.5 (2.2, 2.8)</td>
<td>2.6 (2.1, 3.0)</td>
</tr>
<tr>
<td>Female incidence per 100,000 py</td>
<td>1.8 (1.4, 2.3)</td>
<td>1.6 (1.3, 2.0)</td>
<td>1.7 (1.3, 2.0)</td>
</tr>
<tr>
<td>Male incidence per 100,000 py</td>
<td>2.5 (2.1, 2.8)</td>
<td>2.3 (2.0, 2.9)</td>
<td>2.4 (2.1, 2.7)</td>
</tr>
<tr>
<td>FTD incidence per 100,000 py</td>
<td>9.3 (8.9, 11.1)</td>
<td>9.1 (8.5, 10.4)</td>
<td>9.1 (8.6, 10.3)</td>
</tr>
<tr>
<td>Limbic incidence per 100,000 py</td>
<td>1.2 (1.0, 1.4)</td>
<td>1.1 (1.0, 1.6)</td>
<td>1.2 (1.1, 1.6)</td>
</tr>
</tbody>
</table>

*Age and gender adjusted to the 1996 Irish population. 
*The Irish ALS Registry did not routinely collect age at onset data until 2000. 
*The t-test statistics = -3.2, p = 0.002.

### Table 2 Demographic and clinical features of Irish patients diagnosed with ALS in incident and prevalent cohorts

<table>
<thead>
<tr>
<th>Incident cohort (n = 239)</th>
<th>Prevalent cohort (n = 200)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age (y)</td>
<td>66.0</td>
</tr>
<tr>
<td>Sex (M/F) (%)</td>
<td>269 (117/152)</td>
</tr>
<tr>
<td>Limbic/limbic plus onset (%)</td>
<td>120 (54/66)</td>
</tr>
<tr>
<td>Patients younger than 50 y (n)</td>
<td>62 (26.2)</td>
</tr>
<tr>
<td>Patients older than 65 y (n)</td>
<td>244 (65.9)</td>
</tr>
<tr>
<td>Family history of ALS (%)</td>
<td>21 (5.3)</td>
</tr>
<tr>
<td>Incidence rate per 100,000 py</td>
<td>2.5 (1.8, 3.1)</td>
</tr>
<tr>
<td>Mean disease delay (months)</td>
<td>10.5</td>
</tr>
<tr>
<td>Median survival (moths)</td>
<td>17.2</td>
</tr>
</tbody>
</table>


*1995 December 2030.

ALS, amyotrophic lateral sclerosis; FTD, frontotemporal dementia.
and elderly patients, will be under-represented in the final cohort. In contrast, the UK DNA Bank collects only newly incident cases. Although this approach results in a slower collection rate, the final cohort may more accurately represent the general ALS population.

The observed increase in the rate of familial cases and cases with frontotemporal dementia between the two time periods is almost certainly a result of improved collection of familial aggregation data and a growing awareness among neurologists of cognitive dysfunction associated with ALS.

The multiple sources of information and prospective patient follow-up employed by the Irish ALS Registry mean that an Irish resident diagnosed with ALS is likely to have been ascertained and included in the current study. However, there is currently no diagnostic test for ALS, and relying on clinical details to achieve a diagnosis may impede case ascertainment. The use of probable and definite ALS El Escorial criteria as eligibility criteria for clinical trials and ALS research has been generally criticized as it means that a patient must be quite advanced in their clinical course prior to enrollment. To overcome this, the Irish ALS Registry includes patients with suspected or possible ALS, and the prospective patient follow-up inherent to the Registry’s design allows the diagnosis of ALS to be ultimately confirmed or refuted.

The analysis of the pattern of bulbar and limb onset ALS yields a number of important observations. First, age and gender adjusted incidence among Irish ALS patients is remarkably similar to a recent report of an Italian population of comparable size. The incidence of bulbar and limb onset disease among women is similar for all ages whereas limb onset ALS was consistently more common than bulbar onset disease among men. This observation confirms the clinical perception that bulbar onset disease is proportionately more common among women than among men. Second, our data indicate that the higher incidence of ALS among men that has been consistently reported in ALS epidemiological studies is largely a result of the higher rate of limb onset disease among middle aged men. Finally, the age distribution of bulbar onset and limb onset disease is the same among both genders, increasing rapidly after the age of 65 years, reaching a peak in approximately the eighteenth decade of life and declining rapidly thereafter. This pattern is distinct from that observed with Alzheimer’s disease and Parkinson’s disease, where incidence continues to rise with increasing age, and is consistent with the existence of a genetically susceptible cohort within the general population.

Alternatively, this pattern of disease incidence may reflect poor case ascertainment among the older age groups or a naturally shrinking pool of very elderly in the general population (1% of the Irish population is over the age of 85 years). It will not be possible to determine which of these possibilities is correct until the pathogenesis of ALS is more fully understood.

Acknowledgments: The authors gratefully acknowledge the assistance of all the contributing neurologists, neurophysiologists and primary care physicians who contributed in ascertainment for the Irish ALS Registry. The viability of a single Registry in this manner rests crucially on the excellent work of Ray Ennis (www.medicine.com). Funding: This research was supported by the Intramural Program of the NINDS (U10-NS45803) and by a grant to the ALS Association (19 and 32).

Competing Interests: None.

REFERENCES

Circulating insulin-like growth factors and related binding proteins are selectively altered in amyotrophic lateral sclerosis and multiple sclerosis

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Abstract

Objective: To provide a detailed profile of the peripheral IGF system in the neurological conditions: amyotrophic lateral sclerosis (ALS), post polio syndrome (PPS) and multiple sclerosis (MS). To determine whether subsets of patients within the disease groups could be identified in whom one or more components of the IGF regulatory system are altered compared to healthy control subjects matched for age, sex and BMI.

Design: Three cohorts of patients were recruited, 28 with ALS, 18 with PPS and 23 with MS. Patients were individually matched to a healthy control based on sex, age (±3y), and BMI (±2.5 kg.m⁻²). The concentration (ng/ml) of serum (IGF-I, IGF-II, IGFFBP-3, IGFFBP-2 and IGFFBP-3 and acid-labile subunit (μg/ml)) was determined by IRMA.

