A tropomyosin-receptor kinase-fused gene mutation associates with vacuolar myopathy

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A 44-year-old Caucasian man presented with a 7-year history of upper and lower limb muscle fasciculations and cramps, progressive asymmetric weakness, muscle atrophy, and length-dependent sensory loss. His parents (deceased), 3 older siblings, and 3 children had no history of neurologic symptoms, including weakness and sensory symptoms, with the exception of cramps in the mother. He was not taking any medication known to cause neuropathy or myopathy. On examination, there was right greater than left shoulder girdle muscle weakness and atrophy (Medical Research Council grades 3/5 and 4/5), intrinsic left hand muscle weakness (4+/5) with atrophy, asymmetric pelvic girdle (4/5 right, 3/5 left), and left foot dorsiflexor weakness (4+/5). Strength of neck flexor and extensors, and axial muscles, was normal. He had bilateral calf muscle atrophy and weakness as suggested by his inability to stand on toes (figure, A–C). He was areflexic. Sensory examination revealed distal vibration and pinprick deficits in the upper and lower limbs in a length-dependent fashion. He had bilateral pes cavus.

Serial measurements of serum creatine kinase ranged between 840 and 2,400 U/L (normal <336 U/L) over the preceding 4 years. MRI revealed thigh muscle atrophy (figure, D). Neurophysiologic studies demonstrated reduced motor conduction amplitudes and diffuse fibrillation and fasciculation potentials with large motor unit potentials suggestive of a motor neuronopathy. No small motor unit potentials were recorded. Sensory nerve action potentials were absent in the upper and lower limbs.

A muscle biopsy of the quadriceps, performed a year prior to presentation in our clinic, showed mixed neurogenic and myopathic changes with several fibers containing rimmed and non-rimmed vacuoles, and perivascular inflammation (figure, E–J). Targeted next generation exome sequencing (NGS) of 95 genes associated with hereditary neuropathy and motor neuron disease detected a known pathogenic heterozygous missense variant in exon 8 (c.854C>T, p.Pro285Leu) of the tropomyosin-receptor kinase-fused gene (TFG), located within a carboxy-terminal proline-glutamine (P/Q)-rich domain. NGS of 104 genes associated with myopathies (appendix e-1, links.lww.com/NXG/A118), including myopathies with rimmed vacuoles, revealed no pathogenic variants. Living relatives declined genetic testing.

Discussion

Our patient presented with a classic clinical phenotype and a known pathologic genotype caused by a heterozygous TFG mutation, along with novel histologic evidence of vacuolar myopathy. TFG mutations were first identified in Okinawa and Kansai prefecture patients with autosomal dominant hereditary motor and sensory neuropathy (HMSN-P) manifesting with proximal weakness and distal sensory loss.1 Two of these kindreds carried the same TFG mutation detected in our patient. Limited muscle pathologic findings showing neurogenic changes (fiber type
grouping) have been reported to date. Pathologic studies later demonstrated that HMSN-P is a motor neuronopathy with an associated sensory axonopathy.\textsuperscript{2} TFG-immunopositive inclusions in both sensory and motor neurons suggested pathologic similarities with other neurodegenerative diseases including amyotrophic lateral sclerosis (ALS).\textsuperscript{1,2} TFG-related neurologic disorders are now known to encompass a spectrum of phenotypes that includes autosomal dominant Charcot-Marie-
Tooth disease type 2 (CMT2) and autosomal recessive hereditary spastic paraplegia.  

TFG protein is ubiquitously expressed in a variety of tissues, including normal muscle (data not shown). TFG functions primarily as a chaperone for vesicle trafficking of unfolded and misfolded proteins through the endoplasmic reticulum and into the ubiquitin-proteasome system (UPS). The P/Q domain in TFG specifically regulates the interaction with the UPS, and the TFG p.Pro285Leu variant has been shown to damage the protein quality control system. This could lead to accumulation of abnormal proteins in motor neurons as well as in muscle. Variants in heat shock proteins 22 (HSBP8) and 27 (HSPB1), which are also protein chaperones, have been associated with CMT2, motor neuronopathy, and vacuolar myopathy. Variants in valosin-containing protein (VCP), another protein involved in ubiquitin-dependent protein degradation, lead to a similar phenotypic spectrum.

Low-complexity protein domains enriched for glutamine repeats, with proline (P/Q) and asparagine (N/Q), are also found in prions and are implicated in several degenerative neuromuscular disease models of DNA, RNA, and protein processing that result in cytoplasmic inclusions and templated protein propagation. The p.Glu384Lys mutation in TIA1 cytotoxic granule-associated RNA binding protein, for example, occurs directly within a glutamine-rich prion-related domain and causes distal myopathy with rimmed vacuoles, while an adjacent mutation (p.Pro362Leu) in the same low complexity domain has been reported in a kindred with ALS. Mutations in prion-like domains in hnRNPA2B1 and hnRNPA1 associate with vacuolar myopathy and motor neuron disease. Mutations in matrin-3, another RNA binding protein involved in stress granule formation, also lead to myopathy with rimmed vacuoles and ALS. TFG therefore extends the list of genes associating with vacuolar myopathy, motor neuron disease, and neuropathy.

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References
## Appendix 1 Author contributions

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