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Protection of Motor Neurons in Pre-Symptomatic Individuals Carrying SOD 1 Mutations: Results of Motor Unit Number Estimation (MUNE) Electrophysiology

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1. Introduction

Amyotrophic lateral sclerosis (ALS) is a progressive degenerative disease of motor neurones. There is a family history in approximately 10% percent of cases. Only 20% of such families have point mutations in the Cu, Zn superoxide dimutase 1 (SOD1) gene. Pre-symptomatic loss of motor neurons has been identified prior to the onset of symptoms in SOD1 mice. This loss was biphasic with initial loss in the pre-symptomatic phase followed by a period of stabilisation and then gradual loss at time of weakness to death. (Kong & Xu, 1998).

In order to determine the time course of motor neurone loss prior to symptomatic onset of disease, a longitudinal study of at-risk asymptomatic individuals (i.e. SOD1 mutation carriers with no neurological symptoms or signs as determined by a neurologist) was performed.

There was no detectable difference in the number of motor units in SOD1 mutation carriers compared to their SOD1 negative family controls. (Aggarwal & Nicholson, 2001). This may indicate that mutation carriers have undetectable loss of motor neurones until rapid and widespread cell death of motor neurones occurs, coinciding with the onset of symptomatic features. This implies that the disease is not the end result of the slow attrition of motor neurones. (Aggarwal, 2009).

The longitudinal study was extended on 20 asymptomatic carriers of the Cu, Zn superoxide dimutase 1 (SOD1) point mutation. In 2 of the 20 mutation carriers, there was a sudden reduction in MUNE, several months prior to the onset of weakness. (Aggarwal & Nicholson 2002), which also occurred in another 3 mutation carriers over the course of the study. (Aggarwal, 2009).

This suggests that gradual pre-clinical loss of motor neurones does not occur in asymptomatic SOD1 mutation carriers and supports the observation that sudden, catastrophic loss of motor neurones occurs immediately prior to the onset of symptoms and the development of the disease, rather than a gradual attrition of motor neurones over time. These results suggest that there may be a biological trigger initiating rapid cell loss, just prior to the onset of symptoms. This observation is an important contribution to the current understanding of the pathogenesis of MND.

Regular follow-up of SOD1 carriers with MUNE may lead to early diagnosis, creating an opportunity for future approaches and therapies aimed at preserving motor neurones rather than replacing lost motor neurones. Detecting the onset of motor neurone loss in asymptomatic individuals will identify those who may benefit from early institution of an active management program to improve their quality of life, until more effective treatment modalities are available for this devastating condition.

2. Background

Amyotrophic lateral sclerosis (ALS) is a group of fatal, neurodegenerative disorders, which is characterised pathologically by progressive degeneration and loss of motor neurones in the anterior horn cells of the spinal cord, motor nuclei of the brainstem and the descending pathways within the corticospinal tracts. The term amyotrophic lateral sclerosis (ALS) is used synonymously with motor neurone disease (MND) in the USA, but in the UK and Australia is used only to refer to patients who have a combination of upper and lower motor neurone dysfunction. (Talbot, 2002).

It is primarily a condition of middle to late life, with onset of symptoms between the ages of 50 and 70 and a mean age of onset of 57.4 years. (Ringel et al., 1993). Occasionally, it arises as early as the 2nd decade or as late as the 9th decade. In a natural history study, the overall median survival is 4.0 years from the onset of symptoms, but only 2.1 years from the time of diagnosis. (Ringel et al., 1993). In a study performed at the Mayo clinic, approximately 50% of patients died within 3 years of referral, but 20% were still alive at 5 years and 10% were still alive at 10 years. (Mulder & Howard, 1976).

Aging, motor neurone diseases and many peripheral neuropathies are all associated with loss of motor neurones or axons. When the disorders are recent or rapidly progressive, the extent of the loss may be indicated by weakness and wasting. In slowly progressive denervating conditions, like MND, loss of more than 50-80% of motor units may occur with little or no clinically apparent weakness.

It has been showed that patients with substantial chronic denervation could maintain normal muscle twitch tension until loss of about 70-80% of motor units occurred. (McComas, 1971). The surviving motor neurones enlarge their territories, through collateral sprouting (reinnervation) to keep pace with cell loss, to maintain the muscle maximum compound muscle action potential (CMAP), until late in the disease. At this point, collateral reinnervation is no longer able to provide full functional compensation. (Campbell et al., 1973).

In MND, needle electromyography often reveals evidence of chronic reinnervation (increased motor unit action potential amplitudes and duration with reduced recruitment), but provides little direct evidence to the extent of motor neurone and axonal loss. The supramaximal CMAP amplitude also provides little direct evidence of the extent of motor neurone loss. Normal CMAP amplitudes might mistakenly suggest that motor neurone loss has not occurred yet. (Shefner, 2001).

Motor unit number estimation (MUNE) is a more reliable method for following changes in neurogenic disorders than the CMAP amplitude. It estimates the number of functioning lower motor neurones innervating a muscle or a group of muscles i.e. the number of motor units, which can be excited by electrical stimulation. It is therefore an indirect measure of motor neurone loss, rather than a measure of primary pathology. It can identify that the number of motor units may be well below normal, in the presence of normal CMAP amplitudes. (Brown, 1976).

Pre-symptomatic loss of motor neurones has been identified in an animal model of the disease (transgenic mice expressing mutant human SOD1-G93A). The initial loss in the pre-symptomatic phase related to severe motor axonal degeneration due to vacuolar changes in motor neurones and a slow decrease in CMAP amplitudes. After a period of stabilisation, there was a gradual loss of motor neurones and a rapid decrease in CMAP amplitude, at the onset of weakness due to myelin alteration. At this point, there was a striking loss of motor units. There was also decrease in evoked motor potentials (an indirect measure of the number of motor units), prior to the onset of symptoms. The onset of disease in transgenic G93A mice involves a sharp decline of muscle strength and a transient explosive increase in vacuoles derived from degenerating mitochondria, but little motor neurone death. These did not die until the terminal stage. (Kong & Xu, 1998). The decline exhibited kinetics consistent with both a constant and exponentially decreasing risk of neuronal death. An escalating risk forced by cumulative damage was not responsible for cell death. (Azzouz et al., 1997).

It is possible that the high metabolic activity in motor neurones, combined with the toxic oxidative properties of the mutant SOD1, causes massive mitochondrial vacuolation in motor neurones, resulting in degeneration, earlier than other neurones, triggering the onset of weakness. The involvement of mitochondrial degeneration in the early stages is consistent with a direct effect of toxicity, mediated by properties gained by the mutant enzyme in catalysing redox reactions. (Beckman et al., 1993).

Until recently, it has not been possible to address this in humans, as pre-symptomatic diagnosis was not possible. Now, with the ability to identify Cu, Zn superoxide dismutase 1, (SOD1) mutation carriers, a group of human pre-symptomatic subjects can be studied to determine whether there was gradual lifelong pre-symptomatic loss of motor neurones or whether sudden catastrophic loss of motor neurones occurs just prior to the onset of clinical symptoms.

3. Familial ALS

The only forms of MND in which a clear cause has been established are the genetic variants. 20% of all familial cases are the dominantly inherited adult onset form of MND, which is clinically indistinguishable from the sporadic form of MND. These are due to a point mutation in the cytosolic Cu, Zn superoxide dismutase 1, (SOD1) gene on long arm of chromosome 21 (21q22.1). (Siddique & Deng, 1996). Mutations in other genes, alsin and the heavy subunit of neurofilament (NEFH) can also result in motor neurone degeneration in humans. Two other genes that have been investigated are the other isoforms of SOD. MnSOD (SOD2) maps to chromosome 6q25 and is primarily located in mitochondria and extracellular SOD (SOD3) maps to chromosome 4p15.2. Neither of these genes have yet to be linked to FALS. (Hand & Rouleau, 2002). There is however genetic heterogeneous and other causal genes remain to be found to explain the vast majority of FALS cases. (Siddique et al., 1989).

The initial study to establish a causal link between the SOD1 gene and familial MND (FALS) identified a total of 11 missense mutations in two exons studied in 13 autosomal dominant MND families. (Rosen et al., 1993). This led to an explosion of SOD1 gene screening in MND pedigrees. To date 112 different mutations in the SOD1 have been found which can lead to changes throughout the protein. There have been 99 substitutions, 5 polymorphisms, 3 insertions, 4 deletions and 1 compound mutation types identified. Mutations have been identified in all five exons of the gene. These include 20 on exon 1, 13 on exon 2, 8 on exon 3,

39 on exon 4 and 29 on exon 5 (Figure 1). There have also been 2 non-exon mutations identified on intron 4 and intron 1 and 14 'apparently' sporadic cases described with 6 different SOD1 mutations. (Shaw et al., 1998).

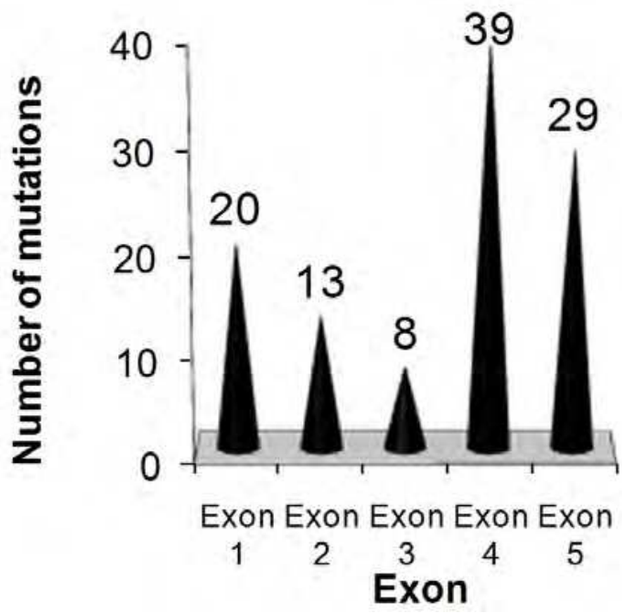


Fig. 1. Number of SOD1 mutations identified for each exon

Most are autosomal dominant in inheritance, but there is one confirmed autosomal recessive mutation, the D90A mutation in exon 4. This is unique in that it exists in dominant families in a heterozygous state, but in a number of pedigrees, specifically those of Scandinavian ancestry, homozygous mutations are required for disease. (Anderson et al., 1997).

Mutations in the heavy polypeptide 200kDa subunit of neurofilaments (NEFH) have been identified in sporadic MND cases, (Figlewicz et al., 1994) and in one FALS case. (Al-Chalabi et al., 1999). Accumulation of neurofilaments in cell bodies and axons of motor neurons is a pathological hallmark of early stages of many neurodegenerative diseases. These mutations lie in the region of the protein involved in cross-linking and thus may disrupt normal aggregation of filaments. Thus far, 1 insertion and 5 deletion mutations have been identified on exon 4. Analysis of the NEFH locus on chromosome 22 however has failed to detect linkage in MND families. (Vechio et al., 1996). Genome search on a large pedigree with autosomal dominant juvenile onset MND found strong evidence for linkage to chromosome 9q34 (ALS4). The average age of onset is 17 years, with slow progression of disease. (Chance et al., 1996). There is also an autosomal recessive, juvenile onset MND, with linkage to a locus on chromosome 15 (ALS5). (Hentati et al., 1998).

The other 90% of all MND patients have the sporadic form. There is no recognisable phenotypic difference between FALS and sporadic MND. The male: female ratio is 1:1 in FALS and 1.7:1 in sporadic MND. (De Belleroche et al., 1995). This decreases with increasing age of onset and approaches 1:1 after the age of 70. (Haverkamp et al., 1995). The site of onset is variable. Survival does not seem to be affected by age or gender, but rather the site of symptom onset. Generally, bulbar onset disease has a worse prognosis, and upper limb onset is more favourable. (Mulder et al., 1986).

It has be postulated that sporadic MND may be the final development of a chain of events that may be set in motion at one or more places in the central nervous system by endogenous and exogenous causes, or both. The aetiology of MND however remains unknown and is probably multifactorial. (Eisen 1995). There is no evidence to support the cause of sporadic MND being due to accumulation of heavy metals in the environment, (Needleman, 1997), deficiencies or excess of essential trace metals, (Mena et al., 1967) or exposure to environmental poisons and industrial solvents. (Leigh, 1997). There is also no evidence to support the cause of sporadic MND being due excessive physical activity or antecedent trauma.

4. Possible patterns of motor neurone loss

In normal healthy individuals, it has been shown that there is little loss of functioning motor neurones before the age of 60. The normal aging process then accounts for loss of approximately 3.9% of the original motor neurone pool per annum after the age of 60. (Brown, 1972). In this situation, the number of motor neurones remain fairly constant up to the age of 60, after which there is a gradual steady decline with age.

MND may be due to a slow attrition of motor neurones over time (Pattern 1 in Figure 2). If this were the case, pre-symptomatic motor neurone loss may be identifiable in SOD1 mutation carriers, as eventually there may be a gradual decline over time (Figure 2).

Another possible course of MND is that normal numbers of motor neurones are maintained until sudden, rapid multi-focal cell death of motor neurones occurs, corresponding with the development of symptoms (Pattern 2 in Figure 2). If this situation, it would be expected that SOD1 mutation carriers have a normal number of motor neurones during the pre-symptomatic phase. In this case, cell death occurs as neurones gradually accumulate damage, secondary to the mutation, which ultimately overwhelms cellular homeostasis. This is the cumulative damage hypothesis. (Clarke et al., 2000).

