Identification of novel *TFG* mutation in HMSN-P pedigree: emphasis on variable clinical presentations

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Running head: *TFG* p.Gly269Val mutation in HMSN-P pedigree

Conflicts of Interest

All authors claim absence of conflicts of interest and have nothing to disclose including not receiving any payment or services from a third party at any time; no relevant financial activities outside the submitted work; no patents, whether planned, pending or issued, broadly relevant to the work; and no other relationships/conditions/circumstances that present a potential conflict of interest relationships.

No. figures: 2

No. tables: 2

No. supplementary files: 0

Abstract

We aimed to identify the genetic cause of neurological disease in an Iranian pedigree whose manifestations suggested hereditary motor and sensory neuropathy with proximal predominance (HMSN-P). Identification of a p.Gly269Val mutation in *TFG*, the known HMSN-P causative gene, provided supportive evidence. Subjective, biochemical, electrodiagnostic, and imaging data were compared with previously reported HMSN-P patients, including patients of an earlier described Iranian pedigree. Although notable clinical variability was found, comparable involvement of proximal and distal muscles was observed in both Iranian pedigrees. Interestingly, the same p.Gly269Val mutation was recently reported as cause of Charcot-Marie-Tooth disease type 2 in a Taiwanese pedigree. The likelihood that the two pedigrees with the p.Gly269Val mutation are not affected with different diseases is discussed. Identification of a second Iranian HMSN-P pedigree further confirms that HMSN-P is not confined to the Far East. Furthermore, p.Pro285Leu that has been the only *TFG* mutation thus far reported in HMSN-P patients is not the only mutation that can cause the disease. It is emphasized HMSN-P is a neuronopathy.

Keywords: HMSN-P, hereditary motor and sensory neuropathy with proximal predominance, *TFG*, p.Gly269Val, neuronopathy

1. Introduction

Hereditary motor and sensory neuropathy with proximal predominance (HMSN-P) is a neuromuscular disease that was described approximately two decades ago (*Takashima et al., 1997*). Its description emphasizes proximal dominant muscle weakness and atrophy, and also includes mild but obvious sensory dysfunction, fasciculation, decreased deep tendon reflexes, axonal degeneration in the peripheral nerves, and autosomal dominant inheritance. The disease appeared to be rare, and was originally reported only in patients of Japanese or Korean descent (*Takashima et al., 1997; Takashima et al., 1999; Takahashi et al., 2007; Miura et al., 2008; Patroclo et al., 2009; Ishiura et al., 2012; Campellone, 2013; Lee et al., 2013*). In 2015, we described a large Iranian HMSN-P affected pedigree and thus showed that the disease is not confined to individuals with Far East ancestry (*Alavi et al., 2015*). As suggested earlier by others, we noted that HMSN-P in other populations may have been overlooked because of erroneous diagnosis of other diseases with partially overlapping phenotypes, including Charcot-Marie-Tooth type 2, spinal and bulbar muscular atrophy (SBMA), spinal muscular atrophy (SMA), and amyotrophic lateral (ALS) (*Maeda et al., 2007; Nakagawa, 2011*). Genetic analyses recently culminated in showing that a heterozygous mutation in *TFG* that encodes the tyrosine receptor kinase (TRK)-fused protein was the cause of HMSN-P in families from two districts in Japan, a family from Korea, and the family from Iran (*Ishiura et al., 2012; Lee et al., 2013; Alavi et al., 2015*). Although the same mutation (c.854C>T) that causes p.Pro285Leu was identified in all the families, haplotype analysis suggested at least 3 independent origins for the mutated alleles (*Alavi et al., 2015*). It was unclear whether association of the same mutation with three different haplotypes reflected a mutation hotspot or the unique effect of this single mutation in causing the HMSN-P phenotype.

TFG was originally identified during genetic analysis of thyroid carcinomas and other cancers (Greco *et al.*, 1995; Hernandez *et al.*, 1999; Hisaoka *et al.*, 2004). The gene was involved in translocation events that created fusion oncogenes. With respect to neurological diseases, a homozygous mutation (c.316C>T) in *TFG* that causes p.Arg106Cys has been identified in an Indian hereditary spastic paraplegia family (Beetz *et al.*, 2013). Two *TFG* missense mutations in sporadic ALS patients were also reported (Kawarai *et al.*, 2013). Finally, a mutation (c.806G>T) in *TFG* that causes P.Gly269Val was observed in a large Taiwanese pedigree that was diagnosed with a form of autosomal dominant Charcot-Marie-Tooth type 2 (Tsai *et al.*, 2014).