Results: In ALS patients, there was an increase of 11% in [IGF(Total)] (p = 0.042) ([IGF(Total)] = [IGF-I] + [IGF-II] and [IGFBP-3]) was decreased by 34% (p = 0.050) compared to matched controls. In "surviving" ALS patients, defined as those ALS patients with long disease duration (+3 SD from the mean survival time for Irish patients post diagnosis), there was an increase in [IGF-I] 36% (p = 0.033) and a large decrease in [IGFBP-1] -38% (p = 0.003) compared to controls. These differences were not evident in pre-asymptomatic ALS patients. The concentration of serum IGF-I was 38% (p = 0.018), acid-labile subunit 17% (p = 0.044) and IGFFBP-2 45% (p = 0.035) higher in MS patients compared to controls. When stratified for Interferon-beta (IFN-β) use, we observed an increase in serum [IGF-I] 52% (p = 0.013) and [IGFBP-3] 19% (p = 0.043) in MS patients undergoing IFN-β treatment, but MS patients not undergoing IFN-β treatment had similar IGF and IGFFBP concentrations to controls. Serum [IGFBP-3] 18% (p = 0.033), [IGFBP-2] 86% (p = 0.015) and (acid-labile subunit) 33% (p = 0.012) was also higher in IFN-β patients compared to controls. Stratifed by stage of disease the most significant increase in components of the peripheral IGF system was attributed to relapsing-remitting MS patients treated with IFN-β. All components of the peripheral IGF system in PPS patients were similar to controls.

Conclusions: The increase in circulating IGF-I and a reduction in regulatory binding protein IGFBP-3 in ALS patients with a "stable" disease profile suggest a potential change in peripheral IGF bioavailability in these subjects. In MS, we report a change in a number of components of the peripheral IGF system, the observed increase in IGF-I in patients treated with IFN-β being of most significance as a potential therapeutic biomarker.

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Keywords: IGF; Amyotrophic lateral sclerosis; Post polio syndrome; Multiple sclerosis; Interferon-β

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1. Introduction

The peripheral insulin-like growth factor (IGF) system has a proven role in maintaining normal neurological function and recovery post-injury [1-3]. IGF-I and IGF-II can cross the blood brain barrier (BBB) [6,7] and uptake of circulating IGF into the cerebrospinal fluid (CSF) has been demonstrated [2,3]. Though high expectation has been placed on the identification of abnormalities in neurotrophic factors in neurodegenerative disease, attempts to demonstrate abnormalities in circulating IGFs have been confounded by the complexity of the IGF regulatory system [5].

Greater than 85% of peripheral IGF (IGF-I and IGF-II) circulates bound in a ternary complex comprising of IGFBP-3 and the acid-labile subunit. Formation of the ternary complex, which is unable to cross the capillary epithelium, ensures a large reservoir of serum IGF available to the tissues. A smaller proportion of IGF is bound to the low molecular weight binding proteins such as IGFBP-1 and 2. These binary complexes readily leave the circulation targeting IGF to the tissues. A further role for IGFBP-2 has also been proposed, substituting as a carrier protein should the binding capacity of the ternary complex be insufficient [5].

Studies investigating changes in the peripheral IGF system in neurological diseases have largely focused on IGF-I, ignoring IGF-II and, to a large extent, the regulatory binding proteins (IGFBPs). Clinical trials of systemically delivered recombinant human IGF-I (rhIGF-I) have been carried out in neurological conditions including amyotrophic lateral sclerosis (ALS) [9,10], post-polio syndrome (PPS) [11] and multiple sclerosis (MS) [12] but clinical benefit has not been conclusively demonstrated. The absence of detailed information on the endogenous peripheral IGF system in disease states may be one of the limitations in the design of these clinical trials. To date, conflicting studies of the circulating IGFs report normal or decreased levels of IGF-I in ALS [13-15] and post-polio syndrome (PPS) [16,17] but unchanged levels of IGF-I in MS [14,19].

The purpose of this study was to provide a detailed profile of the IGF system in three neurological conditions in which data are conflicting regarding disruption of IGF regulation, i.e. amyotrophic lateral sclerosis, post-polio syndrome and multiple sclerosis. ALS is a progressive neurodegenerative disease that leads to loss of both upper and lower motor neurons and is invariably fatal with median survival after onset of 1.6-3.3 years [20-24]. PPS is a condition that can develop in patients previously affected by polio who develop new weakness. PPS has clinical parallels with ALS as motor neurons are affected in both conditions. Multiple sclerosis (MS) is an inflammatory disease of the CNS, characterised by central demyelination and resultant neurodegeneration.

This study is a comparison of IGF-I and IGF-II, and the relevant binding proteins responsible for modulating IGF availability in the circulation in these three neurological conditions. We sought to determine whether subsets of patients within the disease groups could be identified in whom one or more components of the IGF regulatory system are altered compared to healthy control subjects matched for age, sex and BMI.

2. Methods

2.1. Subjects

Three cohorts of patients were recruited from specialist clinics at Beaumont Hospital. These included 28 (10 men and 18 women) with definite or probable ALS (El Escorial criteria) [25], 18 (7 men and 11 women) PPS patients [26] and 23 (8 men and 15 women) with MS (McDonald criteria) [27,28]. The physical characteristics of each population are listed in Table 1. All patients and controls were of Irish descent. ALS patients with spinal (n = 23) and bulbar (n = 5) onset were included and all patients were receiving Riluzole. The ALS functional rating scale (ALSFRS-R) was used as a measure of functional impairment in ALS patients. The mean (SD) ALSFRS-R score was 34 (9), with a range of 21-45.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>ALS Controls</th>
<th>PPS Controls</th>
<th>MS Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>28</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>Female</td>
<td>15</td>
<td>18</td>
<td>11</td>
</tr>
<tr>
<td>Male</td>
<td>13</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td>Mean age (yr)</td>
<td>56.8 (12.9)</td>
<td>56.2 (12.2)</td>
<td>56.7 (8.8)</td>
</tr>
<tr>
<td>Mean BMI (kg/m²)</td>
<td>24.3 (3.7)</td>
<td>24.6 (2.7)</td>
<td>26.5 (2.3)</td>
</tr>
<tr>
<td>Mean age of onset (yr)</td>
<td>40.3 (14.3)</td>
<td>40.3 (14.3)</td>
<td>40.3 (14.3)</td>
</tr>
<tr>
<td>Mean duration of symptoms (yr)</td>
<td>12.7 (4.4)</td>
<td>12.7 (4.4)</td>
<td>12.7 (4.4)</td>
</tr>
<tr>
<td>Mean duration of symptoms (yr)</td>
<td>3.8 (3.2)</td>
<td>3.8 (3.2)</td>
<td>3.8 (3.2)</td>
</tr>
</tbody>
</table>