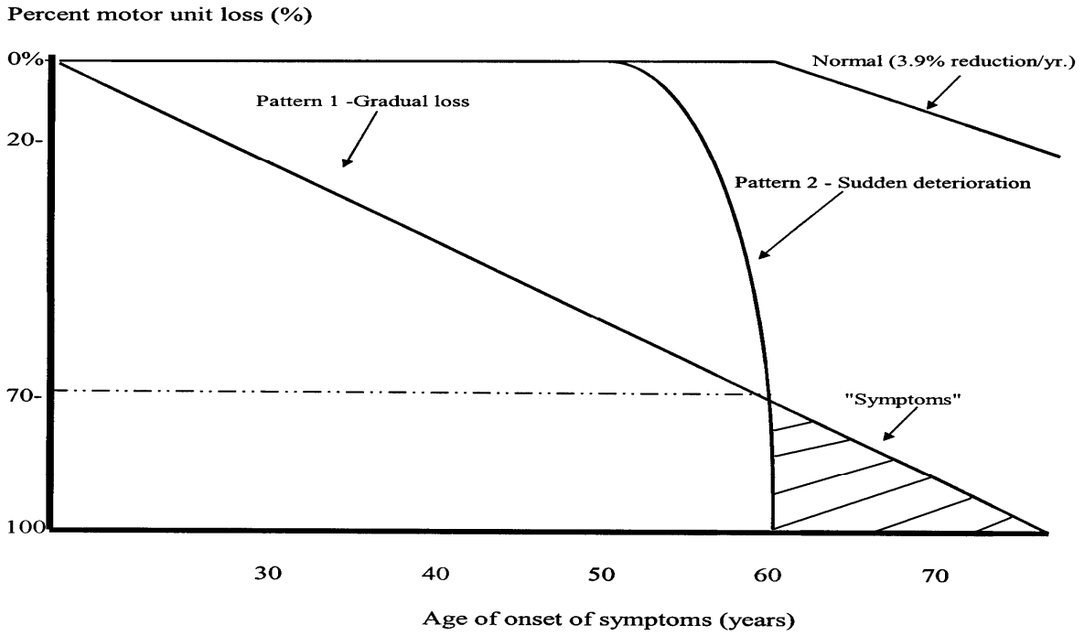


Fig. 2. Diagrammatic representation of possible patterns for motor neurone loss in an individual.

One of the mechanisms most frequently proposed to underlie cumulative damage is oxidative stress, in which an imbalance between the production of reactive oxygen species and cellular antioxidant mechanisms results in chemical modifications of macromolecules, thereby disrupting cellular structure and function. (Robberecht, 2000). A key prediction of the cumulative damage hypothesis is that the probability that any individual neurone will become committed to apoptosis increases as damage accrues within it. A mutant neurone in an older patient will have accumulated a greater amount of damage and is therefore be more likely to die than in a younger patient. Consequently, early in the course of disease, the chance of a cell containing a sufficient amount of damage to initiate apoptosis is small, and the rate of cell loss is correspondingly low. However, as the amount of intracellular damage increases, the chance that a cell will die also increases

It has been shown that the kinetics of neuronal death in a number of inherited neurodegenerative diseases was best explained by models in which the risk of cell death remains constant throughout life of the neurone and that cell death occurred randomly in time and was independent of any other neurone. This implies a "one-hit" biochemical phenomenon in which the mutant imposes an abnormal mutant steady state on the neurone and a single catastrophic event randomly initiates cell death and apoptosis. The principal features of the mutant steady state are that the living mutant neurones function very well for years or even decades and that the predominant feature of the mutant neurones is that they are all at a risk of death. This argues against the multiple environmental factors hypothesis as a cause of MND, as a random process is probably responsible for the initiation of disease. (Clarke et al., 2001).

5. Cu/Zn superoxide dismutase (SOD1) mutations

Linkage studies for familial MND (FALS) on chromosome 21q22.1 led to the identification of point mutations in the gene for Cu/Zn superoxide dismutase (SOD1) as a cause of MND. (Siddique 1991). Superoxide (O2-) is an unstable and highly active molecule, which causes oxidation of cell constituents either directly or through toxic and stable derivatives. The major superoxide dismutase activity in cytoplasm is from SOD1, which consists of 5 small exons that encode 153 highly conserved amino acids with a molecular weight of 16Kda. SOD1 is a homodimer. Within each monomer, there is an active site containing one atom each of copper and zinc. (Radunovic & Leigh, 1996).

The most common SOD1 gene mutation seen in FALS is an alanine to valine shift at codon 4 (Ala4Val). This accounts for 50% of all mutations in the USA. (Rosen, 1993). Of all the clinical variables, only bulbar onset and three specific mutations seem to influence age of onset of MND. Bulbar patients are older when their illness begins, whereas the Gly37Arg and Leu38Val mutations predict an earlier age of onset.). Leu38Val is associated with the earliest onset (mean 35.5 years) and Ile113Thr with the latest onset (mean 58.9 years).

In terms of survival, Ala4Val correlated with the shortest survival of 1.5 years. Whereas, Gly37Arg, Gly41Asp, and Gly93Ala mutation predicted longer survival. The mutations that predict earlier onset are not the same as those that correlate with shortest duration of disease. (Cudkowicz et al., 1997). This suggests that the factors that influence onset of disease differ from those that influence the rate of progression of the disease.

Determining the mechanism by which mutations in the Cu/Zn superoxide dismutase (SOD1) gene triggers the destruction of motor neurones causing MND remains a challenging and complex problem. Five primary hypotheses have been postulated for the

pathogenesis of FALS (Figure 3). (Hand & Rouleau, 2002). At present the favoured hypotheses is that the mutation causes disease as a result of a toxic gain of function by the mutant SOD1 provoking selective neurotoxicity, probably disrupting the intracellular homeostasis of copper and/or protein aggregation. (Clevland, 1999).

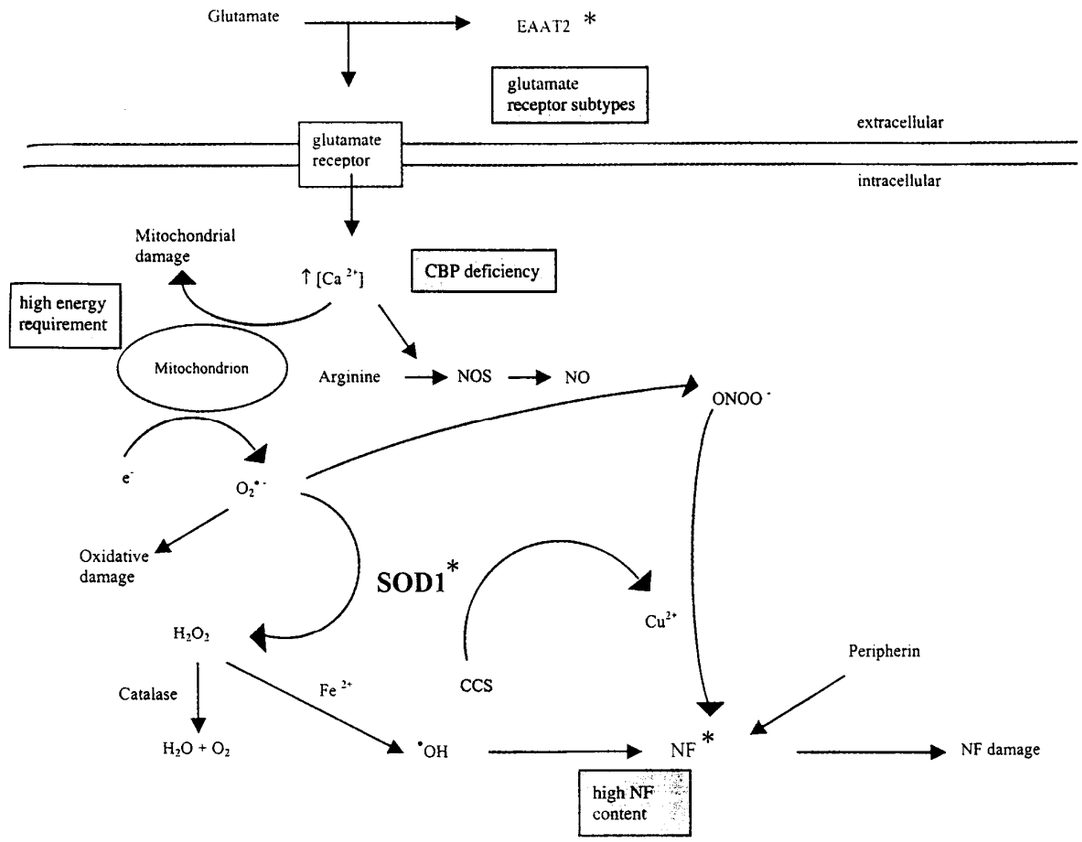


Fig. 3. Pathways that have been implicated in motor neurone cell death in amyotrophic lateral sclerosis (Reproduced from Hand CK. Familial Amyotrophic Lateral Sclerosis. Muscle Nerve 2002; 25:137).

The mutant SOD1 enzyme has altered reactivity with certain substrates, (Noor et al., 2003), in addition to the major superoxide dismutase activity. The SOD1 enzyme catalyses the reduction of hydrogen peroxide (H_2O_2), therefore acting as a peroxidase. This leads to the formation of hydoxyl radicals that can also alter the neurofilament network. Motor neurones have high-energy requirements and thus contain many mitochondria that generate superoxide radials (O_2-) through normal metabolism. SOD1 is an anti-oxidant defence which catalyses conversion of superoxide free radical anion (O2-) to hydrogen peroxide (H_2O_2), which is reduced to H_2O and O_2 by catalse. Mutations at SOD1 binding sites, alter the redox behaviour of the enzyme and destabilise the SOD1 ligand, leading to increased oxidative damage as hydrogen peroxide and its derivatives are toxic to the cell. (Yim et al., 1990).

This supports the hypothesis that the pathogenesis of SOD1 related FALS may be due to increased peroxidase activity of mutant SOD1 resulting in oxidative damage mainly to lipids of the cell membrane.

Mapping of the mutation sites predicted that these mutations destabilise the protein structure, leading to a less active enzyme i.e. "loss of function". This is however not supported by the fact that transgenic mice over expressing SOD1 gene developed disease similar to MND in humans, while those over-expressing normal SOD1 remained unaffected. This suggests that the mutant mice develop the disease independent of the level of SOD1 activity and suggests that the mutant protein itself is selectively toxic to motor neurones and that there is a "gain of toxic function" rather than a "loss of function". (Gurney et al., 1993). Also, although most mutations in SOD1 gene cause decrease in steady state of cytosolic SOD1 activity, Gly37Arg and Asp90Ala, have no significant decrease in SOD1 activity. (Shaw et al., 1998).

As most SOD1 mutations destabilise SOD1 protein (except Asp90Ala), it is possible that the mutant protein, with altered conformation may become unstable and precipitate to form aggregates or inclusions in motor neurons. These aggregates may then disturb normal cell function and lead to cell death. They are easily formed when SOD1 protein stability is decreased because this protein exists in large amounts accounting for 0.5-1% of total cytosolic protein in neurons. Alternations in the length of the coding sequence, folding, solubility or degradation results in the formation of aggregates. (Yim et al., 1990). Structural changes of mutant SOD1 may distort the rim of the electrostatic guidance channel and allow the catalytic site to become exposed and shallow. Molecules that are normally excluded may gain access to the catalytic reactive site. This results in less buffering of copper and zinc, which then become neurotoxic. (Radunovic & Leigh, 1996).

The nitric oxide (NO) produced by nitric oxide synthase (NOS) reacts spontaneously with O_2- to generate peroxynitrite ($ONOO^-$), which nitrosylates proteins leading to damage. Excess NO may also cause an increase in O_2- production by inhibition of mitochondrial electron flow, resulting in further generation of peroxynitrite. This facilitates nitrosylation of tyrosine residues of critical cytosolic proteins thus injuring cells. This reaction is copper dependent. The source of free copper may be mutant SOD1, which cannot accept the ion from the copper chaperone (CCS) protein. Mutant SOD1 possibly exhibit metal mediated cytotoxicities by disrupting the intracellular homeostasis of Cu and Zn, which are potential neurotoxins. (Gurney & Tomasselli, 2000).

The target proteins for nitrosylation include the neurofilament (NF) subunits, which may result in abnormal NF accumulation and subsequent disruption of the NF network and axonal transport, as there is a high neurofilament content in motor neurones. It has also been demonstrated that transgenes encoding mutant NF subunits can directly cause selective degeneration and death of motor neuones. (Cleveland, 1999). Conformational changes have been described in the mutations, Ala4Val, Gly37Arg and His6Arg that may affect the rim of the electrostatic guidance channel coded by exon 3. (Sjalander et al., 1995).

Glutamate is released from the presynaptic terminal activates the glutamate receptor on the postsynaptic cell membrane. It is then cleared from the synaptic cleft by specific glutamate transporters such as EAAT2. (Trotti et al., 1999). Astrocyte (glial cell) dysfunction may result in selective loss of EAAT2, interfering with the normal clearance of glutamate and allowing it to accumulate in the cell membrane and continue to activate the receptor. (Bruijin et al., 1997). Once activated, the glutamate receptor causes a calcium influx and a cascade of toxicity. The neurone does not have the capacity to buffer this efficiently due to a deficiency in calcium binding proteins (CBP's). This results in disturbances in mitochondrial metabolism and as a consequence, motor neurone cell death. (Beal, 1996).

To date, the only effective approved treatment for amyotrophic lateral sclerosis is Riluzole, (Cheah et al., 2010), which has a neuroprotective role, possibly due to pre-synaptic inhibition of glutamate release. (Doble, 1996). Treatment of human ALS patients or transgenic Cu, Zn superoxide dimutase 1 (SOD 1) mice, most commonly produce a modest but significant increase in survival. (Bensimon et al., 1994). It has also been shown to have a small beneficial effect on bulbar function, but not muscle strength. (Miller et al., 2007).
Apoptosis is characterised by a series of cellular changes leading to non-inflammatory cell death. Mitochondrial involvement in the apoptotic pathway also leads to the release of cytochrome c, an activator of the initiator caspase-9, which in turn activates caspase-3, which are executioners in the breakdown of essential cellular proteins. There is evidence that the mutant SOD1 transgene causes motor neurone death in mice through caspase-mediated programmed cell death. (Li et al., 2000). This may then be a target for inhibiting the apoptotic cascade, as it has been shown in a SOD1 transgenic mouse model that a small peptide caspase inhibitor (*zVAD-fmk*), prolonged survival after onset of disease by nearly 70%. (Kosti et al., 1997). It has also been reported that there are elevated levels of *bax* protein in MND spinal motor neurones, which promotes apoptosis. (Mu et al., 1996).