Here, we report finding P.Gly269Val in a newly identified Iranian pedigree affected with HMSN-P. We present clinical, electrodiagnostic (EDX), and muscle magnetic resonance imaging (MRI) data on the Iranian patients. We argue that the Taiwanese and Iranian patients who harbor the same P.Gly269Val mutation in *TFG* should perhaps not be diagnosed with distinct diseases.

2. Materials and Methods

This research was performed in accordance with the Declaration of Helsinki and with approval of the ethics board of the University of Tehran.

The HMSN-160 pedigree studied here includes at least 15 affected individuals distributed in five generations (Fig. 1A). The proband (HMSN-160-IV9) who presented with an atypical motor neuron syndrome with sensory involvement was suspected of possibly being affected with HMSN-P by SN who was very familiar with the clinical features of patients of the earlier Iranian HMSN-P pedigree (HMSN-159). The two pedigrees belonged to different Iranian ethnic groups and geographic regions. The common features of patients in the two pedigrees are described below. The proband of HMSN-160 and several affected and non-affected family members were referred

to us for genetic analysis. Inheritance was autosomal dominant. Individuals HMSN-160-II2 and HMSN-160-II7 had died in separate accidents at ages 22 and 24 years. Presumably, they would have manifested disease had they lived longer.

All exons of *TFG* were polymerase-chain reaction (PCR) amplified from the DNA of peripheral leukocytes of proband HMSN-160-IV9, and the amplicons were sequenced by the Sanger protocol. Sequences were analyzed with Sequencher 5.4 software (Gene Codes Corporation, Ann Arbor, MI). *TFG* reference sequences used were NC_000003.12, NM_001007565.2, and NP_001007566.1. The identified putative disease causing mutation was screened for segregation with disease status in 11 available affected and 3 available unaffected family members by direct sequencing. Many asymptomatic members of the family declined to participate in genetic analysis. The mutation was also screened in 300 Iranian control individuals by an allele specific PCR protocol. Sequences of all primers used are available upon request.

EDX that included nerve conduction studies (NCS) and needle electromyography (EMG) was done according to standard procedures (Dantec Keypoint G4, Natus, CA, USA). Pelvic, thigh, and calf MRI studies were performed using a 1.5-T system (MAGNETOM Avanto 1.5 Tesla, Siemens, Germany). Leg imaging was performed in axial and coronal planes. T1-and T2-weighted spin echo protocols were used.

3. Results

Four sequence variations were observed upon sequencing *TFG* exons in the DNA of the proband of HMSN-160. Two of the variations (c.184+39A>T, rs9824942; c.185-35A>G, rs60550917) are intronic, and one (c.846T>C, rs11353) creates a synonymous (p.Pro282) codon. None of these three variations were considered candidate disease causing mutations; each had reported minimum allele frequencies of at least 0.28, and none were predicted to affect splicing. The fourth variation, c.806G>T that causes p.Gly269Val, was immediately suspected of being the cause of the proband's neurological disease as the same mutation had been recently reported to be cause of dominant axonal CMT in a Taiwanese pedigree (Fig. 1B)(*Tsai et al., 2014*). The mutation co-segregated with disease status in the large Taiwanese pedigree, affected an evolutionarily conserved amino acid residue, was novel and absent in the chromosomes of approximately 500 ethnically matched control individuals (*Tsai et al., 2014*). Elegant cell transfection studies further confirmed that the mutation disrupts functions of the encoded protein (*Tsai et al., 2014*). The p.Gly269Val causing sequence variation (c.806G>T) in *TFG* that was observed in the heterozygous state also segregated with disease status in pedigree HMSN-160, and it was absent in the chromosomes of 300 Iranian control individuals. Given all this, earlier association of *TFG* with HMSN-P, and overlapping clinical presentations of classically defined CMT2 and HMSN-P, the mutation was considered the definitive cause of the neurological disease of pedigree HMSN-160.