Values are presented as mean (SD).
MS patients were classified as relapsing-remitting (N = 11) secondary progressive (N = 10) or primary progressive (PP) (N = 2) by an experienced clinician based on their disease history. Functional impairment was assessed using the Kurtzke expanded disability status scale (EDSS), the mean score 3.4 (1.3) with a range of 1.5-5.0. MS patients who had experienced a relapse within the previous month and those who had received steroids within 1 month were excluded from the study. All relapsing-remitting patients were in the remission phase at time of sampling. Thirteen of the MS patients were undergoing active treatment with interferon-beta (IFN-β). Patients were individually matched to a healthy control based on sex, age (±3y), and BMI (±2.5 kg·m⁻²). Venous blood samples were drawn in the morning after an overnight fast from 10.00. After centrifugation at 4°C, serum was frozen and aliquots stored at −80°C until assay. To reduce analytical variance samples were batch analysed using the same assay lot.

2.2. Immunoassays

The concentration (ng/ml) of serum IGF-1, IGF-II, IGFBP-1, IGFBP-2 and IGFBP-3 was determined by immunoradiometric assay (IRMA; Diagnostic System Laboratories, DSL, Webster, TX) according to the manufacturer's protocol. Measurement of acid-labile subunit was by enzyme linked immunosorbent assay (ELISA; Diagnostic System Laboratories, Webster, TX). Frozen serum with varying concentrations of IGFs/IGFBPs and a pooled serum sample were used as quality control samples to assess intra and inter assay precision. Intra and inter assay CVs were as follows; IGF-1 3.3% and 3.8%, IGF-II 1.0% and 2.6%, IGFBP-1 1.0% and 8.1%, IGFBP-2 10.9% and 18.1%, IGFBP-3 0.7% and 2.6% and acid-labile subunit 9.2% and 6.6%.

2.3. Statistical methods

The sample sizes in the present study ranged from N = 6 to N = 28 and were too small for valid non-parametric tests [24]. Previous studies in these conditions (14,19,20) have used 'mean' data for IGFs and IGFBPs, i.e., suggesting an underlying normal distribution. Therefore, paired t-tests were used to compare patients with matched controls and the resulting p value for each means comparison is reported. Results are reported as mean (SD).

3. Results

3.1. ALS

There was a trend for increases in serum [IGF-I] 10% and [IGF-II] 11% in the ALS patients compared to their matched controls, these did not reach statistical significance. However, the increase in both peptides resulted in a significant increase of 11% in [IGF TOTAL] (p = 0.042) ([IGF TOTAL] = [IGF-I] + [IGF-II]). This was accompanied by a significantly lower circulating level of [IGFBP-1] 34% (p = 0.05). The concentration of IGFBP-2 (p = 0.545), IGFBP-3 (p = 0.137) and acid-labile subunit (p = 0.080) was similar to controls.

Eleven of the ALS patients had a blood sample and ALSFRS-R score taken ≤30 days before death. All patients in this pre-agonal (PA) phase had evidence of respiratory decline however, none had a vital capacity lower than 50% of predicted or a sniff nasal inspiratory pressure of less than 40 cm water. All were clinically well with no evidence of respiratory infection at the time of assessment. A second subgroup of surviving ALS patients was identified (n = 10) these patients had a spinal onset disease of long duration (two standard deviations from the mean survival time for Irish patients post diagnosis) [25]. This surviving group (SP) was used as the best contrasting stage of disease to that of the PA patients.

There was a significant increase in [IGF-1] 36% (p = 0.032) in surviving patients versus matched controls (Fig. 1). With regard to the regulatory binding proteins, there was a large decrease in [IGFBP-1] (−58%) in the SP patients compared to controls (p = 0.026). In pre-agonal patients, these changes were not detected, circulating levels of all peptides were similar to controls (Table 2).

![Fig. 1. Serum concentration of IGF-I (mean ± SD) in surviving (black) and pre-agonal (yellow) ALS patients compared to matched controls (white). Statistical comparison is of pre-agonal vs. matched control.](image-url)
3.2. PPS

The PPS patients had similar [IGF-I] and [IGF-II] to matched controls. The concentration of all binding proteins examined was lower in the PPS patients compared to controls however, none of these decreases reached statistical significance (Table 2).

3.3. MS

The mean concentration of serum IGF-I was 38% (p = 0.018), acid-labile subunit 17% (p = 0.044) and IGF-BP-2 43% (p = 0.035) higher in MS patients compared to controls. Serum [IGF-II] did not differ from controls (p = 0.747). The dominant influence of IGF-II was such that no significant difference in [IGF_TOT] (IGF-I + IGF-II) was found between the MS group and their matched controls (p = 0.268). Serum [IGFBP-1] (p = 0.542) and [IGFBP-3] (p = 0.705) also did not differ significantly from the controls.

When stratified for interferon-β use, patients not undergoing IFN-β treatment (NT) had similar serum IGF-I (p = 0.564), IGF-II (p = 0.132) and IGF_TOT (p = 0.349) compared to controls. There was no significant difference in the concentration of any of the IGF binding proteins between patients not undergoing treatment and controls (Table 3).

In patients undergoing IFN-β treatment significant increases were found in serum IGF-I 52% (p = 0.013) and IGF_TOT 19% (p = 0.043) compared to controls (1, Fig. 2). Serum [IGF-II] was similar to controls (p = 0.167). The concentration of IGF-BP-3 18%

(p = 0.033), IGF-BP-2 86% (p = 0.015) and acid-labile subunit 33% (p = 0.012) were higher in IFN-β patients compared to controls. Serum [IGFBP-1] did not differ from controls (p = 0.981; Table 3).

Though numbers were small, it was noted that relapsing-remitting patients using IFN-β had significant increases in IGF-I 55% (p = 0.009), IGF_TOT 25%

Fig. 2. Serum concentration of IGF-I (mean ± SD) in MS patients not undergoing treatment (red) and patients using interferon-β (blue) compared to matched controls (white). Statistical comparison is of patient group vs. matched control.
4. Discussion

We report a detailed profile of circulating IGF-I and II and their regulatory proteins in these neurodegenerative and neuroinflammatory conditions. The disruption of neurotrophic support provided by IGF has been proposed to contribute to the pathogenesis of ALS. In this model, a reduction in IGF concentration or IGF bioavailability would lead to a decrease in survival signal to motor neurons accelerating the degenerative process. Based on this hypothesis, we further hypothesize that IGF concentration would be reduced in ALS. An equally valid hypothesis would be that the degenerative process in ALS could invoke a compensatory increase in IGF in an attempt to provide additional support to neurons.

