Journal of Human Genetics (2019) 64:171–176
https://doi.org/10.1038/s10038-018-0538-4

BRIEF COMMUNICATION

A novel homozygous mutation of the *TFG* gene in a patient with early onset spastic paraplegia and later onset sensorimotor polyneuropathy

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Received: 12 May 2018 / Revised: 1 November 2018 / Accepted: 2 November 2018 / Published online: 22 November 2018

Abstract

The *tropomyosin-receptor kinase fused gene* (*TFG*) has recently been implicated in several distinct hereditary disorders, including the autosomal-recessive form of complicated hereditary spastic paraplegia called SPG57. Previously, three homozygous variants of the *TFG* gene were reported in five families with SPG57, in which early onset spastic paraplegia, optic atrophy, and peripheral neuropathy were variably identified. Here, we present the first Japanese patient with SPG57, and have added a homozygous p.Ile66Thr variant as the fourth SPG57 genotype.

Introduction

Hereditary spastic paraplegia (HSP) is a group of clinically and genetically heterogeneous neurological disorders. More than 60 spastic paraplegia genes (SPGs) have been identified and HSP is classified into SPG1–79 (https://neuromuscular.wustl.edu/spinal/fsp.html) [1]. The *tropomyosin-receptor kinase fused gene* (*TFG*) was recently implicated in several distinct hereditary disorders, including the autosomal-dominant type of hereditary motor and sensory neuropathy with proximal dominant involvement (HMSN-P) [2, 3], the autosomal-dominant type of Charcot–Marie–Tooth disease type 2 (CMT2) [4], and the autosomal-recessive form of complicated HSP called SPG57 [5–8]. Three homozygous variants of the *TFG* gene have been reported in five families with SPG57, in which early onset spastic paraplegia, optic atrophy, and peripheral neuropathy were variably identified. Here, we present the first Japanese patient with SPG57.

Case report

This 43-year-old Japanese woman was the third child born to healthy parents, who were first cousins (Fig. 1). Her second-oldest brother had a history similar to hers and he could walk short distances using crutches. Her oldest brother had a mild intellectual disability of unknown cause. She was born after a full-term pregnancy without asphyxia. Her early development was normal. She walked alone at the age of 2 years. When she was 3–4 years old, she could climb a tree and ride a tricycle. She was noticed to have difficulty walking at the age of 5 years. She was followed at

Electronic supplementary material The online version of this article (https://doi.org/10.1038/s10038-018-0538-4) contains supplementary material, which is available to authorized users.

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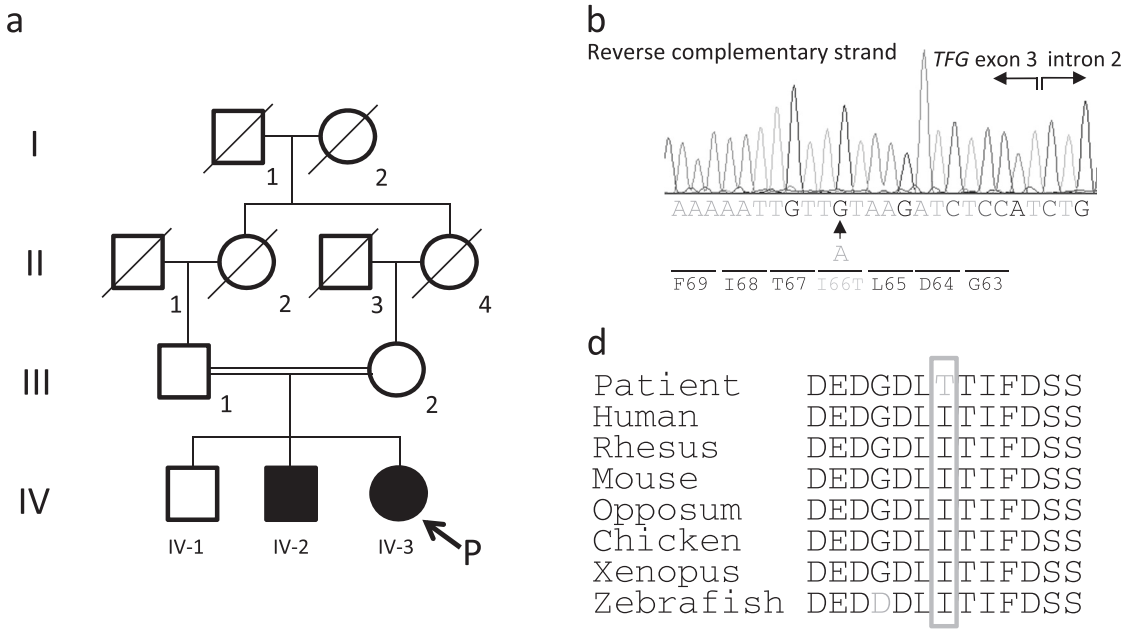

