

## SHORT REPORT

# Detection of preclinical motor neurone loss in SOD1 mutation carriers using motor unit number estimation

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**Objective:** To determine the pattern of motor neurone loss in amyotrophic lateral sclerosis (ALS). In particular, to determine whether there is a gradual life long presymptomatic motor neurone loss or, alternatively, a sudden catastrophic loss just before the onset of symptoms.

**Method:** The statistical motor unit number estimation (MUNE) technique was used in a longitudinal study of 19 asymptomatic carriers of the Cu, Zn superoxide dismutase 1 (SOD1) gene. MUNE results were compared with those of 34 age and sex matched SOD1 negative family controls and 23 population controls. Motor neurone loss was also estimated in 12 patients with sporadic ALS. 84 subjects (43 male and 41 female patients) with an age range from 16–73 years were followed up over three years, both clinically and by MUNE, every six months.

**Results:** In 2 of the 19 mutation carriers, there was a sudden reduction in MUNE several months before the onset of weakness. The patients with symptomatic sporadic ALS also had a reduced MUNE, but there was no detectable loss of motor neurones in the remainder of the subjects.

**Conclusion:** MUNE can be used to detect preclinical loss of motor units in familial ALS. Normal numbers of motor neurones were maintained in 17 SOD1 mutation carriers over the three year period. There was an abrupt loss of motor neurones just before the onset of symptomatic weakness in two SOD1 mutation carriers. These results suggest that some form of trigger may initiate rapid cell loss and death of motor neurones just before the onset of symptoms.

Amyotrophic lateral sclerosis (ALS) is a progressive degenerative disease of upper and lower motor neurones, generally leading to death. Ten per cent of cases are familial and 20% of these families have point mutations in the Cu, Zn superoxide dismutase 1 (SOD1) gene.<sup>1</sup> Normally, there is little motor neurone loss before the seventh decade of life. After this, the number of neurones is reduced by approximately 3% per year.<sup>2</sup> Patients with substantial chronic denervation can maintain normal muscle twitch tension by compensatory mechanisms until about 70–80% of motor units are lost.<sup>3</sup> It has therefore been suggested that there may be a long preclinical period of motor neurone loss that is hidden by compensation before the onset of symptoms.<sup>4</sup> The pattern of motor neurone loss may be either a gradual depletion of motor neurones over time, until a critical threshold is reached when the disease becomes symptomatic, or maintenance of normal numbers of motor neurones until the disease commences with sudden, rapid, widespread cell death of motor neurones with the onset of symptoms.<sup>5</sup>

Presymptomatic loss of motor neurones has been recently identified before the onset of signs of the disease in SOD1 mice. This loss was biphasic with initial loss preceding any signs, followed by a period of stabilisation and then gradual loss at the time of weakness to death.<sup>6</sup> In our previous study,

we detected no difference in the number of motor units between SOD1 mutation carriers and their SOD1 negative family controls.<sup>7</sup> 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 clinical features. This implies that the disease is not the end result of the slow attrition of motor neurones.<sup>8</sup>

To determine the time course of motor neurone loss before the onset of symptoms, we performed a longitudinal study of 19 at risk SOD1 mutation carriers with no neurological symptoms or signs and followed them up for three years.

## METHODS

Family members of known SOD1 positive families were contacted. Eighty eight subjects (45 male and 43 female patients) gave informed consent. There were 19 asymptomatic SOD1 mutation carriers, 34 age and sex matched SOD1 negative family controls, 23 population controls, and 12 patients with sporadic ALS. Four dropped out due to poor tolerability, leaving 84 patients who were followed up over a three year period.

Median and peroneal motor conduction studies were performed, including F wave and repetitive stimulation. Concentric needle and quantitative electromyography (EMG) was also performed on the initial assessment, sampling the tibialis anterior, abductor pollicis brevis (APB), deltoid, and first dorsal interosseous muscles. Needle EMG studies were subsequently omitted because of volunteers' reluctance to continue to participate in the research study. Single fibre EMG was not performed. The statistical technique of motor unit number estimation (MUNE)<sup>9</sup> was performed on APB and extensor digitorum brevis (EDB) every six months. Subjects participated without knowledge of their mutation status and on the understanding that this would not be disclosed to them. The neurologist performing the tests also had no knowledge of their mutation status. The study was approved by the Central Sydney Area Health Service ethics review committee.

In the group of 19 asymptomatic (preclinical) SOD1 mutation carriers, five were found to have a point mutation in exon 4, codon 100, GAA to GGA — Glu100Gly; five were found to have a point mutation in exon 4, codon 113, ATT to ACT — Ile113Thr; and nine were found to have a point mutation in exon 5, codon 148, GTA to GGA — Val148Gly.