The present study reports an increase in the circulating concentration of IGF-I in ALS patients with relatively slowly progressive disease, compared to their matched controls and, as reflected in a lower concentration of the regulatory protein IGFBP-1, a possible decrease in total IGF binding capacity. Our data therefore suggest an increased bioavailability of IGF in surviving ALS patients, and lend support to the concept of a homeostatic response to neuron degeneration. In contrast to these findings, Braunstein et al. reported serum IGF-I in a cohort of ALS patients to be similar to controls and Torres-Alemán et al. a 40% decrease in the mean circulating IGF-I, no change in [IGFBP-3] but an increase in [IGFBP-1] and [IGFBP-2] compared to controls. In none of these studies is the sample size large and the variance of the reported measures within the patient groups suggests the range within a patient group to also be large. In part, the contrast in the data reported in this study can be explained by the chosen method of analysis, e.g. this study measured total IGFBP-1 rather than the lesser and non-phosphorylated form of IGFBP-1 that only accounts for approximately 5% of total IGFBP-1 but it is more likely that the data reported here reflect the changing state of the disease as measured by a potential biomarker of disease progression. A cross-sectional comparison of the ALS patients recruited to the present study and the most widely reported data from Torres-Alemán et al. indicate that the profile of the peripheral IGF system is related to the mean duration of symptoms. The data presented in the previous study is largely consistent with.

\[ p = 0.048 \]

IGFBP-3 22% \( (p = 0.020) \) and IGFBP-2 90% \( (p = 0.032) \) compared to controls. It was noteworthy that patients classified as secondary progressive and continuing treatment with IFN-β had no significant increases in IGF components compared to matched controls (Table 5).

\[ \text{Table 5} \]

<table>
<thead>
<tr>
<th>Measure</th>
<th>Mean (SD)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGF-I</td>
<td>276.2 (109)</td>
<td>0.032</td>
</tr>
<tr>
<td>IGFBP-1</td>
<td>138.6 (259)</td>
<td>0.033</td>
</tr>
<tr>
<td>IGFBP-3</td>
<td>411.7 (65)</td>
<td>0.000</td>
</tr>
<tr>
<td>IGFBP-2</td>
<td>355.2 (25)</td>
<td>0.000</td>
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</table>

Data are expressed as mean (SD). Data are expressed in ng/ml, unless otherwise noted.
Previous studies of the peripheral IGF system in PPS patients are limited. In a pilot study of 10 PPS patients, Shetty KR et al. [14] reported the mean serum [IGF-I] to be approximately 50% lower than age-matched controls. A larger study by Ruo et al. [15] of 124 PPS patients and 261 age-matched controls also reported a 25% lower mean [IGF-I] in the PPS patients. The authors considered this to be indicative of an overall reduction in peripheral IGF bioactivity. In contrast to the above, and in keeping with our observations, Sarnak et al. [16] assessed 87 PPS patients and 392 age-sex-matched controls and found no statistically significant difference in serum [IGF-I]. In the present study, the circulating concentration of IGF-I and IGF-II were similar for the PPS patients when compared to the matched controls. Although the circulating concentration of all binding proteins examined was lower in the PPS patients compared to controls none of these would support a modification of the peripheral IGF system in PPS patients.

One of the major findings of the present study was an increase in specific components of the peripheral IGF system in MS patients treated with the immunomodulatory drug interferon-β: IGF-I 52% (p = 0.013), IGFBP-3 18% (p = 0.033), IGFBP-2 86% (p = 0.015) and acidlabile subunit 33% (p = 0.012) compared to controls. Furthermore, analysis of the sub-groups of MS demonstrated that the increase was principally attributed to patients in the relapsing-remitting stage undergoing IFN-β treatment: IGF-I 39% (p = 0.009), IGFBP-3 22% (p = 0.020), IGFBP-2 90% (p = 0.012). Similar to the compensatory model proposed for surviving ALS patients, the increase in specific components of the peripheral IGF system may be primarily associated with the early stages of MS.

In agreement with previous studies [12, 14, 15, 29], patients not undergoing IFN-β treatment had similar IGF levels to controls. In this study both relapsing-remitting and secondary-progressive MS patients were treated with interferon-β, yet a significant increase in bioavailable IGF-I was only apparent in the patients with relapsing-remitting disease (RRMS). Patients with secondary-progressive disease using IFN-β had similar IGF and IGFBP levels to controls. It is apparent from these data that changes in specific components of the peripheral IGF system in RRMS might parallel the efficacy of IFN-β, resulting from the effect of IFN-β on cell signalling pathways [32] or as the IGF components affected are GH-dependent, hypothalamic-pituitary activity [16].

This study is the first to report an increase in insulin-like growth factors in the circulation associated with interferon-beta use. This may represent a role for IGF peptides as biomarkers of IFN-β responsiveness, as potential mediators of the therapeutic mechanism of IFN-β, or as overall prognostic indicators of disease course.
Further studies are currently underway to examine the change in IGF-I in both relapsing-remitting and secondary-progressing MS patients pre- and post- IFN-β treatment and to determine how the use of IFN-β and the increase in IGF components could be mechanistically linked.

In summary, the present study suggests that ALS patients with slowly progressive disease have increased IGF bioavailability. This is consistent with observations in other conditions in which compensatory increases in serum IGF-I can occur in response to the decreased sensitivity of nerve cells to IGF (21, 22). There is a current emphasis on the identification of biomarkers for ALS that would allow an objective measure of disease progression and could support the clinical assessment. Our data suggest that the finding of elevated IGF-I coupled with decreased IGFBP-1 in ALS may carry a more favourable prognosis. Conversely, a "normalisation" of IGF bioavailability could be viewed as a negative prognostic indicator. The possible role of IGF bioavailability as a biomarker of disease progression warrants further investigation. Although post polio syndrome has certain clinical parallels with ALS, the results of this study demonstrate that alteration in the peripheral IGF system is not associated with all neurological disease. It was not possible to demonstrate a change in IGF-I, IGF-II or any of the IGF binding proteins in PPS patients. In MS, we report a change in a number of components of the peripheral IGF system, including an increase in IGF-I in patients treated with interferon-β. Though the data are largely preliminary, the large increase in IGF-I in response to interferon-β warrants further investigation. Our data adds to the growing body of evidence of an alteration in peripheral IGF bioavailability in neurological disease (37-41), an imperative of future IGF-based therapy.

