CASE STUDY

A novel *VRK1* mutation associated with recessive distal hereditary motor neuropathy

Shu-Yan Feng[1], Liu-Yi Li[1], Shu-Man Feng[2] & Zhang-Yu Zou[3]

[1]Department of Neurophysiology, Henan Provincial People's Hospital, Zhenzhou 450003, China
[2]Department of Neurology, Henan Provincial People's Hospital, Zhenzhou 450003, China
[3]Department of Neurology, Fujian Medical University Union Hospital, Fuzhou 350001, China

Correspondence

Zhang-Yu Zou, Department of Neurology, Fujian Medical University Union Hospital, No. 29, Xinquan Road, Gulou District, Fuzhou 350001, Fujian, China.
Tel: +86 18606021133; Fax: +86 591 87113828;
E-mail: pumczzy@gmail.com

Abstract

Vaccinia-related kinase 1 (*VRK1*) mutations can cause motor phenotypes including axonal sensorimotor neuropathy, distal hereditary motor neuropathy (dHMN), spinal muscular atrophy, and amyotrophic lateral sclerosis. Here, we identify a novel homozygous *VRK1* p.W375X mutation causing recessive dHMN. The proband presented with juvenile onset of weakness in the distal lower extremities, slowly progressing to the distal upper limbs, with bilateral pes cavus and no upper motor or sensory neuron involvement. Nerve conduction studies showed a pure motor axonal neuropathy. Our findings extend the ethnic distribution of *VRK1* mutations, indicating that these mutations should be included in genetic diagnostic testing for dHMN.

Introduction

Distal hereditary motor neuropathy (dHMN), also known as distal spinal muscular atrophy (dSMA), comprises a clinically and genetically heterogeneous group of neuropathies. These are characterized by slowly progressive distal symmetric limb muscle weakness and atrophy that are more pronounced in the lower limbs than the upper limbs, frequently leading to foot deformities such as pes cavus, with no or minimal sensory involvement.[1,2]

HMN is genetically heterogeneous, with more than 30 disease-causing genes identified to date.[2] Autosomal recessive dHMN can be caused by mutations in the *HINT1*,[3] *HSJ1*,[4] *HSPB1*,[5] *IGHMBP2*,[2] *SIGMAR1*,[6] and *PLEKHG5* genes.[7] Homozygous vaccinia-related kinase 1 (*VRK1*) mutations have previously been reported to cause a range

of phenotypes including congenital, early, or adult-onset axonal motor neuropathy,[8] axonal sensorimotor neuropathy,[9] spinal muscular atrophy (SMA),[10] or amyotrophic lateral sclerosis (ALS),[8,11] and some patients had associated features of pontocerebellar hypoplasia or microcephaly.[9,10,12] Here, we describe a novel homozygous mutation in the *VRK1* gene causing recessive dHMN without pontocerebellar hypoplasia or microcephaly in a Chinese pedigree.

Case Report

The consanguineous pedigree is shown in Figure 1A. The 37-year-old proband (V-1) noticed easy fatigability in his legs and some clumsiness on running when he was 15 years old. He had a poor grade in physical

education. The symptoms progressed slowly, and he began to feel a slight weakness of the lower legs. At the age of 22 years he developed right foot drop, followed by left foot drop, and had gait abnormalities but could walk without an aid. The disorder was restricted to the legs until 3 years before presentation, when he developed slight weakness of both hands and had difficulty with buttons. A neurological examination showed intact cranial nerves. He had severe muscle wasting and weakness of the distal lower limbs (3/5) and mild muscle weakness of the distal upper limbs (5⁻/5), with relative preservation of the muscle strength of the proximal limbs. Deep tendon reflexes were absent in

the lower limbs but normal in the upper limbs, with bilateral pes cavus (Fig. 1B). The Babinski reflex was negative bilaterally. Sensations were normal.

