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The first Portuguese family with NEFL-related Charcot-Marie-Tooth type 2 disease

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CMT disease caused by *NEFL* gene mutations is rare. The mode of inheritance can be dominant or recessive and nerve conduction velocities can be normal, reduced (demyelinating) or presenting intermediate values. Two Portuguese adult related members in two successive generations were affected by peripheral neuropathy, one with a chronic ataxic peripheral neuropathy and the other with a classical Charcot-Marie-Tooth phenotype. An axonal sensorimotor peripheral neuropathy was described at neurophysiology. A missense heterozygous mutation, c.794A > G (p.Tyr265Cys), in the *NEFL* gene was found in both patients. This is the first Portuguese family reported with NEFL-related CMT type 2.

Key words: CMT type 2E, *Neurofilament light* gene mutation, *NEFL* gene, NEFL Tyr265Cys mutation

Introduction

The neurofilament light-chain polypeptide (NEFL) is a constituent of neurofilaments, the major intermediate filament of neurons and axons, playing a pivotal role in the maintenance of the cytoskeleton (1). Mutations in the neurofilament light-chain polypeptide gene (*NEFL*) are responsible for 2% of all cases of CMT (2). Dominant axonal (CMT 2E) (3) and demyelinating phenotypes (CMT 1F) (2), and rarely recessive axonal CMT (4), caused by *NEFL* gene mutations have been described. CMT caused by *NEFL* gene mutations is clinical and electrophysiological heterogenous (2, 3, 5, 6).

Herein, we report the clinical, neurophysiologic and molecular findings of the first Portuguese kindred with CMT type 2E, caused by a missense heterozygous mutation, variant c.794A > G (p.Tyr265Cys), in the *NEFL* gene.

Clinical cases

Patient 1

The patient is a 68-year-old woman, born of a second-degree consanguineous marriage. Her mother and one maternal aunt were suspected of having a similar neuromuscular condition, but were not available to examination (Fig. 1). She had a normal motor and intellectual childhood development and experienced an active professional life until retirement.

At the age of 42, she reported the beginning of gait difficulties and numbness in her feet. A few years later, the gait difficulties became worse with occasional falls and she began experiencing pain in the feet with sporadic exacerbations.

Neurological examination at the age of 66, revealed a wide-based ataxic gait, needing support in turns. Walking on heels and tiptoes was difficult, mainly due to ataxia. The Romberg sign was positive. Manual muscle examination did not reveal distal or proximal muscle weakness in the upper and lower limbs. Myotatic reflexes were abolished in the lower limbs and reduced distally in the upper limbs. There was a stocking and glove pattern of diminished tactile and pain sensation, with absent vibratory sensation in the feet and reduced in the upper limbs (10 seconds). Pseudo-athetosis was absent. No cerebellar signs were noted and cranial nerves evaluation was unremarkable. Serum vitamin B12 values were normal and there was no megaloblastic anemia.

Patient 2

The patient is a 47-year-old woman, the single offspring of a non-consanguineous marriage, daughter of Patient 1 (see Figure 1). She presented a normal motor and intellectual development in childhood. At the age of

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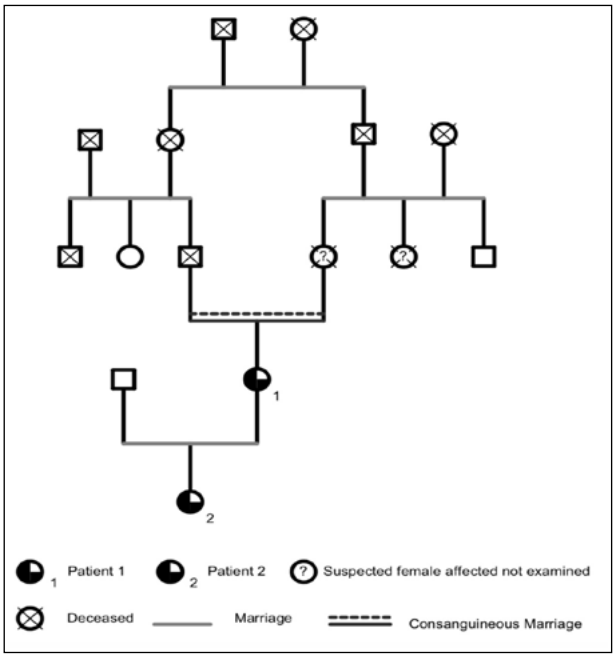

Figure 1. Pedigree of family 1.