6. Methods

The Department of Molecular Medicine at Concord hospital had a large database of family members with a known family history of MND, who had blood samples collected for DNA, as part of a previous linkage study. From this database, family members were contacted by telephone by the department's genetic counsellor and informed about the study.
The regional committees for Ethics in Medical Research from Central Sydney Area Health Service, Royal North Shore Hospital and Prince Charles Hospital, approved this study.
All individuals participated without knowledge of their mutation status and on the understanding that this would not be revealed to them. Subjects were also aware that the results obtained from the study would not be available to them and that the information would only be used for research purposes. New consents were obtained from all individuals who participated in the study. The neurologist performing the MUNE studies also had no knowledge of their mutation status. The mutation status was only used in the final analysis of results. Subsequently, they were divided into "SOD1 negative family controls" and "asymptomatic SOD1 mutation carriers".
In addition, studies were also carried out on normal individuals, such as department technicians, spouses of SOD1 family members and individuals from the general population who attended MND support meeting and had an interest in helping to advance research into MND. This group was used as "population controls", to test the validity and reproducibility of the MUNE technique used.
Sporadic MND subjects were also initially studied once the MUNE technique had been validated to demonstrate that the MUNE technique used was able to detect a loss of motor neurones, when present. These were used as "positive controls".

6.1 Motor unit number estimation

Motor unit number estimation (MUNE) estimates the number of functioning lower motor neurones innervating a muscle or a group of muscles and is a measure of the primary pathologic process of motor neurone loss. The concept of motor unit number estimation

(MUNE) originated in 1967. At the time there was no satisfactory method of assessing the extent of denervation in muscles during life. Analysis of the density of the electromyographic interference pattern during maximal effort was not quantitative, and required the full co-operation of the patient.

The principle of MUNE is that if one can measure the mean single motor unit amplitude (SMUP), it is possible to obtain an estimate of the total number of motor units in the muscle. The results achieved were comparable with estimates of alpha motor fibres obtained by counting axons in specimens of motor nerves. (McComas, 1971).

MUNE has been performed in a number of different ways, each with their advantages and limitations. (Stein & Yang, 1990). The choice of technique depends on the speed and simplicity of the technique, as well as its accuracy and reproducibility. Some methods sample a very small proportion of the number of motor units innervating a muscle (typically 10-20). The coefficient of variation associated with different methods range from 10-45%. (McComas, 1991). If the variability is too large, then the technique cannot be used to follow motor unit loss reliably over time.

The way the average single motor unit potential (SMUP) size is obtained distinguishes the several techniques available. Most employ electrical stimulation of the motor nerve to determine the sizes of the SMUP, but a few use needle EMG.

Each method measures both the average size of the potentials generated by single motor units - single motor unit potentials (SMUP) and the size of the compound muscle action potential (CMAP) obtained with maximal stimulation of a motor nerve.

The motor unit number estimate is calculated by:

$$\text{MUNE} = \frac{\text{Maximum CMAP amplitude (or area)}}{\text{Average single motor unit potential (SMUP) amplitude or area.}}$$

Whereas the methods of measuring the average SMUP differ, they have common assumptions about the measurement of the supramaximal CMAP and the measurement of the average SMUP.

i. Maximal stimulation of any peripheral motor nerve activates all the muscles innervated by that nerve distal to the point of stimulation. Therefore, measurements of the CMAP are the summation of activity from multiple muscles and the MUNE is more accurately an estimate of the number of motor units in a group of muscles rather than in a single muscle.

For example, the median CMAP recorded at abductor pollicis brevis (APB) is more correctly a "thenar MUNE", as it is a summation of the activity of APB, opponens pollicis, flexor pollicis brevis, and to a lesser extent, the lateral lumbricals.

Extensor digitorum brevis (EDB) on the other hand, is a muscle innervated by the deep peroneal nerve. The only source of interfering muscle action potential is from extensor hallucis longus, which can be reduced by correct position of the stimulating electrodes. The muscle belly is flat in profile, eliminating deeper motor units as a cause of small potentials. The recording electrode is placed transversely across the innervation zone, resulting in a simple biphasic negative-positive M wave.

ii. The motor unit potentials used in the calculation of the average SMUP are representative of those generated by the total population of units. All methods, select a subset of the total population of motor units, measure their sizes and calculate an average SMUP for that subgroup.

iii. Finally, there is a phenomenon caused "alternation". This refers to fluctuations in the CMAP amplitude of the same motor unit with similar stimulation intensities. The thresholds of the first few motor axons excited are not sufficiently separate from one another, so that when graded increases in the stimulus intensity occur, the motor axons excited often overlap and add more than one SMUP to the CMAP being recorded. This can result in an underestimation of the mean SMUP size, as it may appear that there are 7 or 8 motor units when there are only 2 or 3 present, which in turn results in an over-estimation of the MUNE.

6.2 Statistical MUNE method

We used the statistical electrophysiological technique of motor unit number estimation (MUNE), (Daube, 1998), was used to estimate the number of motor units in thenar and extensor digitorum brevis muscles. The statistical method estimates the average size of SMUP's and the number of motor units in a group of muscles innervated by the nerve being stimulated, based on the normal variation of the submaximal CMAP evoked with constant stimuli. No attempt is made to identify individual motor unit potentials. The method relies on the known relation between the variance of multiple measures of step functions and the size of the individual steps when the steps have a Poisson distribution. S.D. Poisson was a French mathematician (1781-1840).

Poisson statistics are useful when the distribution arising for events occur randomly in time or when small particles are distributed randomly in space. They have been used to calculate the number of quanta released from a nerve terminal at the neuromuscular junction when the individual quanta are too small to be distinguished, as in myasthenia gravis. (Lomen-Hoerth & Slawnych, 2003).

In pure Poisson statistics, the size of a series of measurements is multiples of the size of a single component. In a Poisson distribution there is a discrete asymmetrical distribution in which responses are found at some levels and others where there are no responses (Figure 4). (McNeil, 1996).

A pure Poisson distribution has decreasing numbers at higher values. In Poisson distribution, the variance of these 30 measurements is equal to the size of the individual components making up each measurement. The variance can thus provide an estimate of the average size of the SMUP's.

The statistical method looks only at variance of the CMAP and does not require identification of individual components. It can be used when the sizes of SMUP's are too small to be isolated. The statistical method assumes that each motor unit has a similar size and that it is the same size each time it is activated.

Sequences of 30 submaximal stimuli are given. The inherent variability of the threshold of individual axons causes variations in the size of the CMAP. The average change in the submaximal CMAP amplitude caused by alternation (addition and subtraction of motor axons) is derived by Poisson statistics.

The occurrence of alternation with changing units that are activated does not modify the accuracy of the statistical method, because the method is a statistical measurement, a different result is found with each series of 30 stimuli. Therefore, multiple trials are needed to obtain the most accurate measurement. (Olney et al., 2000).

Experimental testing with trials of >300 stimuli has shown that repeated measurement of groups of 30 until the standard deviation of the repeated trials is <10% provides a close

estimate of the number obtained with many more stimuli.[86] Estimates of the SMUP size and of the number of motor units are also most reliable if made at multiple different stimulus intensities to test axons with different thresholds.

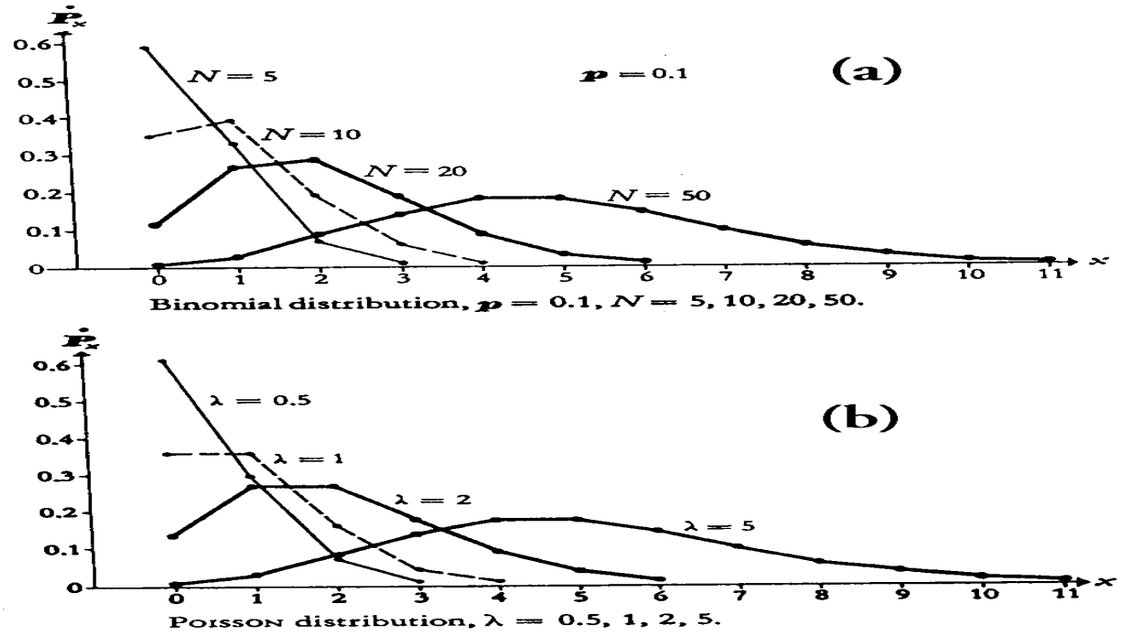


Fig. 4. Graphs illustrating Binomial and Poisson distribution (Reproduced from McNeil D. Statistical Methods. 1996; 184). The top graph (a) illustrates that binomial distribution resembles normal distribution with increasing sample size (n=50). The lower graph (b) illustrates that smaller values (λ=5) result in a normal distribution - Poisson distribution.

MUNE is calculated with the number weighted statistical method, where the mean SMUP amplitude at each level is multiplied by the number of motor units estimated at each level.

The steps in statistical MUNE are as follows:

1. Recording surface electrodes are applied as for standard nerve conduction studies.
2. An initial scan of the CMAP is performed using a series of 30 submaximal stimuli at 1 Hz, increasing in equal increments to identify unusually large steps at which further information is required.
3. On the basis of the scan, three or four 10% stimulus ranges are identified, according to an internal algorithm. Usually, one range includes the smallest step and the other ranges where the steps are >15% (Figure 5).
4. At each intensity, groups of 30 responses are captured at a rate of 3Hz. Estimates are most reliable if 10 groups of 30 responses are recorded. To minimise patient discomfort, however, repetition is repeated until the standard error of the MUNE SMUP size is less than 10%.
5. Statistical MUNE estimates the average size of SMUP's and the number of motor units in a group of muscles innervated by the nerve being stimulated, based on the normal variation of the sub-maximal CMAP evoked with constant stimuli (Figure 6).