References


An all-Ireland epidemiological study of MND, 2004–2005

C. Donaghy, O. O'Toole, C. Sheehan, F. Kee, O. Hardiman and V. Patterson

Keywords: epidemiology, incidence, motor neuron disease, prevalence, prospective

Background and methods: We conducted an all-Ireland population-based prospective epidemiological survey of motor neuron disease (MND) using the Northern Ireland and Republic of Ireland MND registries to examine the incidence and prevalence of the disease over the period 2004–2005. Results and conclusions: Incidence of MND was 1.9 per 100 000 person-years and rates were comparable in both the north and south of Ireland. Prevalence of MND was 5.0 per 100 000 population. When compared with previous published surveys of MND performed in the Republic of Ireland over the last 10 years, rates of disease have remained relatively constant. When standardized to the 1990 US population, the incidence of MND in Ireland was found to be consistent with other European prospective surveys of MND.

Introduction

Motor neuron disease (MND) is a relatively uncommon neurodegenerative condition, and data generated from small populations may yield imprecise estimates of disease. Until recently, most epidemiological studies of MND have been prevalence-based. Disparate methodologies have prevented accurate comparisons to be made between countries and regions. To date, only eight prospective studies of MND exist in the literature [1–8]. However, the recent development of population-based registries in the Republic of Ireland, Scotland and parts of Italy and England [1–4, 6–8] ensures that ongoing prospective data collection is maintained. In this study, we examined the incidence and prevalence of MND over the entire island of Ireland by combining the data from the prospective MND registers of both Northern Ireland (NI) and the Republic of Ireland (ROI) for the period 2004 to 2005. We compared these data with those of previous epidemiological surveys of MND in the ROI and with other population-based prospective surveys.

Methods

The populations of NI and the ROI are similar. The ROI has a younger population, with 35% less than 25 years and 11% more than 65 years compared with 35% and 11% for NI, respectively [9,10]. The large majority of both populations are white, 95% in NI and 94% in the ROI, as indicated by 2001 and 2005 data, respectively [9,10]. 2004 mid-year estimates for NI and the ROI found the total population to be 1 710 322 and 4 043 800, respectively [9,10]. Prevalence day for this survey was taken as 30 June 2005 and incidence was measured from 1 August 2004 to 31 July 2005. This study was approved by local ethics committees.

Cases were ascertained from the NI and the ROI MND registers. Six sources of potential ascertained cases were used. These include: (a) the neurology department, (b) the motor neuron disease associations (MNDAs), (c) acute hospital trust coding system lists, (d) the regional pharmacy unit in the Royal Victoria Hospital, (e) general practitioners (GPs) and (f) neuropathology departments. Once potential cases were identified from any source, medical notes were reviewed to confirm the diagnosis of MND. Patients were then approached for their consent. Before entry onto the disease register, patients were assessed clinically, where possible, in order to confirm their diagnosis and to assign a diagnostic category using the El Escorial Criteria (EEC). The original EEC [11] were used to classify patients into definite, probable, possible and suspected MND. Amyotrophic lateral sclerosis (ALS) patients with diagnoses of progressive bulbar palsy (PBP) and primary lateral sclerosis (PLS) were included and are categorized as ‘possible’ under the original EEC. Patients from all four EEC categories were included in the study.

The denominator for the calculation of prevalence and incidence was the 2004 mid-year estimates for the resident populations of NI and the ROI [9,10]. As 2005 population data were not available for the ROI, disease rates were also measured for the population greater than 15 years, as this group was considered to be the ‘at-risk’ population. MND is considered a disease of adult onset and excluding cases 15 years or younger reduces the likelihood of inclusion of genetic motor...
neurone diseases resembling MND: 95% confidence limits were calculated using the normal approximation of the Poisson distribution. Age- and gender-specific rates were also calculated. Employing the direct standardization method, comparison was made between other prospective incidence surveys of MND using the 1990 US population as the standard, as most of the studies were based on data from the 1990s. The search strategy used to collect relevant prospective articles of MND incidence is described in the Appendix.

Results

There were 32 incident cases ascertained in NI and 77 ascertained from the ROI, providing a total of 109 incident cases for all-Ireland. The age-adjusted male:female (M:F) ratio was 0.7:1 for NI, 1:7:1 for the ROI, and 1:3:1 for all-Ireland. Incidence rates are presented in Table 1 and were comparable for both NI and the ROI. Mean age at onset was 63.2 (SD 11.1) years and was greater in females, 64.6 (SD 9.3) years, than in males, 62.1 (SD 12.2) years. Mean age at onset was lower in the ROI, 62 (SD 12) years, compared with NI, 66 (SD 8) years, but this was not statistically significant ($P = 0.2$). The mean age at diagnosis was 65.4 (SD 10.9) years and was greater in females, 66.1 (SD 9.4) years, compared with males, 64.6 (SD 12.8) years, but was not statistically significant ($P = 0.5$).

There were 83 prevalent cases ascertained in NI and 201 in the ROI, providing a total of 287 incident cases for all-Ireland. The age-adjusted M:F ratio was 1:2:1 for NI, 1:6:1 for the ROI and 1:3:1 for all-Ireland. Prevalence rates are presented in Table 1 and were almost identical for NI and the ROI. Mean age at onset was 58.1 (SD 14) years, being similar in females, 58.2 (SD 14.3) years, and males, 58.0 (SD 13.8) years, and with no difference noted between the ROI and NI. The mean age at diagnosis was 60.1 (SD 10.9) years and was similar in females, 60.4 (SD 13.8) years, and males, 59.9 (SD 13.1) years.