Clinical information and blood chemistry laboratory findings on seven HMSN-160 patients are presented in Table 1. Presentations that portrayed telling commonalities with patients of the Iranian pedigree earlier diagnosed with HMSN-P (pedigree HMSN-159) included considerable proximal and distal muscle weakness, asymmetry at onset, notable fasciculation and cramps in trunk and

limbs, tremor, paroxysmal dry coughing, and occasional urinary dysfunction (Table 1)(*Alavi et al., 2015*). Except for patient HMSN-160-V1 in whom disease duration has been less than one year, muscle force examinations in both families confirmed that proximal and distal muscles are involved. In HMSN-160-V1 with very short disease progression, only mild proximal muscle involvement was detected (Table 1). The average age at onset in HMSN-160 patients was 27.1 years and the range was 22 to 40 years; corresponding ages in HMSN-159 were 32.3 and 25-45 years. As with most HMSN-159 patients, disease progression appeared slow in HMSN-160 patients. Creatine kinase levels were elevated in four of seven HMSN-160 patients, and like the HMSN-159 pedigree, there was no obvious correlation between the amount of elevation and disease duration. Impaired glucose metabolism was observed in only one of the six HMSN-160 patients for whom measurements were obtained. Contrary to this, all the patients had abnormal cholesterol and/or triglyceride levels.

Results of EDX studies on HMSN-160 patients are presented in Table 2. As expected, the nerve conduction findings suggest axonal nerve involvement. Both motor and sensory nerves in both upper and lower extremities were affected in the patients. Abnormally decreased amplitude of motor and sensory action potentials were evidenced in all patients except HMSN-160-V1 for whom less than one year has passed since onset of symptoms. EMG results more consistently suggested chronic denervation and reinnervation process. Abnormal spontaneous activities including fibrillation and/or fasciculation in conjunction with chronic neurogenic patterns of motor unit action potential were detected in all the patients. In addition to EMG abnormalities pertaining to muscles of the extremities that were observed in all the patients, abnormalities of truncal (rectus abdominis and thoracic paraspinal) muscles were evidenced in most patients. Contrary to these

muscles, abnormalities in cranial (tongue, mentalis, and trapezius) muscles was detected in only one (HMSN-160-IV9) of seven patients examined.

Muscle MRI imaging was done on three patients whose disease durations at time of imaging were 3, 14, and 37 years (Fig. 2). Severity of muscle involvement increased with duration of disease. Fat deposition was observed only in the gluteus minimus and gluteus maximus muscles of the patient (HMSN-160-IV3) in early stage of disease progression. Deposition was asymmetric in the gluteus minimus. Asymmetry was also evident in some involved regions of the other two patients with more prolonged duration of disease. Different amounts of fat replacement were observed in muscles with different innervations in these two patients, and this variation was evident in both proximal and distal muscles. In both regions of both patients, some muscles were severely affected and others were less prominently affected. There was no obvious proximodistal gradient of muscle involvement in the MRI images. Specific comparisons of muscle involvements are given in legend of Figure 2.

4. Discussion

Phenotypic features of HMSN-160 patients are consistent with the diagnosis of HMSN-P (*Takashima et al., 1997*). These include proximal muscle weakness, fasciculation and cramps in the extremities and trunk, reduced deep tendon reflexes, evidence of sensory involvement, onset in mid-adulthood, elevated serum CK, evidence of axonal degeneration of peripheral nerves, and autosomal dominant inheritance. Identification of a mutation in *TFG*, the known causative gene of HMSN-P, provides supportive evidence. Hitherto, the only *TFG* mutation found to be causative of HMSN-P has been p.Pro285Leu (c. 854C>T) (*Alavi et al., 2015*). An important finding of the present study is identification of a second *TFG* mutation, p.Gly269Val (c.806G>T), as cause of HMSN-P.

Because the clinical similarities between HMSN-160 and HMSN-159 patients were the initial signal for HMSN-P diagnosis in the HMSN-160 pedigree, these similarities were emphasized above. But within the broad background of similarities, there were some differences (Table 1). For example, subjective complaints of sensory disturbances with progression of disease and even as initial manifestation were reported in some HMSN-159 patients, but none of the HMSN-160 patients. Symptoms and signs of cranial nerve involvement, including dysphagia, dysarthria, tongue atrophy, and bifacial weakness, were observed in most (8/10) HMSN-159 patients, but not in any member of the HMSN-160 family. Fine tremor was more prominent in HMSN-160 patients. Finally, rapid disease progression was observed only among HMSN-159 patients (2/10). The EDX findings in the HMSN-160 and HMSN-159 patients were similar, except that abnormalities in cranial innervated muscles were observed in most of the HMSN-159 patients who were tested. This observation is consistent with clinical evidences of cranial nerve involvement (dysphagia, dysarthria, and tongue atrophy) only in HMSN-159 patients. MRI results showed more severe involvement of anterior thigh muscles as compared to posterior thigh muscles in HMSN-P-160 patients, whereas the reverse was seen in HMSN-P-159 patients.