c

RPM value in each exon

TFG exon	1	2	3	4	5	6	7	8
This patient	6.74	5.74	0.31	2.19	2.11	1.27	2.62	11.64
Disease controls (mean±2SD)	6.39±0.76	5.24±0.67	0.40±0.18	1.88±0.40	1.93±0.40	1.15±0.26	2.50±0.53	11.0±10.5

Disease controls include 95 patients with other neurological diseases whose exome sequencing was conducted in the same runs along with the exome sequencing of this patient. There were no significant differences in the RPM values of individual exons including exon 3 where the mutation (p.I66T) is located.

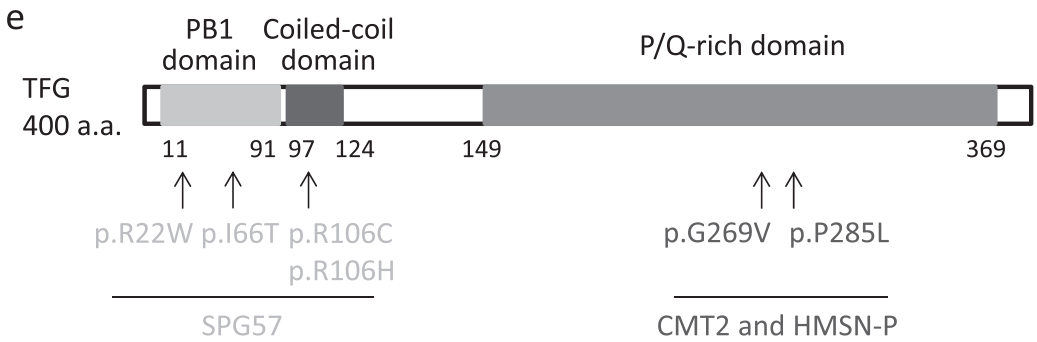

Fig. 1 Pedigree of the patient's family and the mutation detected in the family. **a** Pedigree chart of the family. **b** Sanger sequence analysis revealed a homozygous c.197T>C (p. Ile66Thr) mutation in *TFG*. The result of the direct nucleotide sequence analysis of the reverse complementary strand is shown. **c** Reads per million mapped reads (RPM) were calculated for each exon of *TFG* using the whole exome sequence data obtained in the same runs. Mean and standard deviation (SD) of RPM values were calculated in disease controls ($n = 95$) sequenced in the same runs. **d** The Ile66 of TFG is conserved among species. **e** Schematic representation of TFG isoform 1 and the locations of known mutations in *TFG* are shown. The p.Arg22Trp, p.Ile66Thr, p.Arg106Cys, and p.Arg106His mutations, which cause autosomal recessive SPG57, affect amino acids in PB1 domain and coiled-coil domain of *TFG* near C-terminus. The p.Gly269Val and p.Pro285Leu mutations, which cause autosomal dominant HMSN-P and CMT2, affect amino acids in P/Q-rich domain near C-terminus of *TFG*

a local hospital and underwent rehabilitation with a diagnosis of cerebral palsy. However, her motor deterioration was progressive and she lost the ability to walk alone at the age of 10 years. As she had normal intelligence, she attended regular classes in high school and graduated from vocational school. Since then, she has been working as an office worker. She developed glaucoma at 30 years of age.