33, she reported increasing gait difficulties, without any sensory or balance complaints. At 43 years of age, she underwent bilateral corrective orthopedic surgery of *pes cavus*.

At the age of 46, neurologic examination revealed bilateral *pes cavus*, hammer toes deformity on the left foot and an inverted champagne appearance of the legs. She walked with a steppage gait and walking on tiptoes was possible. In the lower limbs the extensor muscles of the feet were weak bilaterally (3-/5 MRC). No muscle weakness was present in the upper limbs. Myotatic reflexes were abolished throughout. Tactile and pain sensations were apparently normal and vibratory sensation was slightly reduced distally in the lower limbs (10 seconds). No cerebellar signs were observed and cranial nerves evaluation was unremarkable.

Neurophysiological assessment

In Patient 1, motor and sensory nerve conduction studies disclosed a length-dependent axonal sensorimotor neuropathy, with bilateral absent sural and peroneal motor responses (recorded in the feet). Median motor nerve conduction velocity and distal motor amplitude values were of 50 m/s and 4.6 mV, respectively. Somatosensory evoked potentials after median and tibial nerves stimulation did not show sensory responses at peripheral and central levels, which was interpreted as a result of profound peripheral nerve sensory involvement. In Patient 2, the values of median motor nerve conduction velocity and distal motor amplitude were of 56 m/s and 4 mV, respectively. Needle examination of the tibial anterior muscle showed signs of chronic denervation, with a mild reduced muscle recruitment pattern in Patient 1 and severe in Patient 2.

Molecular study

The next generation sequencing (NGS) panel for hereditary peripheral neuropathies, including CMT, was performed through a custom targeted NGS panel. Enrichment was performed by hybrid capture (exons and flanking intronic regions of the 74 target genes) and, after library preparation, the DNA library was subjected to NGS.

A missense heterozygous variant, c.794A > G (p.Tyr265Cys), was detected in the *NEFL* gene in both patients (Fig. 2).

This *NEFL* gene sequence variant was not registered in the Single Nucleotide Polymorphism (dbSNP) or the Genome Aggregation Database (gnomAD), but it is reported as a likely pathogenic variant in ClinVar database (Acession: RCV0001438101). This residue is highly conserved and bioinformatic analysis suggests that this variant is deleterious. Additionally, it has been previously reported in another family (5).

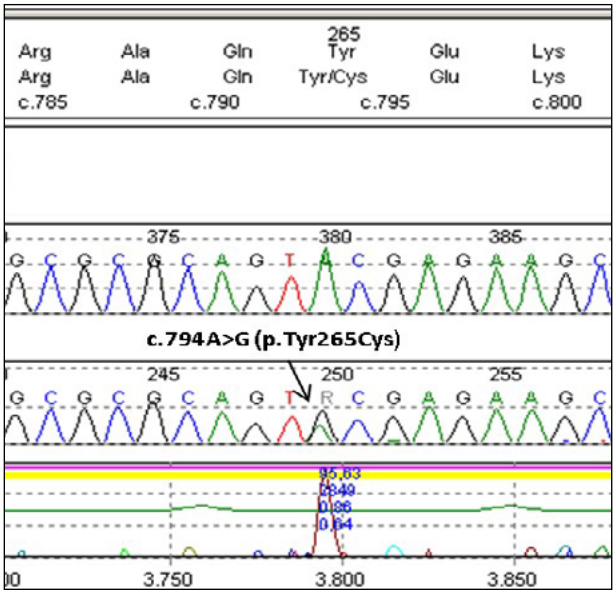

Figure 2. Electropherogram: Molecular study detected a missense heterozygous variant, c.794A > G (p.Tyr265Cys), in the *NEFL* gene in both patients (black arrow).