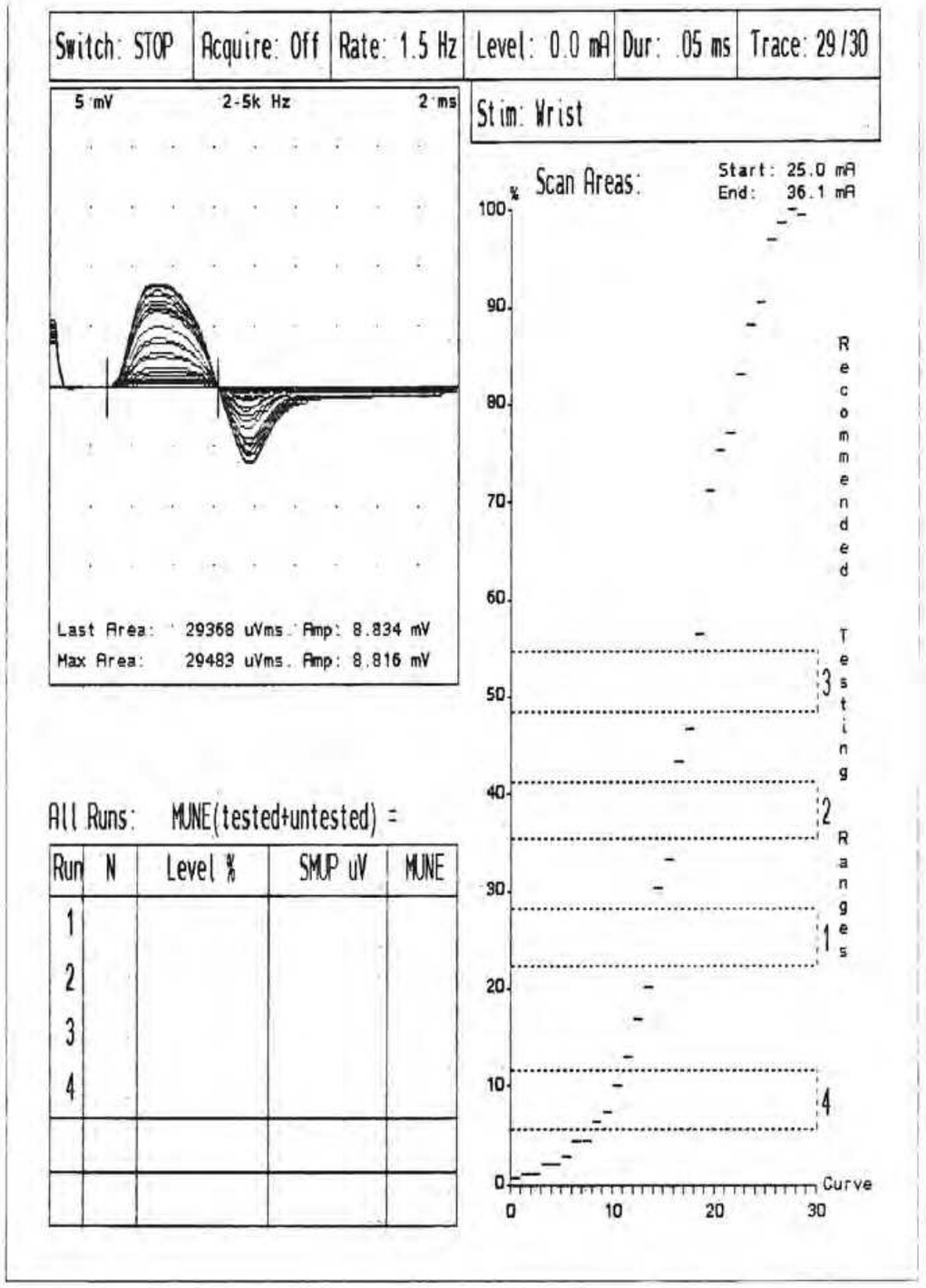
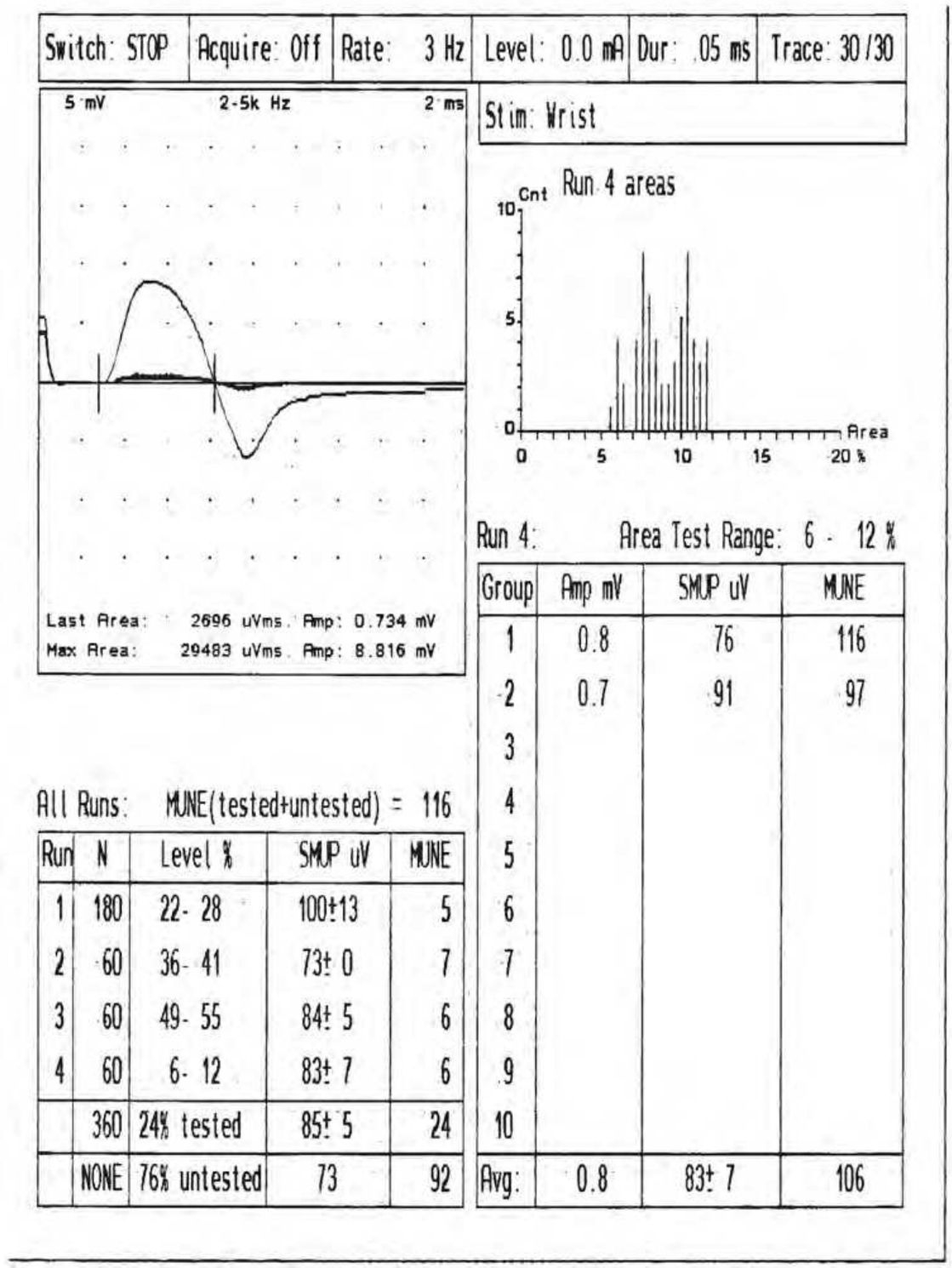


Fig. 5. An initial scan of the CMAP (right) recorded from APB muscles in response to 30 sub-maximal stimuli (x-axis) with equal increments between threshold and maximum stimulation. On the basis of the scan, 10% stimulus ranges are identified, according to an internal algorithm. The CMAP increments are shown at the top left and the eventual table of results in the bottom left corner.



Group	Amp mV	SMUP uV	MUNE
1	0.8	76	116
2	0.7	91	97
3			
4			
5			
6			
7			
8			
9			
10			
Avg:	0.8	83± 7	106

Run	N	Level %	SMUP uV	MUNE
1	180	22- 28	100±13	5
2	60	36- 41	73± 0	7
3	60	49- 55	84± 5	6
4	60	6- 12	83± 7	6
	360	24% tested	85± 5	24
	NONE	76% untested	73	92

Fig. 6. At each intensity level (runs 1-4), groups of 30 responses are captured at a rate of 3Hz. The CMAP amplitudes are shown at the top left, with the histogram of results at the top right. The thenar MUNE results from repeated trials are shown in the bottom left table.

The statistical technique of estimating the size of the SMUP was performed using proprietary software on a Nicolet Viking IV electromyography machine. This technique uses direct stimulation of the motor nerve. The low frequency filter was set at 2 Hz and the high frequency filter at 5 kHz. The gain for extensor digitorum brevis was set at 2 mV/div and for abductor pollicis brevis studies at 5 mV/div. The sweep speed was 2 ms/div. This method had excellent test-retest reproducibility (+/-2.8%). The method was quick to use and well tolerated.

This technique has been greatly modified since its original description, but numerous studies have shown that MUNE can change systematically in ALS patients when used by experienced technicians, even though evaluator bias needs to be taken into account. (Shefner et al., 2004). The statistical MUNE method has also been shown to be unreliable in the presence of clinical weakness due to motor unit instability. (Shefner, 2009).

Our study however was performed on asymptomatic patients, without clinical weakness.

6.3 MUNE technique

Motor unit numbers were estimated in abductor pollicis brevis (resulting in a thenar MUNE) and the extensor digitorum brevis (EDB) muscle. These muscles were used, as both are easily accessible distal muscles. The electrical activity can be recorded without interference, and in the case of EDB, the muscle belly is flat.

Self-adhesive surface recording electrodes (G1) were placed transversely across the innervation zone of each muscle, resulting in a simple biphasic negative-positive M wave, with G2 placed over a bony prominence. The deep peroneal nerve was stimulated just above the ankle and the median nerve at the wrist with a surface stimulator. This was performed by strapping the stimulating electrode onto the surface of the skin, at the point where the threshold of the nerve to electrical stimulation was at its' lowest. A hand-held stimulator was not used, as reproducibility is enhanced when the stimulating electrodes are fixed to the surface of the skin.

Initially, bilateral thenar and EDB MUNE's were obtained from all subjects. After the reproducibility phase of the study, generally only right-sided studies were performed. Once a reduction in MUNE was identified, bilateral studies were once again performed on selected subjects. The protocol was also modified depending on the subjects' tolerance to the procedure.

Median nerve stimulation at the wrist for thenar MUNE was generally well tolerated by most subjects, as the stimulation intensity required to obtain an adequate response was generally less than 20mA with duration of 0.05-0.1ms.

Peroneal nerve stimulation required for EDB MUNE resulted in slightly more discomfort, as the nerve is located further away from the surface of the skin. The stimulus intensity required, in some cases was up to 50-80mA with duration of between 0.1-0.3ms. Some subjects indicated that they were unwilling to continue to participate in the study due to the discomfort caused by performing EDB MUNE. In these subjects, only thenar MUNE's were performed.

To assess the test-retest reproducibility of the technique, SOD1 family members and population controls were followed over a 1-year period, with thenar and EDB MUNE tests repeated every 3 to 6 months. The difference between MUNE results from the first and second study, and if possible, first and third studies were divided by the MUNE of the first study, and expressed as a percentage change. The results were analysed using Pearson and Spearman correlation coefficients.

All results were entered into a database and analysed using a standard statistical software package (SPSS 9.05 for Windows). For the initial part of the study, the MUNE results from asymptomatic SOD1 mutation carriers were grouped together. Although different mutations in SOD1 have different effects on the progression of the disease once symptoms occur, these different mutations do not influence on the age of onset of symptoms.[67]
Motor unit estimates in carriers were compared to age and sex matched family controls without the SOD1 mutation, and sporadic (non-SOD1) MND patients. To determine whether groups had different numbers of motor units, an unpaired t-test was used. Although there were some outlying results, the distributions were not sufficiently skewed to contradict the use of the t-test. Statistical significance was accepted at a p-value of <0.05.
The group of asymptomatic SOD1 mutation carriers were followed over the next 2 to 5 years, depending on the volunteers' motivation, both clinically and by MUNE. Results were compared to their initial baseline MUNE and the date of the study when this reduction was first detected, was used as the date when motor neurone loss commenced.

6.4 Maximal voluntary isometric contraction testing

It has been suggested that the traditional neurological examination is inadequate for documenting motor performance impairment with reliability. (Hanten et al., 1999). Generally, manual motor testing used in a standard neurological motor examination does not allow objective documentation of change in performance, as it may be influenced by the patient's history and progress. Major changes are apparent, but subtle changes are difficult to determine with accuracy.
There are a number of methods that have been developed to quantify maximal voluntary isometric contraction (MVIC). It has been proposed that this is a clinically useful, reliable, reproducible, time efficient and quantitative measure for monitoring disease progression in MND. (Hoagland et al., 1997). This would be surprising, given that in a slowly progressive denervating process, patients with substantial chronic denervation could maintain normal muscle twitch tension until loss of about 70-80% of motor units occurs. (McComas, 1971).
The methods used to quantify maximal voluntary isometric contraction have included an electronic strain-gauge tensiometer and a hand-held Jamar hydraulic dynamometer. In this study, maximum bilateral isometric grip strength was obtained using the Jamar hydraulic dynamometer to determine whether this correlated with the number of functional motor neurones in the thenar group of muscles, as measured by MUNE. Standardised (middle handle) positioning and instructions were given to all subjects. Handgrip force was measured with subjects in the sitting position and with the arm flexed at 90 degrees. Two trials were performed on each hand, and the best result used for analysis. This method was used as previous studies of grip strength reliability showed that there was no significant difference in reliability between one attempt, the mean score of two or three attempts, or the highest score of three attempts. (Hamilton et al., 1994).
Clinical neurological examination was performed, with power of thumb abduction, finger flexion and finger abduction measured according to the Medical Research Council (MRC) grading system and compared to thenar (APB) MUNE.
Felice showed that in twenty one MND patients, changes in thenar MUNE was the most sensitive outcome measure for following disease progression, when compared to other quantitative tests, such as CMAP, isometric grip strength, forced vital capacity and Medical Research Council manual muscle testing. (Felice, 1997).

7. Results

7.1 Demographics

A total of eighty-eight (88) subjects (45 males and 43 females) gave informed consent. The subjects were divided into four test groups.

1. 24 population controls;
2. 32 SOD1 negative (normal) family controls;
3. 20 asymptomatic (pre-clinical) SOD1 mutation carriers (test group),
   a. 5 subjects with point mutation in exon 4, codon 100, GAA to GGA, Glu to Gly) – glu100gly;
   b. 5 subjects with point mutation in exon 4, codon 113, ATT to ACT, Ile to Thr) – ile113thr;
   c. 5 subjects with point mutation in exon 5; codon 148, GTA to GGA, Val to Gly) – val148gly;
   d. 5 subjects with point mutation in exon 5, codon 148, GTA to GGA, Val to Ile) val148ile.
4. 12 sporadic symptomatic MND patients (positive controls).

There was no statistically significant difference in age distribution between these groups, with a range of 16 to 73 years of age.

7.2 Motor units in asymptomatic FALS (SOD1) carriers

For the initial part of the study, the baseline MUNE results were grouped together and the means of the groups were compared. The initial aim of the study was to determine if MND was due to a slow gradual attrition of motor neurones over time. If this were the case, the group of asymptomatic SOD1 mutation carriers, would be expected to have a reduced number of motor units, indicating the presence of pre-clinical motor neurone loss. Motor unit estimates in the group of asymptomatic SOD1 mutation carriers were compared to age and sex matched family controls without the SOD1 mutation, and sporadic (non-SOD1) MND patients. To determine whether groups had different numbers of motor units, an unpaired t-test was used. Statistical significance was accepted at a p-value of <0.05.