Nerve conduction studies showed a pure motor axonal neuropathy of the lower limbs with preserved conduction velocities and no sensory involvement (Table 1). EMG showed acute denervation and chronic reinnervation in the muscles of the lower limbs and distal upper limbs rather than in the thoracic paraspinal muscles and sternocleidomastoid muscles (Table 2). The electrophysiological features were consistent with a distal motor axonal neuropathy. Laboratory testing for diabetes, monoclonal gammopathies, vasculitis, and anti-double-stranded DNA,
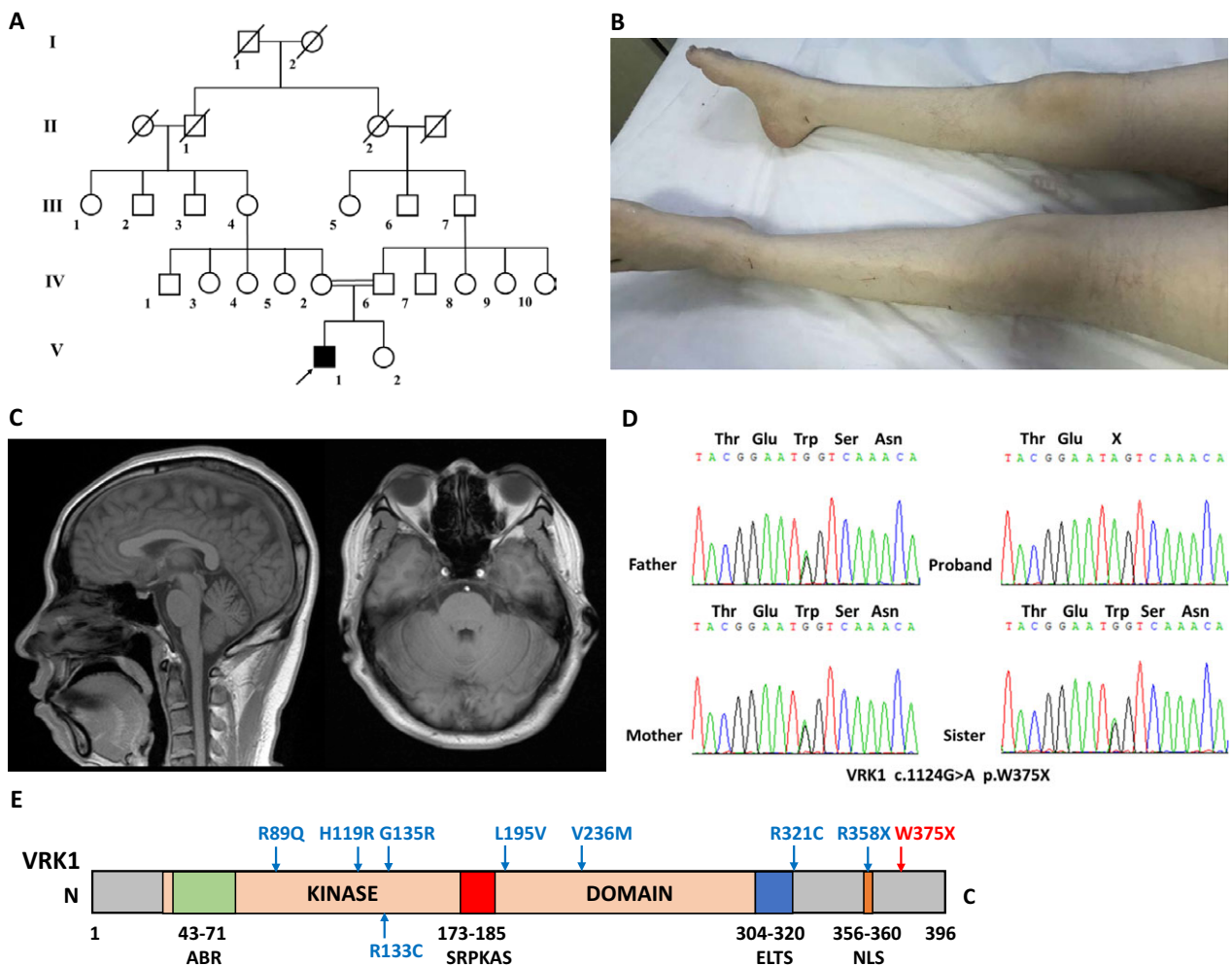

Figure 1. Clinical features of the proband and segregation analysis of the *VRK1* p.W375X mutation. (A) The pedigree of the proband with dHMN. (B) The clinical features of the proband, with obvious atrophy of two-thirds of the legs and pes cavus. (C) Proband brain MRI axial and sagittal T1 images demonstrating the absence of pontocerebellar hypoplasia. (D) Segregation analysis of the *VRK1* p.W375X mutation. dHMN, distal hereditary motor neuropathy; *VRK1*, vaccinia-related kinase 1. (E) Schematic graph of the *VRK1* coding region and the corresponding VRK1 protein, showing the position of mutations identified in previous reports (blue) and our pedigree (red). ABR, ATP-binding region; ELTS, Endosomal-lysosomal targeting sequence; NLS, Nuclear localization signal; SRPKAS, Ser/Thr protein kinases active-site; VRK1, Vaccinia-related kinase 1.