Table 1: Disease rates for MND

<table>
<thead>
<tr>
<th></th>
<th>NI</th>
<th>ROI</th>
<th>All-Ireland</th>
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</thead>
<tbody>
<tr>
<td>Total 19 (CD)</td>
<td>1.9 (1.2 to 2.9)</td>
<td>1.9 (1.3 to 2.3)</td>
<td>1.9 (1.3 to 2.3)</td>
</tr>
<tr>
<td>Female 19 (CD)</td>
<td>2.2 (1.2 to 3.2)</td>
<td>1.4 (0.8 to 2.0)</td>
<td>1.7 (2.2 to 2.7)</td>
</tr>
<tr>
<td>Male 19 (CD)</td>
<td>1.6 (0.7 to 2.4)</td>
<td>2.4 (1.7 to 2.9)</td>
<td>2.4 (1.6 to 2.7)</td>
</tr>
<tr>
<td>SR &gt; 5 years (CD)</td>
<td>2.4 (1.6 to 3.2)</td>
<td>2.4 (1.9 to 2.9)</td>
<td>2.4 (1.9 to 2.9)</td>
</tr>
<tr>
<td>Total 19 (PR)</td>
<td>4.0 (3.8 to 5.9)</td>
<td>5.0 (4.4 to 5.7)</td>
<td>5.0 (4.4 to 5.6)</td>
</tr>
<tr>
<td>Female 19 (PR)</td>
<td>4.5 (3.1 to 5.9)</td>
<td>5.0 (4.0 to 6.0)</td>
<td>4.7 (3.3 to 4.7)</td>
</tr>
<tr>
<td>Male 19 (PR)</td>
<td>3.6 (2.8 to 5.8)</td>
<td>5.0 (4.2 to 6.9)</td>
<td>4.7 (3.1 to 6.9)</td>
</tr>
<tr>
<td>PR &gt; 5 years (CD)</td>
<td>1.4 (1.4 to 7.5)</td>
<td>1.3 (0.5 to 3.3)</td>
<td>1.3 (0.5 to 3.3)</td>
</tr>
</tbody>
</table>

CR, 95% confidence interval; PR, incidence rate per 100,000 person-years; FR, prevalence rate per 100,000 population.

Age- and gender-specific incidence rates were found to increase with age up to a peak, before falling off in the older age groups. Incidence rates peaked in males in the 65-69-year age group, with a second peak in the 80-84-year age group, while those of females peaked in the 75-79-year age group (see Table 2 and Fig. 1). Age- and gender-specific prevalence rates were also found to increase with age. However, prevalence rates in females peaked in the 70-74-year age group while those of males continued to rise (see Table 3 and Fig. 2). When standardized to the 1990 US population, the incidence rate for the 45-74-year age group in all-Ireland increased by 5.2 to 5.7 per 100,000 person-years and was found to be similar to other prospective population-based studies (see Table 4).

Discussion

The incidence of 1.9 per 100,000 person-years and prevalence of 5.0 per 100,000 population for all-Ireland is consistent with figures quoted in the literature over the last 30 years. However, prevalence figures are a less useful epidemiological marker of disease frequency in MND because of the short survival of patients with the disease. Incidence and prevalence are similar in both the north and south of Ireland, with no evidence of a north–south gradient. Traynor et al. [7] noted a higher incidence in the north-western counties of the ROI; however, the findings in this study do not support this. Unfortunately, cluster analysis software such as the spatial scanning statistic [13] could not be employed to further investigate this previous finding by Traynor et al. [7] because of the small numbers. As would be expected from the relatively small numbers, no obvious geographical trends were observed in this study, and although there was a notable reversal in the usual male to female ratio in the NI population, this may also be a consequence of the small size of the study cohort.

No change in incidence was observed in the ROI over a 10-year period from 1995 to 2005 when our results (1.5 per 100,000 person-years) were compared with those of Traynor et al. [7], who found an incidence of 2.1 per 100,000 person-years for the period 1995-1997. A stable secular trend of MND incidence for the ROI was also found by O'Toole et al. [5]. No change in prevalence was found in the ROI over the 10-year period when our results (5.0 per 100,000 population) were compared with those of Traynor et al. [7] who found a prevalence of 4.7 per 100,000 person-years. Similarly, a recent epidemiological study of MND by Forbes et al. [3], found that incidence had not changed over the period 1989-1998 in Scotland.

As might be anticipated, the mean age of onset of MND in patients in the incidence cohort was higher.
Table 2 Age- and gender-specific incidence rates of MND, all-India, 2004-2005

<table>
<thead>
<tr>
<th>Age range (years)</th>
<th>(N)</th>
<th>(X)</th>
<th>95% CI</th>
<th>(N)</th>
<th>(X)</th>
<th>95% CI</th>
<th>(N)</th>
<th>(X)</th>
<th>95% CI</th>
<th>(N)</th>
<th>(X)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-14</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>15-19</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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</tr>
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<td>20-24</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
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<td>0</td>
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</tr>
<tr>
<td>25-29</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>30-34</td>
<td>0</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
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<td>0</td>
</tr>
<tr>
<td>35-39</td>
<td>1</td>
<td>0.46</td>
<td>0.01 to 2.58</td>
<td>1</td>
<td>0.47</td>
<td>0.01 to 2.58</td>
<td>1</td>
<td>0.47</td>
<td>0.01 to 2.58</td>
<td>1</td>
<td>0.47</td>
<td>0.01 to 2.58</td>
</tr>
<tr>
<td>40-44</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
</tr>
<tr>
<td>45-49</td>
<td>1</td>
<td>0.54</td>
<td>0.01 to 2.58</td>
<td>1</td>
<td>0.40</td>
<td>0.01 to 2.58</td>
<td>1</td>
<td>0.24</td>
<td>0.01 to 2.58</td>
<td>1</td>
<td>0.24</td>
<td>0.01 to 2.58</td>
</tr>
<tr>
<td>50-54</td>
<td>6</td>
<td>3.90</td>
<td>1.32 to 7.81</td>
<td>3</td>
<td>3.72</td>
<td>0.88 to 6.36</td>
<td>2</td>
<td>1.65</td>
<td>0.60 to 3.14</td>
<td>1</td>
<td>0.60</td>
<td>0.60 to 3.14</td>
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<tr>
<td>55-59</td>
<td>3</td>
<td>1.94</td>
<td>0.44 to 5.08</td>
<td>8</td>
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<td>0.12 to 2.12</td>
<td>11</td>
<td>3.22</td>
<td>0.72 to 5.72</td>
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<td>7.25</td>
<td>4.31 to 11.50</td>
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<tr>
<td>60-64</td>
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<td>6.43</td>
<td>2.74 to 12.68</td>
<td>10</td>
<td>1.13</td>
<td>0.34 to 2.17</td>
<td>16</td>
<td>16.05</td>
<td>9.16 to 26.05</td>
<td>24</td>
<td>11.49</td>
<td>7.40 to 17.75</td>
</tr>
<tr>
<td>65-69</td>
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<td>7.49</td>
<td>3.23 to 14.73</td>
<td>16</td>
<td>1.12</td>
<td>0.36 to 2.52</td>
<td>16</td>
<td>11.12</td>
<td>5.71 to 21.25</td>
<td>20</td>
<td>11.89</td>
<td>7.40 to 17.75</td>
</tr>
<tr>
<td>70-74</td>
<td>11</td>
<td>11.76</td>
<td>5.87 to 21.04</td>
<td>9</td>
<td>1.12</td>
<td>0.36 to 2.52</td>
<td>14</td>
<td>16.05</td>
<td>9.16 to 26.05</td>
<td>16</td>
<td>11.49</td>
<td>7.40 to 17.75</td>
</tr>
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<td>75-79</td>
<td>10</td>
<td>12.41</td>
<td>5.96 to 22.84</td>
<td>4</td>
<td>0.95</td>
<td>0.31 to 1.95</td>
<td>14</td>
<td>16.05</td>
<td>9.16 to 26.05</td>
<td>16</td>
<td>11.49</td>
<td>7.40 to 17.75</td>
</tr>
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<td>80-84</td>
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<td>0.03 to 3.82</td>
<td>5</td>
<td>13.82</td>
<td>4.45 to 32.37</td>
<td>3</td>
<td>5.17</td>
<td>1.67 to 12.87</td>
<td>5</td>
<td>1.67</td>
<td>1.67 to 12.87</td>
</tr>
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<td>85+</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>48</td>
<td>1.65</td>
<td>1.22 to 2.08</td>
<td>61</td>
<td>2.14</td>
<td>1.64 to 2.75</td>
<td>109</td>
<td>1.99</td>
<td>1.36 to 2.59</td>
<td>150</td>
<td>1.99</td>
<td>1.36 to 2.59</td>
</tr>
</tbody>
</table>

