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

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 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 tip toes 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
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 (Accession: 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 1.** Pedigree of family 1.

**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 (CMT2J), 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 2E. The identified mutation, already described in an Australian family (5), promotes an amino acid exchange of

Conflicts of interest

The Authors declare to have no conflict of interest.

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