As stated in the introduction, the p.Gly269Val causing mutation (c.806G>T) in *TFG* was recently reported to be cause of Charcot-Marie-Tooth type 2 in a Taiwanese pedigree (*Tsai et al., 2014*). Compared to descriptions of HMSN-160 patients presented here, the clinical features of the 16 patients of the Taiwanese pedigree were described in less detail (Table 1). Nevertheless, it appears that diagnosis of CMT rather than HMSN-P rested largely on predominant distal muscle involvement in the patients. The authors distinguished CMT2 in the family from HMSN-P. Interestingly, in a recent proposal for classification of Charcot-Marie-Tooth diseases, HMSN-P was classified as a form of autosomal dominant axonal type CMT and the name AD-CMTax-*TFG*

was proposed for the disorder (*Mathis et al., 2015*). We suggest that diagnosis of different diseases in the Taiwanese and HMSN-160 patients may be inappropriate. A detailed comparison of phenotypic features of earlier described HMSN-P patients, all with the same p.Pro285Leu mutation in *TFG*, emphasized notable clinical variability within the framework of the original description of the disease (*Takashima et al., 1997; Alavi et al., 2015*). Some of the variable features were age at onset (from early 20's to late 60's), rate of disease progression, temporal order of upper and lower limb involvement, proximal only/ proximal and distal muscle weakness, and symmetric/ asymmetric onset (*Maeda et al., 2007; Takahashi et al., 2007; Miura et al., 2008; Patroclo et al., 2009; Ishiura et al., 2012; Lee et al., 2013; Maeda, 2013; Alavi et al., 2015*). Although the very name given to HMSN-P emphasizes proximal dominant involvement, the predominant distal muscle involvement in the Taiwanese patients may simply be an expansion of the range of variability already reported in HMSN-P patients. In this regard, it is again noted that comparable proximal and distal muscle weakness was observed in the Iranian HMNS-159 patients with the p.Pro285Leu (c.854C>T) mutation and the HMSN-160 patients with the p.Gly269Val (c.806G>T) mutation. Although available clinical data may not justify differential diagnosis for patients who carry the p.Pro285Leu or p.Gly269Val *TFG* mutations, results of cellular and molecular studies suggest that the effects of the two mutations at these levels may not be identical (*Lee et al., 2013; Yagi et al., 2014; Tsai et al., 2014; Yagi et al., 2016*). Yagi and co-investigators have emphasized the inhibitory role of TFG in the ubiquitin-proteasome system, enhancement of the inhibitory effect by the p.Pro285Leu mutated protein, and endoplasmic reticulum stress caused by the enhancement (*Yagi et al., 2014; Yagi et al., 2016*). Tsai and co-investigators have shown that the p.Gly269Val mutated TFG protein causes formation of cytoplasmic aggregates that include wild type and mutated TFG proteins (*Tsai et al., 2014; Yagi et al., 2016*). They suggested

that sequestration of the wild type protein deprives cells of normal TFG functions, including functions related to protein secretion. The p.R106C mutated protein causes a complicated form of hereditary spastic paraplegia that is clearly a distinct neurogenic disease (*Beetz et al., 2013*). Consistent with this, whereas p.Pro285Leu and p.Gly269Val affect amino acids within the same domain (proline and glutamine-rich domain) in the *TFG* encoded protein, p.R106 is positioned in a different domain (coiled-coil domain). Furthermore, inheritance pattern of the spastic paraplegia caused by p.R106C is autosomal recessive. The p.R106C mutation disrupts the ability of TFG to self-assemble into an oligomeric complex that ultimately negatively affects the architecture of the endoplasmic reticulum (*Beetz et al., 2013; Yagi et al., 2016*). We believe that further cellular molecular studies with wild type and mutated TFG proteins are needed to gain a fuller understanding of TFG functions (*Yagi et al., 2016*).