At the age of 39 years, she first visited our hospital because of severe lumbago. At that time, she underwent a full neurological work-up. Her speech was clear, fluent, and

comprehensive. No dysphagia was noted. She had exaggerated deep tendon reflexes of all four extremities, except for the Achilles tendon, and marked spasticity of the biceps and quadriceps muscles bilaterally. The Babinski reflex was faintly positive bilaterally and there was a severe foot deformity. Muscle atrophy of both distal lower extremities and thenar/hypothenar muscles was evident. Muscle power was markedly decreased in the lower distal extremities. Her right and left grip power were decreased to 6.5–11.5 and 2–10.0 kg, respectively. Finger dexterity was reduced. No facial weakness was noted. Urinary urgency was observed. She could walk a few meters using crutches, which was characterized by equinus and crouch gait, as well as bilateral planovalgus. The range of motion of both ankle joints was severely limited due to contractures. All toes had pronounced claw toe, but no pes cavus was observed. She had sensory disturbance in the legs, including vibration, pain, and touch. In summary, she had spastic diplegia and distal dominant peripheral sensory and motor disturbances with urinary urgency.

The motor nerve conduction velocities (MCVs) of the median and ulnar nerves were 51 and 62 m/s, respectively. The compound motor action potentials (CMAPs) of those nerves were remarkably reduced to 1.9 and 3.0 mV, respectively. CMAPs of posterior tibial and peroneal nerves were not elicited. Electromyography of the tibialis anterior muscle revealed neurogenic changes with long duration and polyphasic motor unit potentials. The sensory conduction velocity of the sural nerve was not elicited. The F-wave frequency and proximal MCV of the median nerve were normal. Brain magnetic resonance imaging was normal. Ophthalmological examination revealed partial optic atrophy and partial loss of the visual field, with normal intraocular pressure.

She underwent muscle elongation and tendon extension surgery of the bilateral hamstring, gracilis, and psoas muscles at the age of 39 years. Repeat Achilles tendon elongation and bone implantation surgery of the left leg was performed at the age of 41 years because the pes planovalgus had progressed.

To establish the molecular diagnosis, we first conducted Sanger sequencing of *ALS2* as well as mutational analysis of genes for Charcot–Marie–Tooth diseases and related diseases employing a custom designed resequencing microarray (Affymetrix, Inc., Santa Clara, California) that targets *PMP22, MPZ, SIMPLE, EGR2, NEFL, SOX10, GDAP1, MTMR2, SBF/MTMR13, KIAA1985, NDRG1, PRX, GJB1, MFN2, RAB7, GARS, HSPB8, MLNA, GAN1, KCC3, APTX, SETX, TDP1, DNM2, DHH*, and *YARS* [9]. Since causative mutations were not identified in the above analyses, we then conducted whole-exome sequencing. By looking at nonsynonymous rare variants located in causative genes for HSP (Supplementary Data), whose allele frequencies in the in-house database were <0.5% and basecall qualities were ≥20, the only variant whose zygosity was consistent with the mode of inheritance of the corresponding diseases was a homozygous missense variant in exon 3 of *TFG* [c.197T>C:p.Ile66Thr], which was confirmed by Sanger sequencing of the PCR products (Fig. 1b). There were no other pathogenic mutations causing motor neuron diseases and hereditary neuropathies as described in Supplementary Data.

To exclude the possibility of the existence of a deletion in one allele, we evaluated copy number of exons in *TFG*. Whole-exome sequencing of the patient's DNA was conducted in the consecutive 3 runs. In these 3 runs, a total of 96 samples was subjected to whole-exome sequencing. Calculation of RPM (reads per million mapped reads) values of individual samples for each exon of *TFG* were obtained from the whole-exome sequencing data in the 3 runs. There were no significant differences in the RPM values of individual exons including exon 3 where the mutation (p. Ile66Thr) is located, indicating that the copy number of *TFG* was 2 and the mutation was truly homozygous, consistent with parental consanguinity (Fig. 1c).

This variant was neither present in 1261 in-house Japanese control exomes nor in public databases, including the Human Genetic Variation Database (HGVD, http://www.hgvd.genome.med.kyoto-u.ac.jp/), Integrative Japanese Genome Variation Database (iJGVD, https://ijgvd.megabank.tohoku.ac.jp/), and Genome Aggregation Database (gnomAD; http://gnomad.broadinstitute.org/), and conserved among vertebrates (Fig. 1d), and predicted to be probably damaging by PolyPhen-2. The Phred-scaled Combined Annotation Dependent Depletion score was 28.4 (http://cadd.gs.washington.edu/). With these data, we considered the mutation in *TFG* was pathogenic. In silico splice site prediction using 1 kb upstream and downstream sequences of the mutation revealed no significant alteration in predicted splice sites by the mutation (http://www.fruitfly.org/seq_tools/splice.html). Other family members were not available for mutation analysis.