The numbers of motor units in the groups of population controls, SOD1 negative family controls and asymptomatic SOD1 mutation carriers were similar. In population controls the mean thenar MUNE was 148 with a range of 115 - 254, in SOD1 negative family controls was 138 with a range of 106 - 198 and in asymptomatic SOD1 mutation carriers, 144 with a range of 109 - 199. There was no detectable difference in the mean number of thenar motor units in the group of asymptomatic SOD1 mutation carriers compared to the group of SOD1 negative family controls (thenar $p>0.46$), or population controls (thenar $p>0.70$) (Table 1 and Figure 7).

	Thenar (APB) muscle	
	Cases	**MUNE (Range)**
Population Controls	24	148 (115-254)
SOD1 Negative Family Controls	32	138 (106-198)
SOD1 Mutation Carriers	20	144 (109–199)
Sporadic MND patients	12	45 (5–84)

Table 1. Thenar (APB) motor unit number estimates (MUNE number represents mean MUNE).

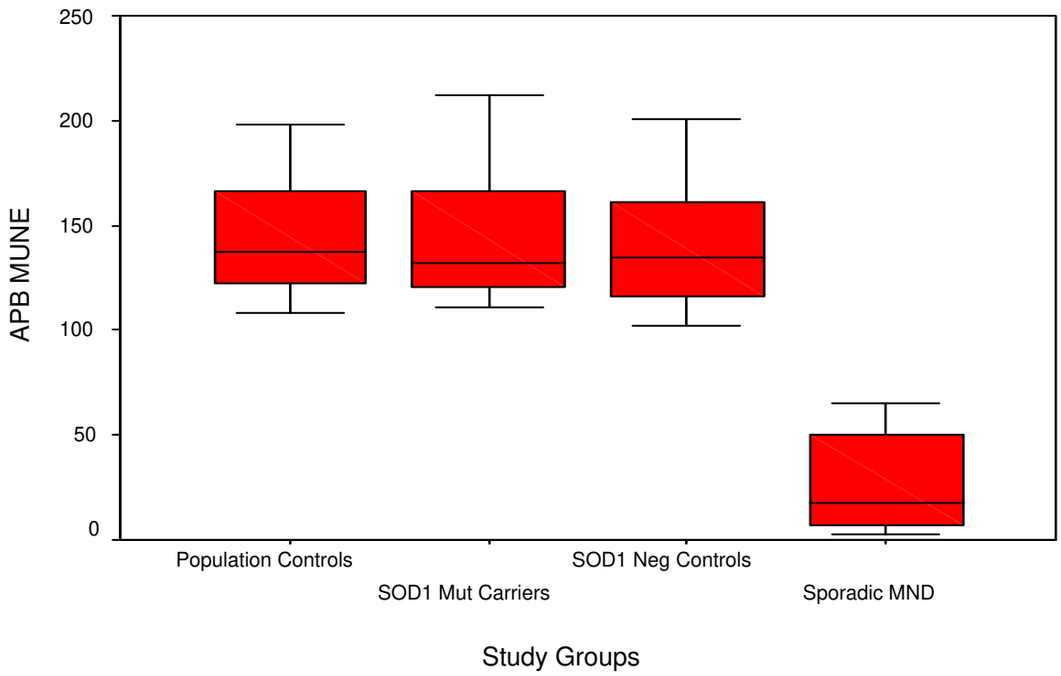


Fig. 7. Baseline thenar (APB) MUNE subdivided into study groups (The lower boundary of the box is the 25th percentile, and the upper border is the 75th percentile of MUNE. The horizontal line inside the box represents the median MUNE. The whispers represent the largest and smallest observed values, i.e. the range). Data is shown in Table 1.

In population controls the mean EDB MUNE was 138 with a range of 119 - 169, in SOD1 negative family controls was 134 with a range of 107 - 180 and in asymptomatic SOD1 mutation carriers, 136 with a range of 111 - 187.
Once again, there was no detectable difference in the mean number of EBD motor units in the group of asymptomatic SOD1 mutation carriers compared to the group of SOD1 negative family controls (EDB $p>0.95$), or population controls (EDB $p>0.50$) (Table 2 and Figure 8).

	Extensor Digitorum Brevis	
	Cases	**MUNE (Range)**
Population Controls	13	138 (119-169)
SOD1 Negative Family Controls	30	134 (107-180)
SOD1 Mutation Carriers	14	136 (111-187)
Sporadic MND patients	9	70 (8-82)

Table 2. EDB motor unit number estimates (MUNE number represents mean MUNE).

Symptomatic sporadic MND subjects showed a definite loss of motor units with fewer motor units compared to all other groups ($p<0001$) with a mean thenar MUNE of 45 with a range of 5 - 84 and a mean EDB MUNE of 70 with a range of 8 - 82 (Tables 1 and 2).
There was no cross over between thenar and EDB MUNE results in symptomatic and asymptomatic subjects.

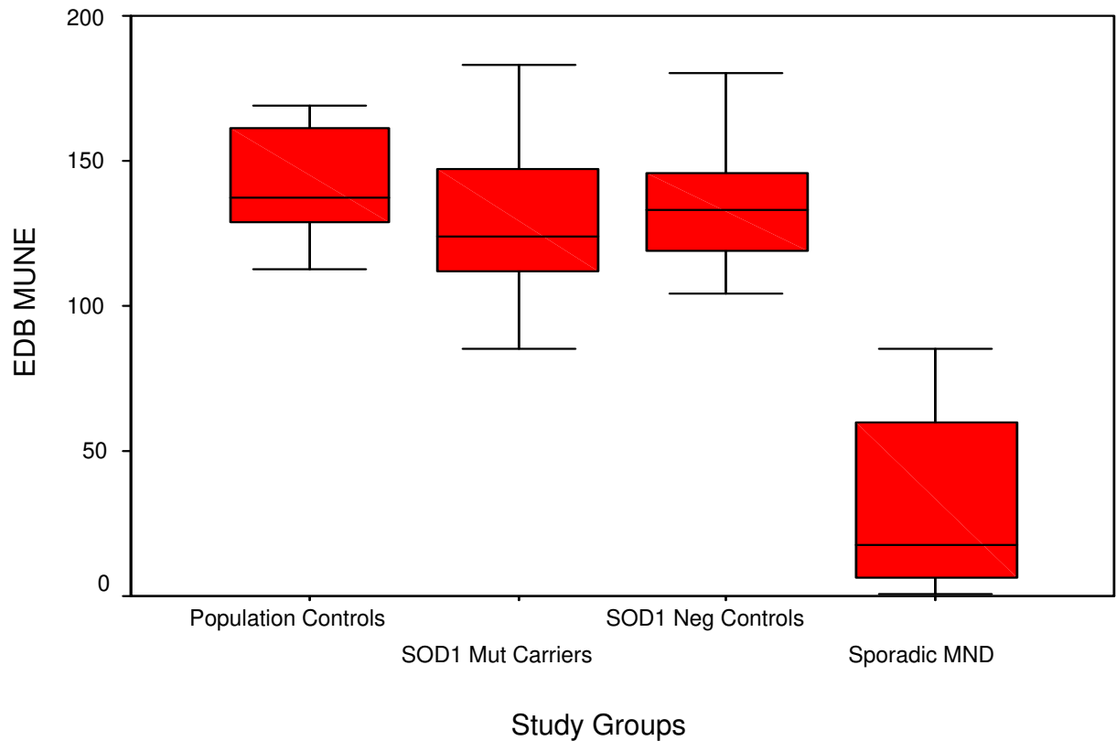


Fig. 8. Baseline EDB MUNE subdivided into study groups (The lower boundary of the box is the 25th percentile, and the upper border is the 75th percentile of MUNE. The horizontal line inside the box represents the median MUNE. The whispers represent the largest and smallest observed values, i.e. the range). Data is shown in Table 2.

7.3 Reproducibility of MUNE technique

To assess the test-retest reproducibility of the technique, 69 of the 88 SOD1 family members and population controls were followed over a 1-year period, with thenar and extensor digitorum brevis (EDB) MUNE tests repeated every 3-6 months, depending on patient availability. The difference between MUNE results from the first and second study, and if possible, first and third studies were divided by the MUNE of the first study, and expressed as a percentage change. The results were analysed using Pearson and Spearman correlation coefficients.

The test-retest correlation of thenar MUNE in asymptomatic subjects was high with a Pearson correlation coefficient of 0.93. The mean difference between MUNE results on separate occasions on the same individual was +/- 3.6%, with a range of 0-11.7% (Table 3).

	Number of Cases	**Mean MUNE**
Thenar 1	88	145.7
Thenar 2	69	140.1
Thenar 3	33	140.0
Thenar Change	**Range (0 - 11.7%)**	**3.6%**

Table 3. Reproducibility of mean thenar (APB) motor unit number estimates in asymptomatic subjects on separate reviews over a one-year period.

For EDB MUNE, the Pearson correlation coefficient was also high, 0.88, with a mean difference between MUNE results on separate occasions on the same individual of +/- 4.6%, with a range of 0-15.7%. The test-retest correlation was high with a Pearson correlation coefficient of 0.91, when groups were broken down into the different study groups.

7.4 Maximal voluntary isometric contraction

Maximal voluntary isometric contraction (MVIC), using the Jamar hand dynamometer was used to measure isometric grip strength to determine whether this correlated with the number of functional motor neurones in the thenar group of muscles as measured by MUNE. Isometric grip strength tests, thenar MUNE and MRC power were performed on 69 asymptomatic subjects twice within a 3-6 month period to assess the test-retest reproducibility of this technique. Pearson correlation coefficients between study 1 and study 2 of right hand grip strength was 0.941, left hand grip strength 0.910 and thenar MUNE results 0.937. These results indicate that the reproducibility of these techniques was high.

Right hand grip strength correlated with left hand grip strength, with Pearson correlation coefficients of 0.959 and Spearman correlation coefficients of 0.956 Two-way analyses of variance showed a no significant difference between the right and left hands (Figure 9). There was no correlation between right grip strength and right thenar motor unit number, with Pearson correlation coefficients of 0.483 and Spearman correlation coefficient of 0.34 (Figure 10).

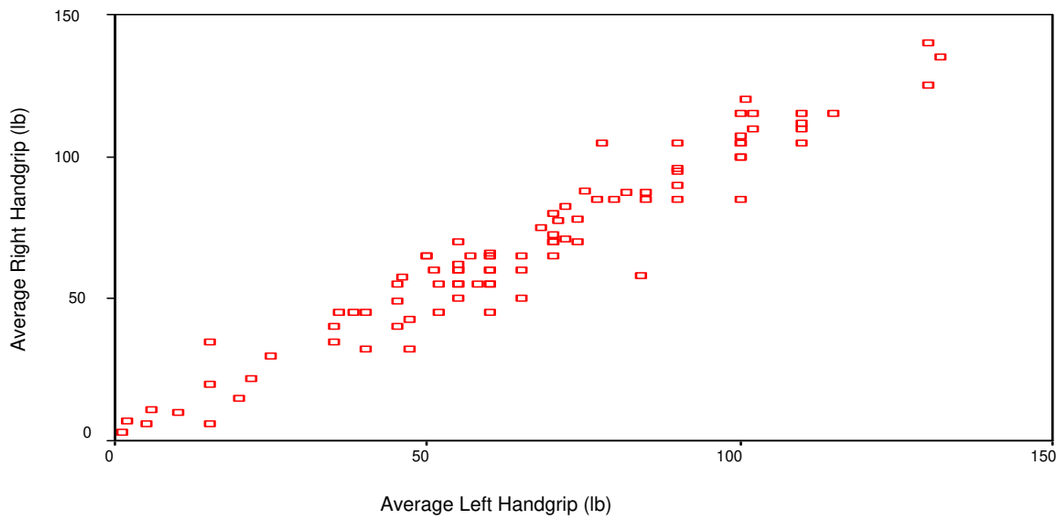


Fig. 9. Graph showing the correlation between right and left handgrip

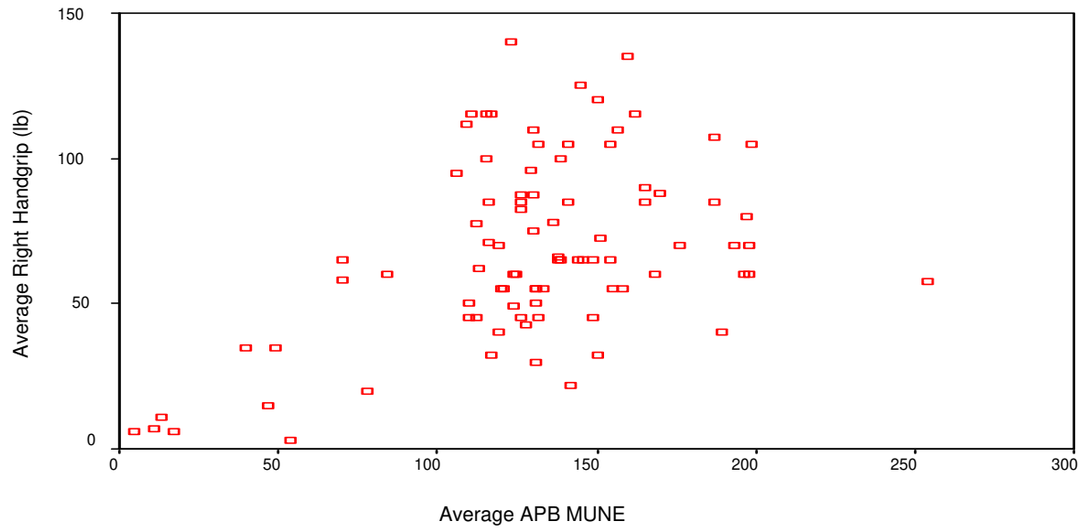


Fig. 10. Scatter graph showing the lack of correlation between right handgrip and right thenar (APB) MUNE

7.5 Detection of pre-symptomatic motor neurone loss in SOD1 mutation carriers

The MUNE results, after validating their reproducibility, were used as a baseline to follow the number of motor units over time in individual pre-symptomatic SOD1 mutation carriers over the next 2-5 years, to determine whether pattern of motor neurone loss is either a slow attrition of motor neurones over time or whether normal numbers of motor neurones are maintained until sudden, rapid multi-focal cell death of motor neurones occurs, corresponding with the development of symptoms.