Discussion

Charcot-Marie-Tooth disease (CMT) is the most common inherited motor and sensory neuropathy and is divided into demyelinating (CMT1) and axonal (CMT2) forms using electrophysiological and pathological criteria. CMT1 is characterized by demyelination and slow nerve conduction velocities (NCVs), whereas CMT2 is characterized by signs of axonal regeneration and normal or slightly reduced NCVs.

To date, mutations in as many as 14 different genes have been implicated in CMT2 (9), including KIF1B (CMT2A1), MFN2 (CMT2A2), RAB7 (CMT2B), TRPV4 (CMT2C), GARS (CMT2D), NEFL (CMT2E), HSPB1 (CMT2F), MPZ (CMT2I/J), GDAP1 (CMT2K), HSPB8 (CMT2L), DNM2 (CMT2M), AARS (CMT2N), LAMIN A (AR-CMT2A) and MED25 (AR-CMT2B). Among them, mutations in MFN2 have been found in approximately 11-24.2% of CMT2 patients, whereas AARS and TRPV4 mutations were only recently identified in limited CMT2 families, and mutations in other genes were found in only a few patients.

Mutations in the neurofilament light chain polypeptide (*NEFL*) gene are present in CMT2E and CMT1F neuropathies, with variable clinical and pathological expressions. Codon 22 is one of the mutational hot spots in the *NEFL* gene. Three types of Pro22 mutations have been previously reported: Pro22Ser in CMT2E with giant axons, Pro22Thr in CMT1F and Pro22Arg in a Korean CMT1 family, associated with demyelinating neuropathy features in CMT1F. Histopathological findings showed onion bulb formations but no giant axons (10-15). Pro22 mutations may influence not only the Thr-Pro phosphorylation site by proline-directed protein kinases but also impact the structure of the NEFL protein in a different way.

We report the first Portuguese family with CMT type 2E. The identified mutation, already described in an Australian family (5), promotes an amino acid exchange of tyrosine by cysteine (Tyr265Cys) and occurs in a highly conserved residue of the *NEFL* gene. Two related affected subjects in two generations carried the mutation. Moreover, it is probable that two more family members were clinically affected by this mutation (Patient 1's mother and maternal aunt).

It has long been recognized that *NEFL* gene mutations are associated with intra-familial phenotypic variability (5, 6), which is present in this Portuguese family, with the oldest patient presenting an ataxic sensory peripheral neuropathy and the youngest one a classical CMT phenotype.

Significant inter-familial phenotypic variability regarding onset, clinical presentation and severity of the disease has already been described (3, 7, 8) and it is obvious when comparing the Australian and Portuguese families with the same *NEFL* gene mutation. The proband of the Australian family presented a more complex and severe neurological involvement, combining central and peripheral nervous system symptomatology with significant clinical disability. In the Australian and Portuguese families, the mode of inheritance was dominant and the peripheral nerve involvement was of the axonal type. Cases of peripheral nerve involvement of the demyelinating type (CMT1F) (2), as well as of the intermediate type (6), have been described, and the mode of inheritance is dominant in the majority of cases, but recessive inheritance has already been reported (4).

Some components of the peripheral nervous system can be clinically more severely affected than others and some patients present central nervous system involvement, with compromise of the pyramidal tract (5) or of the brainstem and cerebellum (6). NEFL-associated CMT nerve pathology is primarily of the axonal type, with focal accumulations of neurofilaments, axonal swellings and significant secondary demyelination (9) and electrophysiology can present axonal, demyelinating and intermediate nerve conductions values, to which correspond CMT type 2E, CMT type 1F and Intermediate NEFL-associated CMT, respectively.

Conflicts of interest

The Authors declare to have no conflict of interest.

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