IR, incidence rate; CI, confidence interval.

Figure 1 Line graph of age- and gender-specific incidence of MND for all-India, 2004-2005.

than that in the prevalence cohort. This is consistent with the observation that that an older age of onset is associated with a poor prognosis [17]. By extension, a higher proportion of less severely affected patients with an earlier age of disease onset would be expected to be found in the prevalence study.

Consistent with the majority of epidemiological studies of MND, age-specific incidence was noted to decline in the older age groups and does not directly support the 'aging theory' for MND. With the exception of studies from the Mayo Clinic (Rochester, MN, USA) [15,16], age-specific incidence and mortality rates in published studies repeatedly show a decline in MND after approximately 75 years in both males and females. It is noteworthy that the Rochester studies were retrospective and drawn from a relatively smaller population, and that the study did not disaggregate the age groups beyond 75 years. Moreover, a further study using Mayo Clinic records for Olmsted County, Minnesota in 2002 [17] suggested that age-adjusted incidence peaked in the seventh decade. Nonetheless, there is a theoretical risk in the elderly of poorer ascertainment. A study, based in Scotland, assessing MND patients over 80 years [18], found that these patients received fewer routine treatments and specialist neurologist consultations. Bulbar-onset disease, which has a poorer prognosis [19], was also considerably more common among the elderly. It is possible therefore that these factors could contribute to reduced ascertainment in older age groups. However, the consistent finding of a decline in disease incidence in the very old in all published population-based studies suggests that the observation is robust. The decline in incidence in the very old may be explained by the death of the susceptible population, as MND is generally fatal in 3-5 years. Interestingly, peak incidence in both this study and that by Traynor et al. [7] occurred in the 60-69-year age group, suggesting no change over the last 10 years.

Prevalence was noted to decline sharply in females after the age group 70-74 years, while continuing to rise in males. Other studies have demonstrated that females are more likely to present with progressive bulbar palsy (PBP) [7,20,21] and that survival in this clinical subtype of MND is associated with a poorer prognosis [20]. It is possible that the decline in age-specific prevalence among older females is due to increased mortality from PBP, as has been also shown in a previous Irish study [7].

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Table 3: Age- and gender-specific prevalence rates of MND: all-Ireland, 30 June 2005

<table>
<thead>
<tr>
<th>Age ranges (years)</th>
<th>N females PR per 100,000</th>
<th>95% CI</th>
<th>N males PR per 100,000</th>
<th>95% CI</th>
<th>Total PR per 100,000</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-14</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0.45</td>
<td>0</td>
<td>0.01 to 2.76</td>
</tr>
<tr>
<td>15-34</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.32</td>
<td>1</td>
<td>0.23</td>
</tr>
<tr>
<td>25-49</td>
<td>5</td>
<td>0.02</td>
<td>0.11 to 3.31</td>
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<td>0</td>
<td>0.06 to 1.65</td>
</tr>
<tr>
<td>40-59</td>
<td>5</td>
<td>0.9</td>
<td>0.10 to 3.24</td>
<td>4</td>
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<td>0.30 to 4.65</td>
</tr>
<tr>
<td>50-64</td>
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<td>0.01 to 2.26</td>
<td>3</td>
<td>1.14</td>
<td>0.38 to 4.95</td>
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<td>1</td>
<td>1.09</td>
<td>0.35 to 4.95</td>
<td>7</td>
<td>3.46</td>
<td>1.30 to 7.13</td>
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<tr>
<td>75-84</td>
<td>1</td>
<td>4.31</td>
<td>1.66 to 8.50</td>
<td>5</td>
<td>4.36</td>
<td>1.85 to 8.56</td>
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<tr>
<td>85-94</td>
<td>11</td>
<td>6.58</td>
<td>3.28 to 11.77</td>
<td>14</td>
<td>8.23</td>
<td>4.55 to 13.00</td>
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<tr>
<td>105-114</td>
<td>31</td>
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<td>3.65 to 44.72</td>
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<td>36.46</td>
<td>19.90 to 57.59</td>
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<tr>
<td>65-74</td>
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<td>18.72</td>
<td>11.44 to 28.91</td>
<td>21</td>
<td>21.04</td>
<td>13.03 to 32.16</td>
</tr>
<tr>
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<td>21</td>
<td>30.1</td>
<td>20.76 to 44.33</td>
<td>16</td>
<td>19.68</td>
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</tr>
<tr>
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<td>17</td>
<td>26.99</td>
<td>27.59</td>
<td>15</td>
<td>26.06</td>
<td>14.89 to 47.09</td>
</tr>
<tr>
<td>95-104</td>
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<td>4.95</td>
<td>1.03 to 14.48</td>
<td>16</td>
<td>44.24</td>
<td>25.30 to 71.84</td>
</tr>
<tr>
<td>105-114</td>
<td>31</td>
<td>6.22</td>
<td>1.25 to 18.18</td>
<td>8</td>
<td>38.9</td>
<td>16.75 to 76.66</td>
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<tr>
<td>Total</td>
<td>116</td>
<td>3.99</td>
<td>3.62 to 4.72</td>
<td>71</td>
<td>6.16</td>
<td>5.10 to 8.30</td>
</tr>
</tbody>
</table>