Discussion

Our patient had early onset spastic paraplegia, which later progressed to diplegia associated with sensorimotor polyneuropathy. Previously, three homozygous variants of the *TFG* gene were reported in five SPG57 families, which included p.Arg106Cys in two families, p.Arg22Trp in two families, and p.Arg106His in one [5–8]. This report adds a homozygous Ile66Thr variant as the fourth mutation causing SPG57.

The mutation identified in the study (p.Ile66Thr), as well as p.Arg22Trp, p.Arg106Cys, and p.Arg106His, all of

Table 1 Clinical summary and results of genetic testing of the present case and reported cases

Mutation	Family 1 [7]			Family 2 [5]		Family 3 [8]		Family 4 [6]			Family 5 [6]		Family 6 (present case)
	c.64C>T (p.Arg22Trp)			c.64C>T (p.Arg22Trp)		c.316C>T (p.Arg106Cys)		c.317G>A (p.Arg106His)			c.316C>T (p.Arg106Cys)		c.197T>C (p.Ile66Thr)
Individual number	1	2	3	1	2	1	2	1	2	3	1	2	1
Gender	F	F	M	M	F	M	M	F	M	F	F	F	F
Origin	Eastern Sudan			Pakistan		India		British Pakistan			India		Japan
Age at onset of motor symptoms	1 y	10 mo	14 mo	congenital	congenital	NA	NA	NA	8 y	13 y	8 mo	12 mo	5 y
Age at initial examination	15 y	8 y	14 mo	5 y	4 y	12 y	8 y	26 y	24 y	20 y	10 y	5 y	39 y
Spasticity UL/LL	++/++	+/++	++/++	−/++	−/++	NA/++	NA/++	NA	−/+	−/+	+/+++	+/+++	++/++
Motor deficit PUL/DUL	+/+	−/−	+/+	NA	NA	NA	−/+	−/−	−/−	−/−	−/−	−/−	−/++
Motor deficit PLL/DLL	++/++	++/+++	++/++	NA	NA	++/++	++/++	-/-	+/++	+/+	++/++	++/++	+/+++
Tendon reflexes UL/LL patellar	++/+	++/++	++/++	++/++	++/++	NA	++/++	+/++	+/++	N/++	++/++	++/++	++/++
Ankle reflex/Plantar response	++/+	++/+	N/+	++/+	++/+	NA	++/+	++/−	++/+	++/+	++/+	++/+	−/+
Ataxia	−	−	−	−	−	NA		NA	NA	NA	NA	NA	NA	−
Dysarthria	−	−	−	−	−	NA	NA	NA	NA	NA	NA	NA	NA	−
Muscle atrophy DUL/DLL	−/−	−/−	−/−	NA	NA	NA	+/+	NA	NA	NA	NA	NA	NA	+/+
Facial atrophy	−	−	−	−	−	NA	NA	NA	NA	NA	NA	NA	NA	−
Cog/Psych	+/−	+/−	+/−	++/−	++/−	NA	−/−	−/−	−/−	−/−	−/−	−/−	−/−	−/−
Optic atrophy	−	−	−	NA	NA	+	+	−	−	−	+++	+++	+
Extrapyramidal Signs	−	−	−	+	+	NA	−	NA	NA	NA	NA	NA	NA	−
Sensation	Intact	Intact	Intact	Intact	Intact	NA	Intact	Intact	Disturbed	Intact	Disturbed	Disturbed	Disturbed
Microcephaly	+	−	+	−	−	NA	NA	NA	NA	NA	NA	NA	NA	−
Peripheral Neuropathy	NA	+	NA	−	−	NA	++	+	−	NA	+	NA	+++
Brain MRI	n.d.	Thin corps callosum, mild cerebellar atrophy	n.d.	Reduced cerebral white matter and abnormal myelination, thin corps callosum	n.d.	n.d.	Subtle increase of signal intensity of parietal white matter	NA	Normal	NA	NA	NA	Normal
Self walk	NA	NA	NA	−	−	+	+	NA	+	+	NA	NA	+

Table 1 (continued)