A yet more basic consideration of the very nature of the disorder being called HMSN-P may be in order. When the disorder was first identified in patients from Okinawa in Japan, the authors considered classifying it as a motor neuron disease (MND), but ultimately categorized it as a subtype of hereditary motor sensory neuropathy (HMSN) because the consensus for MND included no sensory involvement (*Takashima et al., 1997; Takashima, 2013*). Several authors, based on neuropathologic studies and clinical similarities with familial amyotrophic lateral sclerosis (FALS), have since explicitly or implicitly suggested that HMSN-P is a misnomer and that the associated disease is a neuronopathy (*Fujita et al., 2011; Nakagawa, 2011; Maeda, 2013*). Nevertheless, recent literature does not reflect general adherence to this proposal. The findings relating to patients of Iranian pedigrees HMSN-159 and/or HMSN-160 strongly suggest that their underlying pathology is a motor and sensory neuronopathy. Asymmetry at presentation, involvement of proximal, truncal, and cranial muscles early in disease course, prominent

fasciculation and cramps, and rapid progression to major disability within a few years after onset are not typically seen in HMSN. Presentations of truncal cramp and fasciculation are more in favor of neuropathy. Electrophysiologic findings of denervation and reinnervation in cranial and truncal muscles early in disease course also implicate neuropathy. Interestingly, sensory involvement has now been proposed for ALS (*Isaacs et al., 2007*). More generally, while prototypical neuropathies including ALS and spinal muscular atrophy are predominantly motor neuropathies, sensory neuropathy (ganglionopathy) that affects sensory neurons in the dorsal root ganglia has been described. To emphasize distinctive features of the disease under consideration, the name TFG-related motor and sensory neuropathy (TMSNMP) can be considered.

Ethical approval

This research was performed in accordance with the Declaration of Helsinki and with approval of the ethics board of the University of Tehran.

Abbreviations

HMSN-P, hereditary motor and sensory neuropathy with proximal predominance; EDX, electrodiagnostic; MRI, magnetic resonance imaging; NCS, nerve conduction studies; EMG, electromyography.

Acknowledgements

We acknowledge the Iran National Science Foundation for funding the research and thank the patients and their family members for participating in the study.

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Figure legends

Figure 1. Iranian HMSN-P pedigree (HMSN-160) with p.Gly269Val mutation in *TFG*. A- *TFG* genotypes of individuals tested are presented. Present age of individuals is provided when known. Filled circles and squares, HMSN-P affected; unfilled circles and squares, asymptomatic at time of examination; m, mutated *TFG* allele; wt, wild-type *TFG* allele. B- DNA sequence chromatograms showing the heterozygous c.806G>T mutation in *TFG*, and the wild type sequence.

Figure 2. Axial view of T1 weighted muscle MRI of lower extremities of HMSN-160 patients. Pictures show nonhomogeneous involvement of muscles with different innervations in both proximal and distal regions. For example in patient HMSN-160-1V3, gluteus minimus (arrow in A1) and gluteus maximus (arrow in A2) muscles are involved, but distal regions are spared. Asymmetry is most conspicuous in HMSN-160-1V3 who is in early stage of disease progression. In later stages of the disease, muscles of both distal and proximal regions are involved, and a proximodistal gradient of involvement is not evident. For example, in HMSN-160-III4: some distal muscles such as the tibialis anterior muscles (arrow in B4) are less involved than some proximal muscles such as the vastus lateralis (arrow in B3) and gluteus maximus muscles (arrow in B2), while other distal muscles such as the medial gastrocnemius muscles (arrow head in B4) are more severely involved than some proximal muscles such as the gluteus medius muscles (arrow head in B1). In HMSN-160-III2: the distal extensor hallucis longus and extensor digitorum longus muscles (arrow in C4) are less involved than the proximal vastus lateralis (arrow in C3) and gluteus maximus (arrow in C2) muscles, while the distal peroneal muscles (arrow head in C4) are more severely involved than the proximal gluteus medius muscles (arrow head in C1).