Our results were similar to other prospective studies, providing no evidence of a latitudinal gradient within populations of European extraction. Standardizing rates from eight studies to the 1990 US population, it was found that rates tended to be lowest in southern European countries; however, the range of standardized rates was reasonably narrow and confidence intervals overlapped across studies, implying no significant difference. The original EEC [11] were employed in all studies except two, including all four diagnostic categories as well as cases of primary lateral sclerosis. The study from Western Washington state [5] was excluded because of primary lateral sclerosis while the study from northwestern Italy [8] was most restrictive, including only

Table 4: Incidence (per 100,000) of MND from prospective published surveys 1990-2004

<table>
<thead>
<tr>
<th>Country/Region</th>
<th>Study period</th>
<th>No. cases</th>
<th>Grade incidence rates (95% CI)</th>
<th>Adjusted incidence for the 45-14-year age group</th>
<th>Male to female ratio</th>
<th>Ref</th>
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</thead>
<tbody>
<tr>
<td>Ireland</td>
<td>1995-1997</td>
<td>231</td>
<td>2.1 (1.8-2.4)</td>
<td>6.3 (4.6-7.9)</td>
<td>1.41</td>
<td>[1]</td>
</tr>
<tr>
<td>Ireland</td>
<td>2002-2004</td>
<td>465</td>
<td>2.8 (2.0-2.2)</td>
<td>3.7 (2.4-2.5)</td>
<td>1.41</td>
<td>[8]</td>
</tr>
<tr>
<td>Scotland</td>
<td>1989</td>
<td>114</td>
<td>2.2 (1.8-2.7)</td>
<td>5.2 (4.0-5.3)</td>
<td>1.21</td>
<td>[1]</td>
</tr>
<tr>
<td>Scotland</td>
<td>1989-1996</td>
<td>152</td>
<td>2.4 (2.2-2.6)</td>
<td>5.8 (4.5-6.0)</td>
<td>1.21</td>
<td>[3]</td>
</tr>
<tr>
<td>Nova Scotia, Canada</td>
<td>2005</td>
<td>21</td>
<td>2.2</td>
<td>n/a</td>
<td>2.01</td>
<td>0.102</td>
</tr>
<tr>
<td>Northern Italy</td>
<td>1985-1996</td>
<td>231</td>
<td>2.5 (2.2-2.9)</td>
<td>5.8 (4.6-6.5)</td>
<td>1.21</td>
<td>[8]</td>
</tr>
<tr>
<td>Southern Italy</td>
<td>1998-1999</td>
<td>130</td>
<td>1.6 (1.2-1.9)</td>
<td>4.1 (2.8-3.7)</td>
<td>1.61</td>
<td>[4]</td>
</tr>
<tr>
<td>Lombardy, Italy</td>
<td>1998-2002</td>
<td>147</td>
<td>2.1 (1.2-5.2)</td>
<td>4.7 (3.8-5.1)</td>
<td>1.31</td>
<td>[2]</td>
</tr>
<tr>
<td>Western Washington State, USA</td>
<td>1990-1995</td>
<td>235</td>
<td>1.8 (1.3-2.4)</td>
<td>5.6 (3.5-7.6)</td>
<td>1.21</td>
<td>[9]</td>
</tr>
<tr>
<td>All-Ireland [current study]</td>
<td>2001</td>
<td>109</td>
<td>1.9 (1.6-2.3)</td>
<td>5.7 (4.3-7.6)</td>
<td>1.31</td>
<td></td>
</tr>
</tbody>
</table>

*Adjusted to the 1990 US population.*

*Taken directly from published articles; i.e., not available.*
definite and probable cases and excluding primary lateral sclerosis and primary muscular atrophy. It can therefore be noted that compared with the other studies, MND incidence from the north-western Italian study [6] is likely to be an underestimate.

From current prospective epidemiological data it appears that MND frequency is stable within European populations. It is important that further epidemiological surveys adhere to robust methodologies, employing prospective incidence-based disease registers. The newly developed European incidence-based registry of MND (EURALS) [22] will facilitate the collation of data on European patients with MND. The provision of a pan-European registry will not only help provide large-scale accurate epidemiological data, but will also enhance recruitment for observational studies of risk and for enrolment in therapeutic trials.

Acknowledgements

We would like to thank all the patients and volunteers who participated in this study. We would also like to thank Mrs Alison Dick and Ms Bernie Corr who help support the MND registers.

Funding

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Appendix

Search strategy

Databases MEDLINE and EMBASE were chosen and a search was performed for all articles relevant to the topic ‘prospective studies of incidence in MND’. Within MEDLINE, three separate searches were performed. The first set of searches pertained to the population under study. A search was made using the medical subject heading (MeSH) terms ‘Amyotrophic Lateral sclerosis’ and ‘Motor Neurone Disease’ with subheading ‘epidemiology’. Secondly, ‘Amyotrophic lateral sclerosis’, ‘Motor neurone’s disease’ and their abbreviations (ALS, MND) were searched for as keywords. All six separate searches were combined using the boolean operator ‘OR’. The second set of searches pertained to the measurement of incidence. The MeSH term ‘incidence’ was searched for as was ‘incidence’ as a keyword. Both these searches were combined using ‘OR’. Thirdly, a search was performed for the MeSH term ‘prospective studies’ as well as ‘prospective’ as a keyword and both were combined with ‘AND’. Because of reference searches of already acquired articles it was discovered that a small number of relevant articles were missed because the term ‘prospective’ had not been indexed or used in the title or abstract. The search was then redesigned excluding the use of ‘prospective’ and ‘incidence’ as a keyword. This yielded significantly more articles, all of which had to be searched separately. Nine articles that described prospective incidence studies of MND were found. A similar search strategy was employed for the EMBASE database and no further articles were found.

References


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