During the course of the study, 5 of the SOD1 mutation carriers developed leg weakness. A significant fall in motor unit number was detected in these 5 SOD1 mutation carriers, were there was a detectable reduction of motor units, 4-10 months prior to the onset of weakness and the diagnosis of familial ALS being made. There was no detectable loss of motor units in the other 15 SOD1 mutation carriers or in the group of SOD1 mutation negative relatives, during the study period.

In individual cases, there was:

51% loss of motor units, 4 months prior to onset of weakness in Case 1
37% loss of motor units, 10 months prior to onset of weakness in Case 2
28% loss of motor units, 6 months prior to onset of weakness in Case 3
46% loss of motor units, 6 months prior to onset of weakness in Case 4
68% loss of motor units, 8 months prior to onset of weakness in Case 5

There was further motor unit loss as weakness progressed, at which point the diagnosis of MND was confirmed.

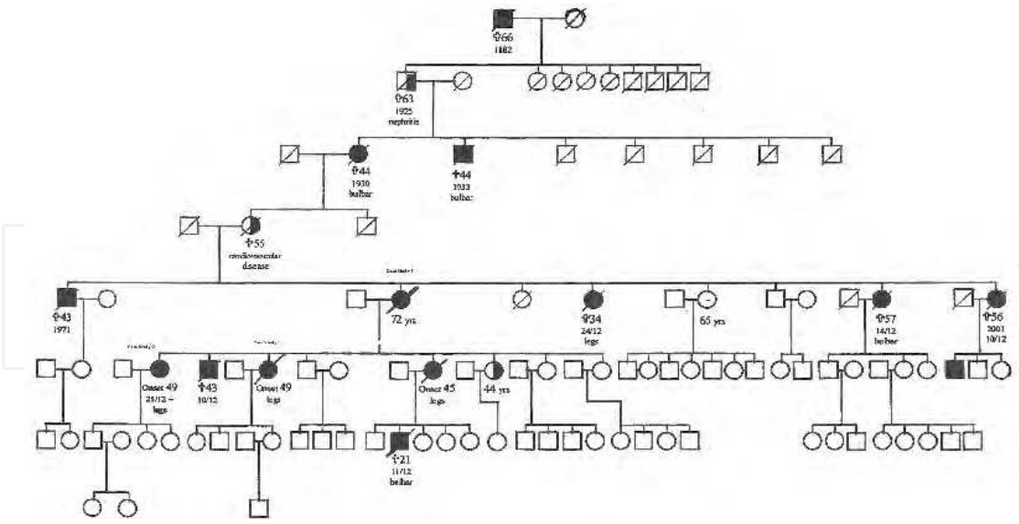


Fig. 11. Pedigree of cases 1, 2 and 3

7.5.1 Case study 1

A 48-year-old lady from a family with a strong history of familial ALS. Pedigree is shown in Figure 11. This family has a point mutation in the SOD1 gene at val148gly. At the time of recruitment, October 1998, the subject was asymptomatic, with a normal neurological examination and no evidence of wasting, weakness or fasciculations. Progress MUNE results are shown in Table 4 and Figure 12.

Months before and after weakness	**-26**	**-24**	**-21**	**-13**	**-7**	**-4**	**0**	**+3**	**+5**	**+8**	**+17**
Date of study	**Oct-98**	**Dec-98**	**Mar-99**	**Nov-99**	**Jul-00**	**Nov-00**	**Mar-01**	**Jun -01**	**Aug-01**	**Nov-01**	**Aug-02**
R Handgrip	60	65	68	60	60	65	65	62	60	45	Died
R Thenar MUNE	130	131	126	131	115	113	115	110	83	49	
L Handgrip	60	60	60	60	60	60	55	45	37	18	
L Thenar MUNE	122		114	110		122	83	52	40	0	
R EDB power	5/5	5/5	5/5	5/5	5/5	5/5	5/5	3/5	0/5	0/5	
R EDB MUNE	124	120	132	116	116	114	114	72	53	0	
L EDB power	5/5	5/5	5/5	5/5	5/5	5/5	0/5	0/5	0/5	0/5	
L EDB MUNE	130			125		64	0	0	0	0	

Table 4. Case 1 progressive handgrip, dorsiflexion power and thenar and EDB MUNE results

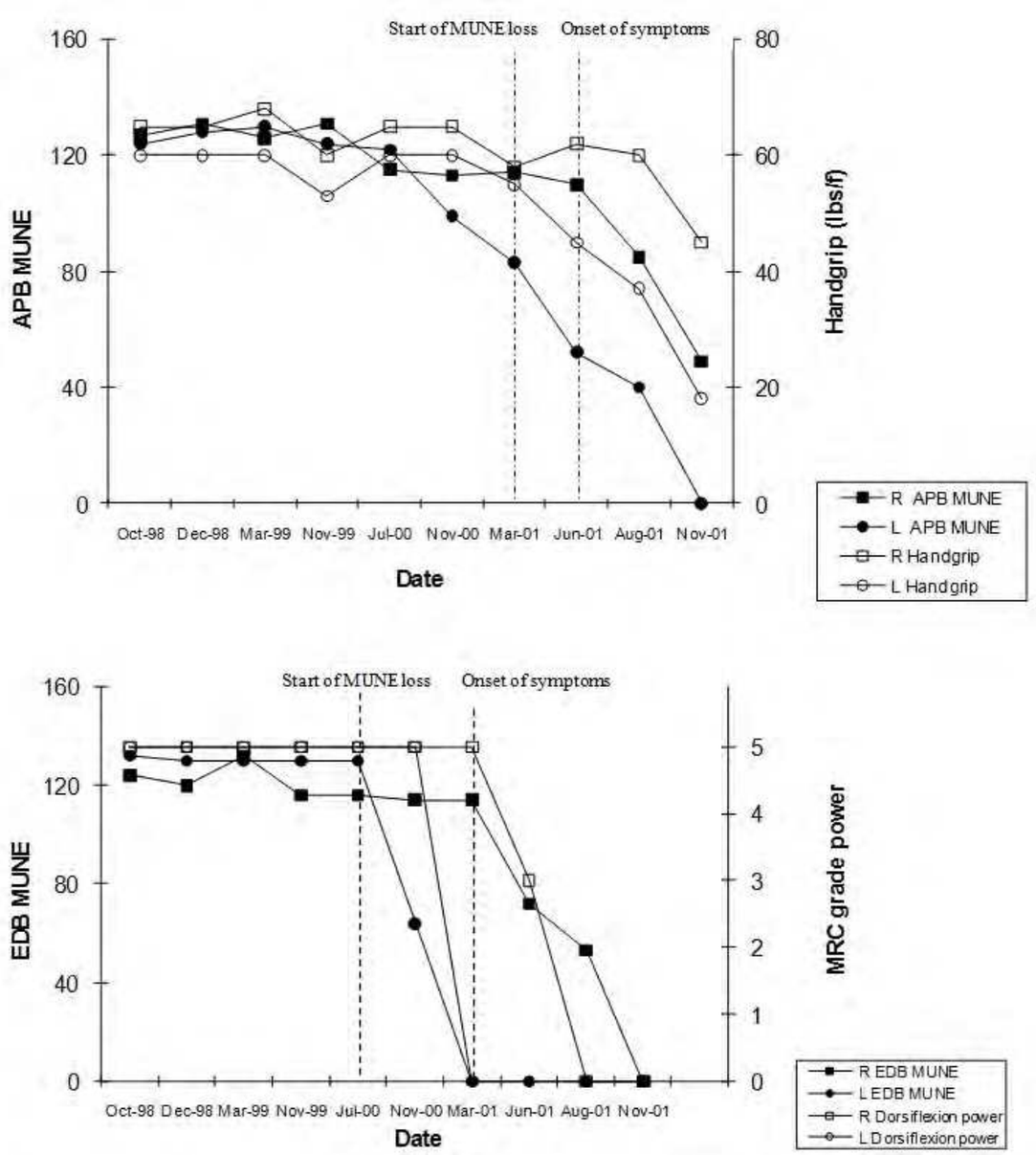


Fig. 12. Progressive results of case 1 showing the change in APB and EDB motor unit estimates over time in relation to handgrip strength and power. There is a reduction of APB and EDB MUNE prior to the onset of weakness.

The MUNE results remained stable over the first 2 years of the study. By November 2000, her left EDB MUNE had dropped to 64 (total reduction of 51%), but she only developed wasting and weakness of the anterior compartment muscles of her left leg, 4 months later, when she had MRC grade 2/5 of dorsiflexion and eversion, 3/5 plantarflexion and inversion 3/5. Proximal muscles, upper limb and right leg muscles were normal. Deep tendon reflexes were brisk in the upper and lower limbs. Over the next 6 months, her right thenar MUNE from 130 to 115 (12%) She had no detectable right arm weakness at the time and her handgrip strength remained at around 65 pounds.

An independent neurologist performed needle EMG, which showed extensive denervation in left leg muscles with fibrillation potentials in the left tibialis posterior, gastrocnemius, vastus medialis and L5/S1 paraspinal muscles. There were no changes in the right vastus medialis, biceps brachialis, triceps, left deltoid and the tongue. It was felt that these changes were not enough to make a diagnosis of ALS. She went on to have a MRI scan of her lumbar spine showed degenerative disc disease of L4/5 and L5/S1, with no evidence of neural compression.

Over the next 6 months, her right EDB MUNE continued to drop from 114 to 72 and subsequently to 53, a 54% reduction. Her left thenar MUNE also dropped from 122 to 83 motor units, a 36% reduction. Her right foot power remained normal until August 2001, 7 months after the reduction was noted. At that time, she developed upper limb weakness, with a reduction of left handgrip strength to 45 pounds.

Repeat needle EMG examination showed severe denervation in the left tibialis anterior and gastrocnemius, but still no changes in proximal right lower limb or upper limb muscles. Once again, this was considered not to be diagnostic for ALS. Her EDB and thenar MUNE however continued to drop and she developed upper and lower limb weakness and became wheelchair bound. She subsequently died with respiratory failure in August 2002.

7.5.2 Case study 2

A 43-year-old sister of case 1. She had the same strong family history of ALS, with a point mutation in SOD1 gene at val148gly. Her pedigree is shown in Figure 11. She was asymptomatic at the time of recruitment with a normal neurological examination, and no evidence of wasting, weakness or fasciculation. Her right and left thenar MUNE's remained stable for the first 2½ years of the study at around 115-120 motor units. Progress MUNE results are shown in Table 5 and Figures 14.

Months pre and post weakness 1st detected	**-42**	**-40**	**-37**	**-29**	**-20**	**-10**	**0**	**+11**	**+21**	**+27**
Date of study	Oct-98	Dec-98	Mar-99	Nov-99	Jul-00	Jan-01	Nov-01	Oct-02	Aug-03	Feb-04
R Handgrip	60	60	65	65	60	70	65	65	65	65
R Thenar MUNE	111	111	117	119	120	114	96	97	86	85
L Handgrip	60	55	60	65	63	65	65	60	60	60
L Thenar MUNE	117				119	111	89	86	79	81
R EDB power	5/5	5/5	5/5	5/5	5/5	5/5	4+/5	4+/5	4+/5	4+/5
R EDB MUNE	104	111	119	108	104	92	71	75	75	65
L EDB power	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
L EDB MUNE	112						89	80	80	81

Table 5. Case 2 progressive handgrip, dorsiflexion power and thenar and EDB MUNE results

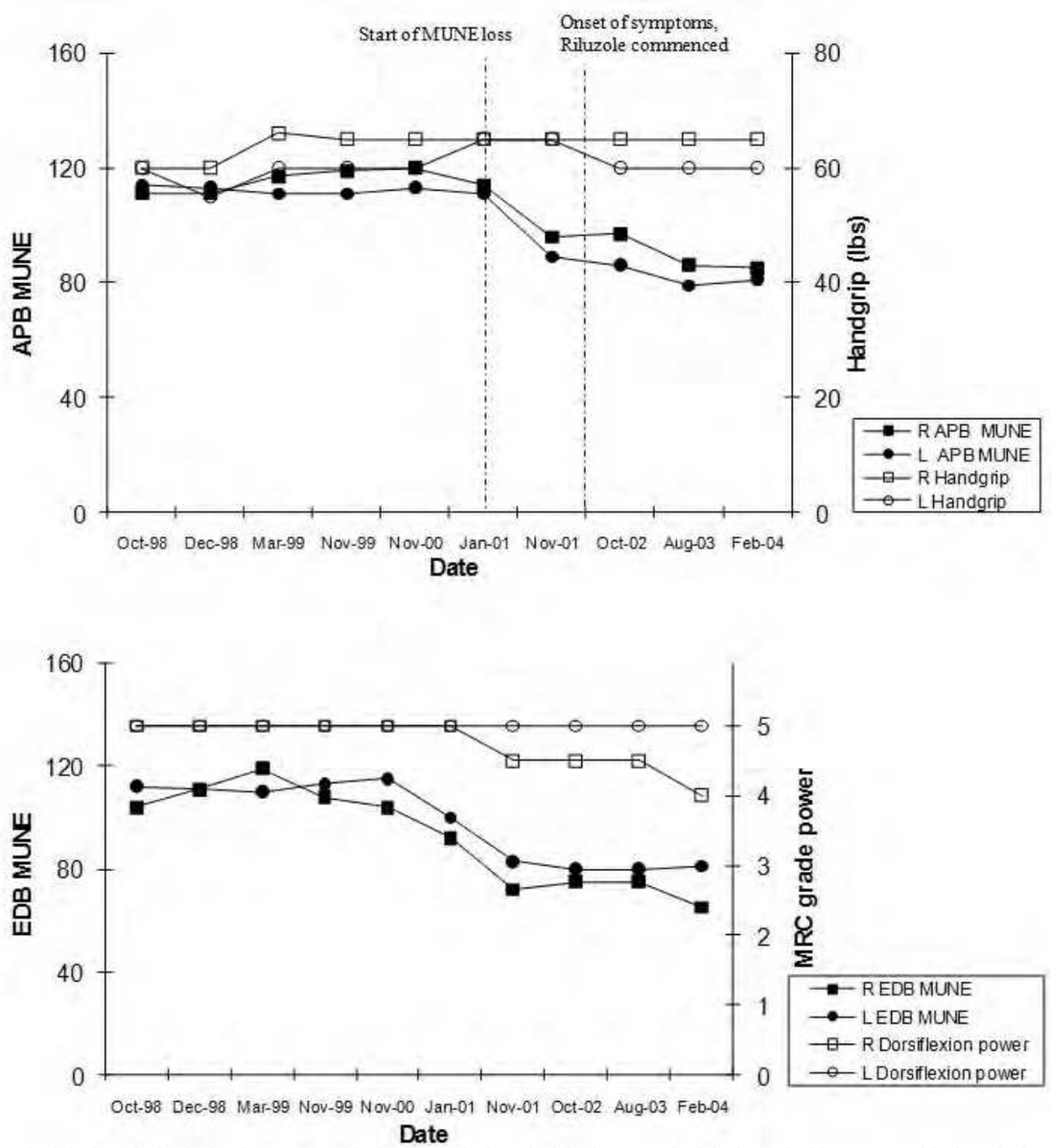


Fig. 14. Progressive results of case 2 showing the change in APB and EDB motor unit estimates over time in relation to handgrip strength and power. There is a reduction of APB and EDB MUNE even though strength has remained stable.