Table 1

Table 1- Phenotypes of HMSN-160 patients^a

Patient ID	HMSN-160-V1	HMSN-160-IV3	HMSN-160-IV9	HMSN-160-IV7	HMSN-160-III4	HMSN-160-IV5	HMSN-160-III2	HMSN-159 ^b	CMT patients ^c
Sex	F	F	M	M	F	F	M		
Age at onset	22	30	25	25	40	23	25	25-45; Av: 32.3	28-40; Av: 35.2
Present age (Yrs) ^d	22	33	33	37	54	40	62		
Years between onset and recent examination	0	3	8	12	14	17	37	3 to 31	
Initial manifestation:									
Motor/sensory	Motor	Motor	Motor	Motor	Motor	Motor	Motor	8/10: motor; 2/10: sensory	Predominatly distal limbs
Upper limb/lower limb	Lower limb	Lower limb	Lower limb	Lower limb	Lower limb	Lower limb	Lower limb	10/10: lower limb	Mild distal sensory
Proximal/distal	Mild proximal ^e	Mild proximal ^e	Proximal and distal	Proximal and distal	Proximal and distal	Proximal and distal	Proximal and distal	Motor: proximal; sensory: distal	
Assymetry	-	-	+	+	+	+	+	8/10 +	All symetrical
Sensory findings									
Paresthesia & numbness	Absent	Absent	Absent	Absent	Absent	Absent	Absent	5/10 +	
Pinprick sensation	Normal	Normal	Impaired ^f	Impaired ^f	Impaired ^f	Impaired ^f	Impaired ^f	5/8 +	
Vibration	Normal	Normal	Impaired ^f	Impaired ^f	Impaired ^f	Impaired ^f	Impaired ^f	3/8 +	
Fasciculation & cramp	+	+	+	+	+	+	+	9/10 +	
Tremor	+	+	+	+	+	+	+	2/10 +	
Cranial nerve involvement	-	-	-	-	-	-	-	8/10 +	
Paroxysmal dry cough	-	-	+	+	+	+	-	10/10 +	
Urinary dysfunction	-	+	-	-	-	-	-	3/10 +	
Deep tendon reflex	Normal	Normal	Reduced	Reduced	Absent	Reduced	Absent	10/10 +	
Ambulatory state	Normal	Walks independently, without facility	Walks independently, without facility	Walks independently, without facility	Walks independently, without facility	Walks independently, without facility	Uses walking device for walking		
Blood chemistry data									
CK (normal: 20-159 U/L)	80	89	1233	746	84	195	1054	5/6 elevated	
FBS (normal: 82-115 mg/dl)		99	112	106	192	78	104	0/5 impaired	
Cholesterol (normal: <200 mg/dl)		195	191	238	291	206	255	5/5:impaired lipid metabolism	
LDL (normal: < 130 mg/dl)		107	119		179	140	146		
HDL (normal: 30-60 mg/dl)		38	30		53	47	46		
Triglyceride (normal: <150 mg/dl)		251	209	156	293	97	345		

a, Patients arranged left to right on basis of increasing years since onset; b, summary of data on 10 patients of Iranian HMSN-159 pedigree described in [Alavi et al., 2015](#);
c, summary of data on patients with same *TFG* mutation as patients of HMSN-160 as described in [Tsai et al.,2014](#); d, and age at examination; e, evidenced only while performing gymnastic excersises;
f, in all cases: distal>proximal, lower>upper; CK, creatine phosphokinase; FBS, fasting blood sugar; LDL, low density lipoprotein; HDL, high density lipoprtein