Mutation	Family 1 [7]		Family 2 [5]		Family 3 [8]		Family 4 [6]		Family 5 [6]		Family 6 (present case)
	c.64C>T (p.Arg22Trp)		c.64C>T (p.Arg22Trp)		c.316C>T (p.Arg106Cys)		c.317G>A (p.Arg106His)		c.316C>T (p. Arg106Cys)		c.197T>C (p. Ile66Thr)
Sleep disturbance	NA	NA	NA	+	+	NA	NA	NA	NA	NA	–
Facial dysmorphism	NA	NA	NA	–	–	NA	–	NA	NA	NA	–
Epilepsy	NA	NA	NA	NA	NA	NA	–	NA	NA	NA	–
Bladder dysfunction	NA	NA	NA	NA	NA	NA	–	NA	NA	NA	++

+ positive, – negative, *Cog* cognition, *DUL/DLL* distal upper/lower limbs, *LL* lower limbs, *N* normal, *Psych* psychological, *PUL/PLL* proxial upper/lower limbs, *UL* upper limbs; Tendon reflexes: ++ Increased, – Decreased, *n.d.* not done, *NA* not applicable due to lack of information; Plantar response: + extensor, *y* year, *mo* month

which cause autosomal recessive complicated HSP involving mainly upper motor neurons, are located in the PB1 and coiled-coil domains near the N-terminus of TFG. In contrast, in HMSN-P and CMT2 mainly involving lower motor neurons, p.Pro285Leu [2, 3] and p.Gly269Val [4] located in the P/Q-rich domain near the C-terminus of TFG have been identified, respectively (Fig. 1e). These observations further support a phenotype-genotype correlation of *TFG* [6].

The clinical phenotypes of SPG57 are relatively consistent and are characterized as complex HSP. However, detailed clinical descriptions indicate that spasticity of the upper extremities was present in the patients with three variants (p.Arg106Cys, p.Arg22Trp, and p.Ile66Thr) and optic atrophy in two variants (p.Arg106Cys and p.Ile66Thr) (Table 1). Microcephaly, reduced white matter volume, and cognitive deficit were reported only in the patients with the p.Arg22Trp variant. Axonal neuropathy was noted in all variants. Unaided walking was not attained in the family with the p.Arg22Trp variant. Even among patients with the same variant, phenotypic differences were reported, such as in the families with p.Arg22Trp [5, 7], in which the disease severity and comorbidity differed in each family. Age at examination, environmental epigenetic factors, or other modifier genes may have affected the phenotypic differences, which require further delineation.

TFG is ubiquitously expressed across tissues and the full-length transcript is predominantly expressed in neural tissues (brain, spinal cord, and dorsal root ganglia) [10]. The reported phenotypes of patients harboring variants in the *TFG* gene indicate that peripheral axons and lower and upper motor neurons are variably affected. These findings indicate that TFG has critical functions in neurons. The functions of native TFG include vesicle biogenesis on the endoplasmic reticulum (ER), the formation of a matrix between ER exit sites and the ER-Golgi intermediate compartment, a sorting hub for secretary cargoes [11]. A TFG inhibition study revealed slow protein secretion from the ER and altered ER morphology, disrupting the organization of peripheral ER tubules and causing collapse of the ER networks onto the underlying microtubule cytoskeleton [8]. The mutant protein lacks the ability to self-assemble into an oligomeric complex, which is critical for normal TFG function [8]. Therefore, TFG-related disorders are caused by impaired ER function [10].

To understand the clinical spectrum of *TFG* variants and genotype–phenotype correlation, the accumulation of more cases is needed.

Acknowledgements The authors thank Japan Spastic Paraplegia Research Consortium for the genetic analysis of the present patient. This work was supported by Grants-in-Aid from the Research

Committee for Ataxic Disease (YT), the Ministry of Health, Labor and Welfare, Japan, and grants for AMED under Grant Numbers JP16kk0205001h0001 (ST) and JP17kk0205001h0002 (ST).

Author contributions KH and TM: study conceptualization, design, and manuscript preparation. TO, NS, TI, YO, RS, and NT: clinical evaluation and operation, HT, HI, and KK: gene analysis and interpretation of genetic analysis data. ST, MA, and YT: critical revision of the manuscript and study supervision.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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