Over the next 6 months, there was a reduction in her right thenar MUNE to 96 (20%) and her left thenar MUNE to 89 (19%), with no detectable weakness. Her right EDB MUNE also dropped from 111 to 92 (17%), but she only had detectable weakness 10 months later of MRC grade 4+/5 in right dorsiflexors, at which time her right EDB MUNE had dropped further to 71 motor units (35%). The left EDB MUNE also dropped from a baseline of 112 (2 years previously) to 89 (20%), but with no detectable weakness.

An independent neurologist performed needle EMG examination, which showed high amplitude motor units with reduced recruitment in vastus medialis, tibialis anterior and

extensor carpi radialis longus, bilaterally but no fibrillation potentials were seen. It was felt that these changes were not enough to make the diagnosis of ALS.
In view of her strong family history, a presumed diagnosis of familial ALS was made and she was commenced on Riluzole in February 2002.
Over the next 3 years, her EDB MUNE results have stabilised. Her weakness has not progressed significantly. In February 2004, she still had MRC grade 4+/5 power of her right dorsiflexors and no symptomatically apparent weakness in her left dorsiflexors or upper limbs.

7.5.3 Case study 3

A 68 year-old mother of case 1 and 2. She had the same family history of ALS, with a point mutation in SOD1 gene at val148gly. Her pedigree is shown in Figure 12. She was also asymptomatic at the time of recruitment. Her right and left thenar MUNE's remained stable at around 130 motor units. Due to her age, she was not followed as regularly as her daughters.
On her review in November 2001, there had been a reduction of her right thenar MUNE to 98 (23%) and her left thenar MUNE to 98 (25%), with no detectable weakness. Her right EDB MUNE also dropped from 147 to 106 (28%), but she did not have any detectable weakness. She subsequently developed voice change 6 months later in May 2002, and died of respiratory failure in August 2002. Progress MUNE and handgrip results are shown in Table 6.

Months pre and post weakness 1st detected	**-43**	**-41**	**-38**	**-6**	**0**	**+3**
Date of study	Oct-98	Dec-98	Mar-99	Nov-01	May-02	Aug-02
R Handgrip	55	55	55	55	Voice	Died
R Thenar MUNE	127	135	130	98	Change	
L Handgrip	60	50	50	45		
L Thenar MUNE	130			125		
R EDB power	5/5	5/5	5/5	5/5		
R EDB MUNE	147	153	150	106		

Table 6. Case 3 progressive handgrip, dorsiflexion power and thenar and EDB MUNE results

7.5.4 Case study 4

A 48-year-old lady with a strong family history of ALS. This family had a point mutation in SOD1 gene at glu100gly. At the time of recruitment, the subject was asymptomatic, with a normal neurological examination and no evidence of wasting, weakness or fasciculations. Progress MUNE results are shown in Table 7.
The MUNE results remained stable over the first 2½ years of the study. Her right EDB MUNE results remained stable at around 120 motor units. It was then noted that her left EDB MUNE had dropped from 118 to 64 (46% reduction) and her right thenar MUNE had also dropped from 153 to 123 (20% reduction). She did not have any detectable weakness of her upper or lower limbs. Needle EMG showed neurogenic changes, but not sufficient to fulfil the criteria for ALS.

She subsequently developed left lower leg weakness with inversion and eversion of MRC grade 3/5 and dorsiflexion and plantarflexion 4/5, 6 months later. Her disease progressed rapidly and died within the 4 months of diagnosis, in March 2002 of respiratory failure.

Months pre and post weakness 1st detected	**-33**	**-30**	**-25**	**-14**	**-6**	**0**	**+4**
Date of study	Apr-98	Jul-98	Dec-98	Nov-99	Apr-01	Oct-01	Mar-02
R Handgrip	60	66	70	80	60	70	Died
R Thenar MUNE	149	144	142	151	153	123	
L Handgrip	50	60	65	60	55	58	
L Thenar MUNE	128						
R EDB power	5/5	5/5	5/5	5/5	5/5	5/5	
R EDB MUNE	126	132	137	119	118	118	
L EDB power	5/5	5/5	5/5	5/5	5/5	3/5	
L EDB MUNE	123			118	64	42	

Table 7. Case 4 progressive handgrip, dorsiflexion power and thenar and EDB MUNE results

7.5.5 Case study 5

A 44-year-old man with a strong family history of ALS and a 2nd cousin once removed of case 4. His family also had a point mutation in SOD1 gene at glu100gly. At the time of recruitment, the subject was asymptomatic, with a normal neurological examination and no wasting, weakness or fasciculations. Progress MUNE results are shown in Table 8.

Months pre and post weakness 1st detected	**-50**	**-48**	**-44**	**-35**	**-22**	**-8**	**0**
Date of study	May-98	Jul-98	Nov-98	Aug-99	Sep-00	Nov-01	Jul-02
R Handgrip	115	110	120	115	110	115	120
R Thenar MUNE	127	117	107	116	138	116	121
L Handgrip	100	100	105	105	105	100	100
L Thenar MUNE	135					122	120
R EDB power	5/5	5/5	5/5	5/5	5/5	0/5	0/5
R EDB MUNE	125	114	129	106	115	0	0
L EDB power	5/5	5/5	5/5	5/5	5/5	5/5	4/5
L EDB MUNE	127				114	36	0

Table 8. Case 5 progressive handgrip, dorsiflexion power and thenar and EDB MUNE results

The MUNE results remained stable over the first 3 years of the study. The right EDB MUNE dropped from 115 down to being not recordable. Thenar MUNE remained stable at about 130 motor units. He had occasional fasciculations in the right quadriceps region, weakness of the right quadriceps and MRC grade 0/5 weakness of right dorsiflexion. He was unsure

as to when weakness developed. Needle EMG showed changes of active and chronic denervation limited to the right quadriceps muscle, which was not considered diagnostic for ALS.

Over the next 6 months, there was a reduction of the left EDB MUNE as well from 114 to 36 (68% reduction), but with no detectable weakness. In July 2002, 8 months later, he was reviewed by his neurologist and was found to have only slightly reduced left ankle power with MRC grade 4/5 and weakness of knee flexion bilaterally. He progressed rapidly after that and by October 2002 had bilateral lower limb weakness to a point were he was unable to stand without assistance and became wheelchair bound. He commenced Riluzole, in May 2003, but over the next year there was progression of upper limb weakness. Currently, his forced lung capacity is around 30% and he is using BiPAP ventilation at night and receives PEG feeding.

8. Conclusion

Motor neurone disease (MND) is a group of fatal, progressive neurodegenerative disorders, with an overall median survival is approximately 4.0 years from the onset of symptoms. By the time most patients with MND are aware of clinical weakness and seek review by their primary physician or neurologist, a significant proportion of motor units have already been lost. Early detection of motor neurone loss in clinically apparently unaffected muscles is therefore important to establish an early diagnosis of the condition.

Motor unit number estimates in the group of asymptomatic SOD1 mutation carriers were compared to age and sex matched family controls without the SOD1 mutation, and sporadic (non-SOD1) MND patients. There was no detectable difference in the number of thenar motor units in the group of asymptomatic SOD1 mutation carriers compared to the group of SOD1 negative family controls (thenar $p>0.46$), or population controls (thenar $p>0.70$).. In addition, there was no detectable difference in the number of EBD motor units in the group of asymptomatic SOD1 mutation carriers compared to the group of SOD1 negative family controls (EDB $p>0.95$), or population controls (EDB $p>0.50$). Symptomatic sporadic MND subjects showed a definite loss of motor units with fewer motor units compared to all other groups ($p<0.001$). There was no overlap between MUNE results in symptomatic and asymptomatic subjects.

These results indicate that the group of asymptomatic carriers of the SOD1 mutation have no significant difference in the number of motor neurones, when compared to age and sex matched controls. All carriers had a full complement of motor neurones during the asymptomatic phase, indicating that mutation carriers have normal survival of motor neurones and that symptomatic MND is not the end result of a slow attrition of motor neurones. This implies that gradual pre-symptomatic loss of motor neurones does not occur in asymptomatic SOD1 mutation carriers. This supports the observation that sudden, catastrophic loss of motor neurones occurs immediately prior to the onset of symptoms and the development of the disease, rather than a gradual attrition of motor neurones over time. These results suggest that there may be a biological trigger initiating rapid cell loss, just prior to the onset of symptoms. This observation is an important contribution to the current understanding of the pathogenesis of MND. (Aggarwal & Nicholson, 2001).

The statistical MUNE technique was used for the study. This technique has been greatly modified since its original description, but numerous studies have shown that MUNE can

change systematically in ALS patients when used by experienced technicians, even though evaluator bias needs to be taken into account. Shefner demonstrated that the statistical MUNE was unreliable in the presence of clinical weakness due to motor unit instability. The difference is that our study was performed on asymptomatic patients, without clinical weakness.

It also showed that MUNE may be used as a reliable method of pre-symptomatic detection of motor unit loss in SOD1 mutation carriers. Following 69 SOD1 family members and population controls over a 1-year period, with thenar and EDB MUNE tests repeated every 3 to 6 months, assessed the test-retest reproducibility of the technique. The mean difference between thenar MUNE results on separate occasions in asymptomatic subjects was +/- 3.6%, with a range of 0-11.7%, and +/- 4.6%, with a range of 0-15.7% in EDB MUNE. These results indicate that the reproducibility of this technique and the results achieved was high, so that individual results could be used as a baseline for serial MUNE studies. (Aggarwal, 2009).

During the course of the study, however, a significant fall in motor unit number was detected in 5 of the SOD1 mutation carriers, several months before the onset of weakness and the diagnosis of motor neurone disease (MND) being made. There was no detectable loss of motor units in the other 15 SOD1 mutation carriers or in the group of SOD1 mutation negative relatives. From the study, a threshold MUNE of less than 100 was considered to imply that symptoms were imminent.

In individual cases, there was a reduction of 68% 8 months prior, 51% 4 months prior, 46% 6 months prior, 35% 10 months prior and 28% 6 months prior to the onset of weakness. Further motor unit loss occurred as weakness progressed and the diagnosis of MND being made.

Case 1 was a 48-year-old lady from a family with a strong history of familial MND, with a point mutation in the SOD1 gene at val148gly. At the time of recruitment in October 1998, she was asymptomatic. Her MUNE results remained stable over the first 2½ years, after which her left EDB MUNE dropped by 51%, and she only had detectable weakness of her left foot 4 months later with wasting and weakness of the anterior compartment muscles of her left leg of MRC grade 2-3/5. Over the next 6 months, her right EDB MUNE dropped by 56%, but she only developed right foot weakness 3 months later in June2001.

Her 43-year-old sister also showed a reduction in MUNE prior to the onset of symptoms. About 3 years into the study, there was a reduction in her right thenar MUNE to 96 (20%) and her left thenar MUNE to 89 (19%), with no detectable weakness. Her right EDB MUNE also dropped by 17%, but she only had detectable weakness 10 months later of MRC grade 4+/5 in right dorsiflexors, at which time her right EDB MUNE had dropped by a total of 35%. In view of her strong family history, a presumed diagnosis of MND was made and she was commenced on Riluzole in February 2002. Over the last 2 years, her EDB MUNE have not shown any decline. Her weakness has not progressed significantly, as on her last review in February 2004, she still had MRC grade 4/5 power of her right dorsiflexors and no clinically apparent weakness in her left dorsiflexors or upper limbs. It is possible that since "treatment" was commenced prior to the loss of a significant number of motor neurones, this may have slowed down the progression of the disease in this individual case. (Aggarwal & Nicholson, 2002).

Her mother also had a detectable reduction right thenar MUNE of 23% and left thenar MUNE of 25%, with no clinically apparent weakness. Her right EDB MUNE also dropped by 28%, with no detectable weakness. She subsequently developed bulbar symptoms 6 months and died of respiratory failure.