Table 2

Table 2- Nerve conduction and EMG studies on HMSN-160 patients^a

Patient ID	HMSN-160-V1	HMSN-160-IV3	HMSN-160-IV9	HMSN-160-IV7	HMSN-160-III4	HMSN-160-IV5	HMSN-160-III2
Sex	F	F	M	M	F	F	M
Age at onset	22	30	25	25	40	23	25
Present age (Yrs) ^b	22	33	33	37	54	40	62
Years between onset and tests	0	3	8	12	14	17	37
CMAP (mV) (left/right)							
Median (normal: ≥ 4)	7.4/6.1	nd/15.6	nd/1.6	8.1/9.5	6.2/6.3	4.6/4.6	0.9/3.4
Ulnar (normal: ≥ 6)	7.7/8.9	8.4/nd	2.3/nd	4.8/3.4	1.5/3.6	3.7/3.8	1.4/1.0
Peroneal (normal: ≥ 2)	3/4.5	nd/3.8	1.6/nd	1.7/0.4	2.6/2.1	0.4/0.3	NR/NR
Tibial (normal: ≥ 4)	11.7/11.2	12.0/nd	nd/0.6	0.7/2.3	2.4/3.4	2.1/0.9	NR/NR
MCV (m/s) (right/left)							
Median (normal: ≥ 49)	55.0/54.2	nd/62.5	nd/44.7	50.8/52.5	58.0/52.4	55.3/51.9	51.7/51.2
Ulnar (normal: ≥ 49)	75.2/65.7	62.2/nd	57.1/nd	58.5/50.6	56.1/63.9	60.4/54.6	56.0/53.1
Peroneal (normal: ≥ 44)	56.9/55.0	nd/56.0	49.7/nd	38.0/40.2	43.9/44.4	47.6/38.4	nd/nd
Tibial (normal: ≥ 41)	53.1/53.5	46.9/nd	nd/52.6	39.6/41.6	40.2/42.5	50.9/50.3	nd/nd
SNAP (μV) (right/left)							
Median (normal: ≥ 20)	30.0/30.1	19.0/17.4	nd/5.3	8.3/6.1	NR/NR	2.2/6.3	NR/NR
Ulnar (normal: ≥ 17)	21.8/27.3	25.5/18.3	5.6/nd	9.7/2.5	NR/NR	3.3/3.4	NR/NR
Radial (normal: ≥ 15)	nd/23.9	nd/nd	nd/nd	nd/nd	nd/nd	nd/nd	nd/nd
Sural (normal: ≥ 6)	6.3/7.0	NR/NR	NR/nd	NR/NR	NR/NR	NR/NR	NR/NR
Superficial peroneal (normal: ≥ 6)	nd/nd	NR/NR	NR/nd	nd/nd	nd/nd	nd/nd	nd/nd
SCV (m/s) (right/left)							
Median (normal: ≥ 50)	69.8/59.8	54.5/55.0	nd/65.2	46.8/48.1	NR/NR	46.5/41.7	NR/NR
Ulnar (normal: ≥ 50)	53.4/70.9	67.9/64.0	51.0/nd	43.7/42	NR/nd	47.2/47.0	NR/NR
Radial (normal: ≥ 50)	nd/68.5	nd/nd	nd/nd	nd/nd	nd/nd	nd/nd	nd/nd
Sural (normal: ≥ 40)	57.9/67.6	NR/NR	NR/nd	NR/NR	NR/NR	NR/NR	NR/NR
Superficial peroneal (normal: 40)	nd/nd	NR/NR	NR/nd	nd/nd	nd/nd	nd/nd	nd/nd
EMG							
Spontaneous activity^c							
Cranial innervated muscles	Absent	Absent	Fib	Absent	Absent	Absent	Absent
Cervical innervated muscles	Fascic	Fascic	Fib/Fascic	Fib/Fascic	Fascic	Fascic	Fib/Fascic
Thoracic innervated muscles	Absent	Absent	nd	Fascic	Fascic	Fascic	Fascic
Lumbosacral innervated muscles	Fascic	Fascic	Absent	Fib/Fascic	Fib/Fascic	Absent	Fascic
Chronic neurogenic MUAP							
Cranial innervated muscles	-	-	+	-	-	-	-
Cervical innervated muscles	-	+	+	+	+	+	+
Thoracic innervated muscles	-	-	nd	+	+	+	+
Lumbosacral innervated muscles	+	+	+	+	+	+	+

a, Patients arranged left to right on basis of increasing years since onset; b, and age when tests were done; c, including fibrillation & fasciculation;

nd, not done; NR, no response; CMAP, compound muscle action potential; MCV, muscle conduction velocity;

SNAP, sensory nerve action potential; SCV, sensory conduction velocity; EMG, electromyography; MUAP, motor unit action potential;

Fib, fibrillation; Fascic, fasciculation.

Figure 1A

Figure 1B

• affected - E7 Fragment base #97. Base 251 of 473 •

G	T	A	T	G	T	T	A	T	T	C	A	G	T
U	A	T	A	U	A	A	T	A	A	U	T	U	A

Mutated

• unaffected - E7 Fragment base #97. Base 251 of 469 •

G	T	A	T	G	G	T	A	T	T	C	A	G	T
U	A	T	A	U	U	A	T	A	A	U	T	U	A

Wild type

Figure 2