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Novel *SOD1* mutation discovered in atypical ALS by whole exome sequencing

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Patients with familial amyotrophic lateral sclerosis (f-ALS) typically have asymmetric onset weakness, upper motor neuron signs, absent sensory involvement and relatively rapid disease progression with fatality typically in 2–5 years of onset.¹ In contrast, distal hereditary motor neuropathies (dHMNs) and hereditary motor and sensory neuropathies type-2 (HMSN2) have symmetric length-dependent motor axonal loss and slow disease onset and progression. Hereditary motor neuropathies and motor neuron diseases have extremely diverse genetic causes,¹² and within each group, distinct phenotype–genotype correlations are uncommon for f-ALS, dHMN and HMSN2.

This report illustrates a varied phenotypic presentation from *SOD1*-associated f-ALS and illustrates whole exome sequencing (WES) as an effective approach for diagnosing genetically heterogeneous disorders with atypical phenotypes.

Case report

The proband was evaluated at ages 39, 40, 43, 65, 67 and 73 years. He had symmetrical progressive distal weakness of legs with foot drop and foot paraesthesias. Weakness progressed to his hands. Ankle reflexes were absent, and vibration sensation was reduced at the toes. Babinski and Chaddock signs were absent. From 39 to 65 years of age, he was suspected to have dHMN or HMSN. By age 67, spinobulbar muscular atrophy (SBMA) and HMSN2C were also considered in the differential diagnosis because of weakness in bulbar musculature, flaccid dysarthria and dysphagia. His reflexes became brisk at patellar and biceps brachii tendons despite the prominent weakness in the subserved muscles. At the age of 73, he has severe flaccid dysarthria and diet restriction from aspiration risk. He can transfer, stand and walk with a cane or walker. His first nerve conduction studies at 39 years

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of age were normal including peroneal, tibial and sural responses, and needle EMG showed fasciculations limited to tibialis anterior and medial gastrocnemius muscles with large motor units showing reduced recruitment. By age 67, the peroneal, tibial and median motor compound motor action potentials were absent and the ulnar motor was 4.2 mV (normal >6.0 mV) with conduction velocity 45 m/s (>51 m/s). His facial motor response was preserved 3.6 mV (normal >1.8 mV). The sensory nerve action potentials were normal with sural at 2 uV (normal >0 uV) and median at 29 uV (normal >15 uV). Needle EMG now had prominent neurogenic motor unit potentials with fibrillations without fasciculations in distal greater than proximal muscles including orbicularis oris and masseter.

The patient's affected sister had similar progressive symptoms beginning in her 30s. The first examination at our clinic when she was 59 showed her ankles and hands were weak symmetrically, although weakness was worse at the left ankle initially in her 30s. She had foot paraesthesias with vibration detection reduction in the feet. Nerve conductions and needle EMG results were similar to those of her brothers. She was also initially diagnosed with dHMN or HMSN. She progressed to quadriparesis with bulbar weakness. In her 60s, she had dysphagia and dysarthria with brisk reflexes at the patella and biceps brachii. She had neuromuscular respiratory arrest and died at the age of 66. Their father had unexplained progressive leg weakness beginning in his late 40s evolving to loss of ambulation; he died at the age of 56 from lung cancer.

During their clinical evaluations, genetic testing for various forms of dHMN2, HMSN2 and SBMA (for the brother) yielded negative results. Candidate genes tested included *TRPV4*, *BSC12*, *MPZ*, *MFN2*, *CX32* and *AR* nucleotide repeats.

Exome sequencing and data analysis

Exome sequencing was applied using SureSelect All Exon Kit V4 and HiSeq2000. Variant calls were carried out using an in-house developed pipeline incorporating multiple, large variant databases.

RESULTS

We identified a novel missense mutation in *SOD1* p.Phe64Leu (c.T193>C) tracking with disease (figure 1). This novel mutation is absent in dbSNP137, 1KGenome, BGI-Danish-200Exomes, EPS6500-Exome databases and our in-house Exome database, totalling >8000 controls. SIFT (sorting intolerant from tolerant, deleterious, p=0.02), Mutation Taster (disease causing probability score=0.999) and Polyphen2 (probably damaging PSIC:0.967) all predicted the mutation as damaging. This amino acid is highly conserved in vertebrates, in juxtaposed known mutations and at the critical metal binding site (figure 1). We also analysed other f-ALS-associated genes, only exon1 of *ATXN2* had less than 10X coverage and was examined by Sanger sequencing. Fragment analysis showed that *C9ORF72* repeat numbers were normal (<20).

DISCUSSION

This study identified a novel *SOD1* mutation in an ALS family with slow disease progression and prolonged survival highlighting the heterogeneity of phenotype-genotype correlation of f-ALS by *SOD1* mutations. Importantly, WES assisted in the genetic diagnosis. Had WES been available earlier, improved patient and family counselling would have been possible. Atypical clinical presentation and broad genetic differential hindered causative gene discovery. Both affected siblings had prolonged course (>3 decades), symmetrical length-dependent weakness, sensory symptoms and initial absence of upper

motor neuron signs. All these features have been noted to contribute to the delay in clinical diagnosis of f-ALS.¹ The eventual development of bulbar weakness, brisk reflexes in weak muscles and fatality ultimately were consistent with f-ALS diagnosis. We note that prolonged survival in f-ALS, although very rare, has been described in patients with *SOD1* mutations. One patient was reported surviving four decades³; in contrast to our patient, that patient had typical asymmetric upper and lower motor neuron components.

Mutations in *SOD1* have been associated with a broad range of clinical presentations among patients with ALS.¹ *SOD1* gene codes for a highly conserved sequence of 153 amino acids with a Cu–Zn binding motif. Remarkably, in this relatively small protein, more than 150 missense mutations have been reported in f-ALS.⁴ Additionally, in a large variant database including 6500 exomes (NHLBI-ESP6500), *SOD1* gene does not have any non-synonymous polymorphisms. *SOD1* appears to function at high fidelity and any amino acid alterations are likely to cause ALS with varied penetrance and expression.

The application of WES identified a novel *SOD1* p.Phe64Leu mutation and underlined the diagnosis of f-ALS over previously considered inherited motor predominant neuropathies. In cases where atypical clinical presentations obscure the selection of specific candidate gene or require a long list of genetic testing, WES can rapidly evaluate diverse genetic causes.⁵ As the speed of new gene discoveries continues to accelerate, the application of WES in neuromuscular diagnosis is predicted to increase.

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Figure 1.

(A) The pedigree of described family with amyotrophic lateral sclerosis and initial lower motor neuron presentation. Asterisks indicate persons who had genetic testing. (B) Chromatogram of SOD1 p.Phe64Leu (c.T193>C) mutation identified by whole exome sequencing in the proband and confirmed in his affected sister by Sanger sequencing, not present in the unaffected sister and absent in >8000 controls. (C) The position of the mutation is highly conserved. It juxtaposed the known mutations flanked by amino acids important in Cu^+ and Zn^{2+} binding for catalytic and stabilising protein functions. (D) The monomer structure of wild-type SOD1 (PDB# 2XJK) and mutant SOD1 with p.Phe64Leu generated by Swiss Model server (swissmodel.expasy.org), suggesting conformational change. The images were created by PyMol (<http://www.pymol.org>) with 64Phe and 64Leu mutation sites shown in yellow.