Case 4 was a 48-year-old lady with a strong family history of MND and a point mutation in SOD1 gene at glu100gly. At the time of recruitment, she was asymptomatic and her MUNE results remained stable over the first 3 years of the study. It was then noted that her reduction of her left EDB MUNE of 46% reduction, but with no detectable weakness. She subsequently developed left lower leg weakness of MRC grade 3/5 about 6months later.
Finally, her 2nd cousin, once removed, is a 44-year-old man who also had a point mutation in SOD1 gene at glu100gly. His MUNE results also remained stable over the first 3 years of the study. The right EDB MUNE dropped from 115 down to being not recordable over a 12-month period. At that time, he had occasional fasciculations in the right quadriceps region and MRC grade 0/5 weakness of right dorsiflexion. He was unsure as to when the weakness had developed. Over time, his left EDB MUNE has reduced by 68%, but his left ankle power remained normal until 8 months later, when it reduced slightly to MRC grade 4/5.
This study also shows that there can be substantial loss in MUNE and still have an essentially normal EMG with minimal signs of acute denervation or motor unit potential remodelling, as one would expected that at a minimum, the muscles with transiently reduced MUNE numbers should have reduced recruitment during EMG studies.
Four of the five SOD 1 mutation carriers who had a pre-symptomatic loss of motor neurones had needle EMG studies performed by an independent neurologist, which showed neurogenic changes but not sufficient to fulfil the criteria to make the diagnosis of MND. All had a reduction in MUNE at the time of the EMG study of which the independent neurologist was not aware. This implies that MUNE may be a more reliable and sensitive method for diagnosing MND than needle EMG. MUNE can be used as a non-invasive method of predicting impending decline in motor neurones and estimating the rate of neuronal death in asymptomatic subjects. This indicates that loss of motor neurones is detectable in the pre-symptomatic phase and this loss was detectable before significant needle EMG changes of pathology occur. McComas showed that patients with substantial chronic denervation could maintain normal muscle twitch tension until loss of about 70-80% of motor units, before collateral reinnervation was unable to provide functional compensation, and this is the probable explanation of this finding.
As MUNE is a measure of the primary pathologic process of motor neurone loss and can identify that the number of motor units are reduced, even in the presence of a non-diagnostic needle EMG. Needle electromyography may reveal evidence of chronic reinnervation, but provides little direct evidence to the extent of motor neurone and axonal loss.
This lack of corroboration with needle EMG in the pre-symptomatic stage requires a paradigm shift in the traditional concept that needle EMG is the "gold" standard for the diagnosis of ALS. We are aware that traditional neurologists and neurophysiologists will find this difficult to accept, as it would be expected that at a minimum, the muscles with transiently reduced MUNE numbers should have reduced recruitment during EMG studies. It is hard to understand physiologically how there can be substantial loss in MUNE and still have normal EMG with no signs of acute denervation or motor unit potential remodelling. These cases clearly indicate that loss of motor neurones is detectable in the pre-symptomatic phase and this loss was detectable before significant needle EMG changes of pathology occur. Even though some may argue that a reduction in MUNE cannot be used to support or diagnosis FALS, once changes occur on conventional EMG studies, the window of opportunity to influence the progression of this condition has been missed.

Maximum isometric grip strength using the Jamar hydraulic dynamometer also does not correlate with the number of functional motor neurones in thenar group of muscles as measured using the statistical method of MUNE, indicating that MUNE is a more sensitive test than MVIC for monitoring disease progression in MND. It has also been shown that MUNE is able to identify deterioration in functional motor units before handgrip maximal voluntary isometric contraction (MVIC).

This confirms McComas' observation that patients with substantial chronic denervation could maintain normal muscle twitch tension until loss of about 70-80% of motor units occurs. This suggests that handgrip MVIC is not as sensitive as thenar MUNE for monitoring disease progression, as it is unable to detect early motor neurone loss due to the presence of compensatory mechanisms. The surviving motor neurones enlarge their territories, through collateral sprouting (reinnervation) until late in the disease, when collateral reinnervation is no longer able to provide full functional compensation. Thenar MUNE however does examine all of the motor units that are involved in handgrip MVIC, as forearm flexors and ulnar-innervated muscles are involved in the generation of handgrip MVIC. It also confirms Felice's study which showed that in patients with MND, changes in thenar MUNE was the most sensitive outcome measure for following disease progression, when compared to other quantitative tests, such as CMAP, isometric grip strength, forced vital capacity and Medical Research Council manual muscle testing.

As motor neurone loss once it occurs is rapid and precipitous, any potential treatment will need to be given early to SOD1 mutation carriers. Once the disease progresses, resulting in functional impairment and disability, restorative treatments to replace lost motor neurones becomes less feasible. To date there have been a number of drugs which have undergone clinical trials in MND, for which there is no evidence of benefit. These include creatinine, high dose vitamin E, Gabapentin and nerve growth factors such as brain derived neurotrophic factor and insulin-like growth factor-1. If effective treatment for MND were to be developed to arrest the process of degeneration, therapies aimed at preserving functional motor neurones would be more feasible. This requires the ability to be able to identify individuals at risk of developing the disease, which currently are SOD1 mutation carriers.

Currently, the only effective approved treatment for MND is Riluzole, which has a neuroprotective role, possibly due to pre-synaptic inhibition of glutamate release. Riluzole is an anti-glutamate agent that has been approved for the treatment of patients with amyotrophic lateral sclerosis in most countries. There have been a least three large randomised trials involving hundreds of patients that have been unable to show that Riluzole is a disease altering agent nor does it have any restorative reports.

In one of the cases in the study, Riluzole was commenced once she developed mild weakness. At the time, there was a slight reduction in MUNE, but conventional needle EMG examination did not fulfil the criteria to make the diagnosis of MND. In view of her strong family history and positive genetic testing, a presumed diagnosis of MND was made. Since commencing Riluzole there has been no significant reduction in her EDB MUNE over the last 2 years, and her weakness of right dorsiflexors has only progressed marginally from MRC grade 4+/5 to 4/5 power. It is possible that since "treatment" was commenced prior to the loss of a significant number of motor neurones, this may have slowed down the progression of the disease in this individual case. Early in the course of ALS, the rate of cell death is low as the amount of neuronal damage caused by the mutation is small. As the amount of intracellular damage increases, a critical threshold is reached, which overwhelms

cellular homeostasis, resulting in rapid apoptosis and cell death. The increase in MUNE numbers may be either due to reinnervation of the damaged muscle or repair of poorly functioning synapses, at the early stage of the disease, without resulting in a change in CMAP.

We would argue that previous trials have all be performed in the symptomatic phase of the disease when 70-80% of motor units have already been lost, rather than in the pre-symptomatic phase of the disease, when the therapeutic benefit might change, as "treatment" is commenced prior to significant motor neurone loss occurring and therefore, the progression of disease can be slowed down. MUNE numbers are believed to reduce because of remodelling of the motor unit and in our study, the compound muscle action potential amplitudes (CMAP) were retained as early in the course of the disease, the rate of cell death is low. The increase in MUNE numbers may be either due to reinnervation of the damaged muscle or repair of poorly functioning synapses, at the early stage of the disease, without resulting in a change in CMAP.

This longitudinal study showed that it was possible to detect loss of motor neurones in the pre-symptomatic stage of MND in humans. This study provided further evidence that considerable motor neurone loss occurred just before the onset of symptoms or weakness. (Aggarwal, 2009).

This study indicates that SOD1 mutation carriers have normal survival of motor neurones, with as carriers had a full complement of motor neurones during the asymptomatic phase. Significant pre-symptomatic loss of motor neurones did not occur in asymptomatic SOD1 mutation carriers. Sudden and widespread motor neurone death occurs at the time development of the symptomatic symptoms, rather than life-long motor neurone loss. Sudden, catastrophic and multifocal loss of motor neurons occurs immediately prior to the onset of symptoms and the development of MND. This suggests that there may be a biological trigger initiating rapid cell loss, just prior to the onset of symptoms, rather than life-long motor neurone loss. Also, if the trigger initiating motor neurone loss can be identified, it may be possible to prevent motor neurone loss in familial ALS and develop treatments for sporadic MND. The mutant SOD1 protein itself cannot be the trigger, as it is constantly expressed. There may however be a gradual accumulation of a toxic product, possibly SOD1, which has changed into a new toxic conformation or aggregate, resulting in neuronal damage. The possibility of an individual neuron undergoing apoptosis increases as damage accumulates . This cumulative damage may be due to oxidative stress, resulting in disruption of the cellular structure and function.

Neurofilament heavy polypeptide (NF-H) is an abundant stable cytoplasmic protein located in neuronal cells in large axons and may be used as a cell type marker. Abnormal accumulation of NF-H in motor neurones is associated with ALS, but it is unclear to what extent these contribute to human disease. Analysis of blood serum markers looking for increased levels of NF-H was not performed in this study, but would be interesting to be done in the future to the compare levels of NF-H in the carriers.

The results of this study indicate that the risk of cell death probably remains constant throughout life of the neurone and that cell death occurs randomly in time and is independent of that of any other neurone. This suggests a "one-hit" biochemical phenomenon in which the mutation imposes an abnormal mutant steady state on the neurone and a single catastrophic event randomly initiates cell death and apoptosis. Early in the course of MND, the rate of cell death is low as the amount of neuronal damage caused

by the mutation is small. The delay in clinical onset was thought to reflect the gradual accumulation of damage within the neurones, as a result of the mutation, which ultimately overwhelms cellular homeostasis leading to cell death. The living mutant neurons function very well for years or decades but the probability that an individual neurone undergoes apoptosis increases as damage accumulates within it. A mutant neurone in an older patient will have accumulated a greater amount of damage and will therefore be more likely to die than in a younger patient. Consequently, early in the course of disease, the chance of a cell containing a sufficient amount of damage to initiate apoptosis is small, and the rate of cell loss is correspondingly low. The mutant neurones appear to function normally for decades, with weakness only occurring once apoptosis and cell death occurs due to a gradual accumulation of damage within the cell. Therapies aimed at preserving motor neurones may be more feasible than trying to replace lost motor neurones. A number of treatment or preventative strategies arise, such as measures to diminish SOD1 aggregation or interactions to specifically reduced apoptosis in motor neurones. As motor neurone loss at this stage is rapid and precipitous, any potential treatment will need to be given much earlier in SOD1 mutation carriers.

Determining the mechanism by which mutations in the Cu/Zn superoxide dismutase (SOD1) gene triggers the destruction of motor neurones causing MND remains unknown. At present, the favoured hypothesis is that the mutation causes disease as a result of a toxic gain of function by the mutant SOD1 provoking selective neurotoxicity, probably disrupting the intracellular homeostasis of copper and/or protein aggregation. However, as the amount of intracellular damage increases, the chance that a cell will die also increases. This cumulative damage may be due to oxidative stress, in which an imbalance between the production of reactive oxygen species and cellular antioxidant mechanisms results in chemical modifications of macromolecules, thereby disrupting cellular structure and function. It is possible that the high metabolic activity in motor neurones, combined with the toxic oxidative properties of the mutant SOD1, causes massive mitochondrial vacuolation in motor neurones, resulting in degeneration, earlier than other neurones, triggering the onset of weakness. Prominent cytoplasmic intracellular inclusions in motor neurones and within astrocytes surrounding them developed by the onset of clinical disease and in some cases represented the first pathological sign of disease. These aggregates increased in number as the disease progressed. This indicates that the mutant SOD1 toxicity is mediated by damage to mitochondria in motor neurones and this damage triggers the functional decline of motor neurones and the clinical onset of symptoms. The absence of motor neurone death in the early stages of the disease indicates that the majority of motor neurones could be rescued after early clinical diagnosis.

Regular follow-up of SOD1 carriers with MUNE may lead to early diagnosis, creating an opportunity for future novel approaches and therapies aimed at preserving motor neurones rather than replacing lost motor neurones. If the trigger initiating motor neurone loss can be identified, it may be possible to prevent motor neurone loss in familial ALS. At this stage, detecting the onset of motor neurone loss in asymptomatic individuals will identify those who may benefit from early institution of an active management program to improve their quality of life, until more effective treatment modalities become available for this devastating condition This observation is an important contribution to the current understanding of the pathogenesis of MND, as it shows that motor neurone disease does not seem to be the end result of slow attrition of motor neurones. MUNE may be able to be used

as a method of pre-symptomatic testing of individuals who on genetic testing are SOD1 mutation carriers. Regular follow-up of SOD1 carriers with MUNE may lead to early diagnosis, creating an opportunity for future novel approaches and therapies aimed at preserving motor neurones rather than replacing lost motor neurones.

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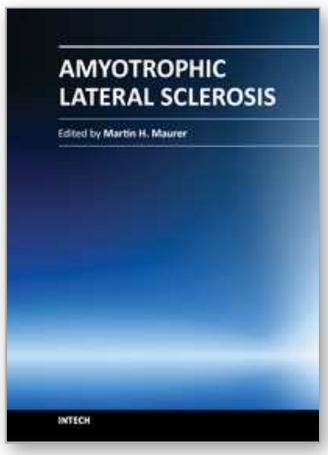
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Amyotrophic Lateral Sclerosis

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Though considerable amount of research, both pre-clinical and clinical, has been conducted during recent years, Amyotrophic Lateral Sclerosis (ALS) remains one of the mysterious diseases of the 21st century. Great efforts have been made to develop pathophysiological models and to clarify the underlying pathology, and with novel instruments in genetics and transgenic techniques, the aim for finding a durable cure comes into scope. On the other hand, most pharmacological trials failed to show a benefit for ALS patients. In this book, the reader will find a compilation of state-of-the-art reviews about the etiology, epidemiology, and pathophysiology of ALS, the molecular basis of disease progression and clinical manifestations, the genetics familial ALS, as well as novel diagnostic criteria in the field of electrophysiology. An overview over all relevant pharmacological trials in ALS patients is also included, while the book concludes with a discussion on current advances and future trends in ALS research.

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