Maximum isometric grip strength was measured with the Jamar hydraulic dynamometer (Sammons Preston Inc, Bolingbrook, Illinois, USA). Clinical neurological examination was performed, with power recorded according to the Medical Research Council grading system.

**Abbreviations:** ALS, amyotrophic lateral sclerosis; APB, abductor pollicis brevis; EDB, extensor digitorum brevis; EMG, electromyography; MRC, Medical Research Council; MUNE, motor unit number estimation; SOD1, Cu, Zn superoxide dismutase 1

**Table 1** Abductor pollicis brevis and extensor digitorum brevis and mean motor unit number estimates (MUNE)

	Abductor pollicis brevis		Extensor digitorum brevis	
	No	MUNE (range)	No	MUNE (range)
Population controls	23	148 (115–254)	12	138 (119–169)
SOD1 negative family controls	34	138 (106–198)	32	134 (107–180)
SOD1 mutation carriers	19	144 (109–199)	14	136 (111–187)
Patients with sporadic ALS	12	45 (5–84)	9	70 (8–82)

SOD1, Cu, Zn superoxide dismutase 1.

**RESULTS**

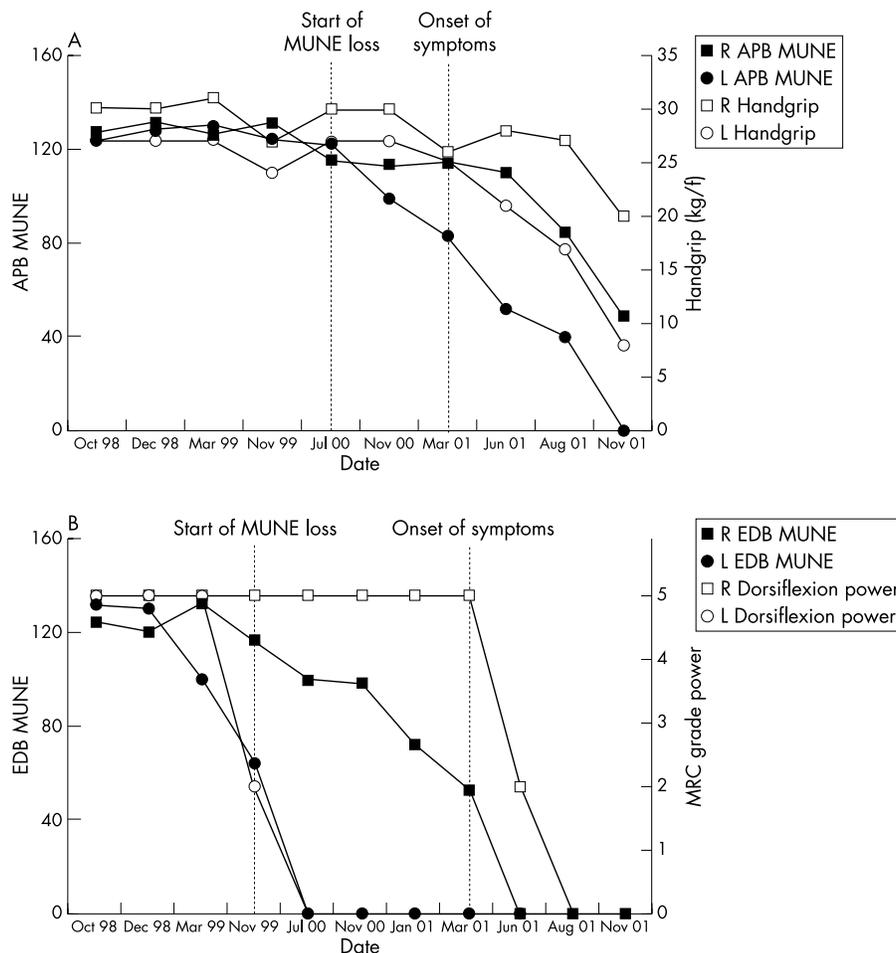
In our previous study,<sup>7</sup> there was no detectable difference in the number of motor units in 19 SOD1 mutation carriers as a group compared with their 34 SOD1 negative family controls (APB  $p > 0.46$  and EDB  $p > 0.95$ ) or with 23 population controls (APB  $p > 0.70$  and EDB  $p > 0.50$ ). The 12 patients with symptomatic ALS had fewer motor units than all other groups ( $p < 0.001$ ; table 1). Test-retest correlation was high, with Pearson correlation coefficients of 0.93 for APB MUNE and 0.78 for EDB MUNE.

There was no significant loss of motor units in 17 of the 19 SOD1 mutation carriers and the 32 population controls over the three years of the study. The average difference between MUNE results on separate occasions for the same person was  $\pm 5\%$ . In two SOD1 mutation carriers, there was a detectable

reduction of 51% in one and 37% in the other before the onset of clinical symptoms.

**Case 1**

A 48 year old woman with a strong history of familial ALS (Val148Gly) was asymptomatic at the time of recruitment, with a normal neurological examination. She had no evidence of wasting, fasciculations, or weakness. Initial nerve conduction studies were normal, as was concentric needle and quantitative EMG of the upper and lower limb muscles. Her left EDB MUNE dropped from 130 to 100 (23% reduction) within six months (fig 1). At the time, she had no detectable weakness. Over the next six months, her left EDB MUNE dropped further to 64 (total reduction of 51%), when she had wasting and weakness of the anterior compartment muscles



**Figure 1** Progressive results of case 1 showing the change in (A) abductor pollicis brevis (APB) and (B) extensor digitorum brevis (EDB) motor unit number estimates (MUNE) over time in relation to handgrip strength and power. APB and EDB MUNE are reduced before the onset of weakness. L, left; MRC, Medical Research Council; R, right.

of her left leg of MRC grade 2/5. Proximal muscles, upper limb, and right leg muscles were normal. Tone was normal but her upper and lower limb deep tendon reflexes were brisk. Plantar responses were flexor. Her right EDB MUNE and right APB MUNE dropped over the next months, even though there was no detectable weakness of these limbs. Her handgrip strength remained about 30 kilograms force. Concentric needle EMG showed evidence of active denervation in both tibialis anterior, left tibialis posterior, left gastrocnemius, left medial hamstring, and left L5/S1 paraspinal muscles. There were no changes in the right vastus medialis, biceps brachialis, triceps, first dorsal interosseous, deltoid, extensor carpi radialis, and left deltoid muscles and the tongue. Magnetic resonance imaging of her lumbar spine showed degenerative disc disease of L4-5 and L5-S1, but with no evidence of neural, in particular S1, compromise.

Her right EDB MUNE continued to drop over the next six months to 53 motor units. Her left APB MUNE also dropped significantly to 83 motor units. She became wheelchair bound with bilateral lower limb weakness, requiring assistance with transfers.

### Case 2

A 43 year old sister of case 1 was also followed up over three years. She had the same very strong family history of ALS, with a point mutation in the SOD1 gene at Val148Gly and was asymptomatic at the time of recruitment. Her right and left APB MUNE remained stable at around 115–120 motor units for the first 2.5 years of the study. Over the last six months of the study, her right APB MUNE dropped to 96 (20%) and her left APB MUNE dropped to 89 (19%). Her right EDB MUNE also started to drop from 104 to 92 (12%). Weakness of MRC grade 4+/5 in the right tibialis anterior was only apparent three months later, when her right EDB MUNE had dropped further to 72 motor units (37%). The left EDB MUNE had also dropped from a baseline of 112 (two years previously) to 89 (20%) but there was no detectable weakness. There were no upper motor neurone signs on clinical examination. Lower limb motor and sensory conduction studies were normal. Needle EMG was abnormal in the vastus medialis, tibialis anterior, and extensor carpi radialis longus bilaterally, with excessively high amplitude motor units with reduced recruitment. No spontaneous activity was present apart from very few fasciculations in the right vastus medialis and tibialis anterior. She remained independent with only mild lower limb weakness.

### DISCUSSION

During the course of this three year longitudinal study, we were able to detect motor neurone loss before the onset of symptoms in two of 19 SOD1 mutation carriers. All carriers had a full complement of motor neurones during the asymptomatic phase, indicating that SOD1 mutation carriers have normal survival of motor neurones until sudden catastrophic rapid cell death of motor neurones occurs, coinciding with the onset of clinical features. Previous clinical observations in patients with motor neurone disease have suggested that there may be a long preclinical phase during which the disease is not symptomatic because of a period of relative tolerance and compensation.<sup>4</sup> This is the first study to detect loss of motor neurones in the presymptomatic stage of ALS in humans. Before this, motor neurone loss had been identified only in the Gly93Ala SOD1 mouse model before the onset of signs of the disease.<sup>6</sup>

There may be a gradual accumulation of a toxic product, possibly SOD1, that is transformed into a new toxic conformation or aggregate, resulting in neuronal damage. This cumulative damage may be due to oxidative stress, resulting in disruption of the cellular structure and function. As the amount of intracellular damage increases, a critical threshold may be reached, which overwhelms cellular homeostasis, resulting in rapid apoptosis and cell death.<sup>8, 10</sup>

The mutant neurones appear to function normally for decades, with weakness occurring only once apoptosis occurs. As motor neurone loss at this stage is rapid and precipitous, any potential treatment would need to be given much earlier in SOD1 mutation carriers. Treatments 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 measures specifically to reduce apoptosis in motor neurones.

This study showed that it is possible to identify factors causing motor neurone loss before the development of symptoms. It may then be possible to develop effective preventions for familial ALS and possible treatments for sporadic ALS.

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