Table 1. Nerve conduction studies of the proband.

Motor nerves	Distal motor latency (msec)			CMAP amplitude (mv)			MCV (m/sec)		
	Left	Right	RV	Left	Right	RV	Left	Right	RV
Median(APB)	3.4	3.0	<4.4	12.6	7.9	>4.0	68.8	59.1	>49
	6.2	6.8		11.7	7.5				
Ulnar(ADM)	2.9	3.0	<3.3	8.5	8.2	>6.0	64.6	65.1	>49
	6.8	6.9		7.8	7.7				
Peroneal(EDB)	3.5	4.4	<6.7	1.4	0.1	>3.0	47.9	43.2	>44
	9.6	6.1		1.2	0.1				
Tibial(AHB)	3.5	3.8	<5.8	0.8	0.7	>4.0	56.3	54.3	>41
	10.0	10.4		0.7	0.6				

Sensory nerves	Distal motor latency (msec)			SNAP amplitude (μv)			SCV (m/sec)		
	Left	Right	RV	Left	Right	RV	Left	Right	RV
Median	2.3	2.2	<3.5	47.9	43.0	>20	61.4	61.9	>50
Ulnar	1.8	1.9	<3.1	44.6	41.3	>17	62.5	62.1	>50
Peroneal	2.3	2.2	<4.4	24.2	31.2	>6	46.5	49.5	>40
Sural	2.6	2.5	<4.4	17.1	18.9	>6	44.6	47.9	>40

ADM, abductor digiti minimi; APB, abductor pollicis brevis; CMAP, compound motor action potential; EDB, extensor digitorum brevis; MCV, motor conduction velocity; NE, not elicited; RV, reference value; SCV, sensory conduction velocity; SNAP, sensory nerve action potential.

Table 2. EMG of the proband.

Muscle	Sharp waves	Fibrillation	Fasciculation	Duration (msec)	Amplitude (uv)	Recruitment
Left sterno-cleidomastoid m.	−	−	−			Reduced
Right deltoid m.	−	−	−			Reduced
Right first interosseous m.	+	+	−			Reduced
Right T9 paraspinal m.	−	−	−			
Right T10 paraspinal m.	−	−	−			
Left quadriceps femoris m.	+	+	−	19.3(↑↑)	1286(↑↑)	Reduced
Right quadriceps femoris m.	+	+	−	19.1(↑↑)	886(↑)	Reduced
Left tibialanterior m.	++	++	−			Reduced
Right tibialanterior m.	++	++	−			Reduced
Left gastro-cnemius m.	−	+	−			Reduced
Right gastro-cnemius m.	−	−	−			Reduced

anti-Ro/SSA, anti-La/SSB, and anti-nuclear antibodies were negative. Brain MRI was normal, and pontocerebellar hypoplasia or microcephaly, as reported in other patients previously described with *VRK1* mutations, were absent (Fig. 1C).

Screening of the proband's DNA for genes related to ALS and Charcot-Marie-Tooth disease (CMT) (Table S1) using next-generation sequencing revealed one novel homozygous nonsense variant in *VRK1*, c.1124G>A; p.W375X. The variant was Sanger sequenced for confirmation. The subsequent parental studies demonstrated that both asymptomatic parents carry a heterozygous p.W375X variant, supporting autosomal recessive inheritance. The proband's asymptomatic sister also carried the *VRK1* heterozygous p.W375X variant (Fig. 1D).

Discussion

The juvenile onset of weakness in the distal lower extremities slowly progressed to the distal upper limbs (in a length-dependent fashion), along with bilateral pes cavus, the absence of upper motor and sensory neuron involvement, and the absence of electrophysiological denervation changes in the muscles of the proximal upper limbs, thoracic paraspinal muscles and sternocleidomastoid muscles, supporting a diagnosis of dHMN rather than ALS. Next-generation sequencing of the proband identified a homozygous nonsense variant, p.W375X, in the *VRK1* gene.

As per the guidelines of the American College of Medical Genetics and Genomics for sequence variant interpretation,[13] the c.1124G>A; p.W375X variation was

interpreted as pathogenic, as (1) this variation led to a null allele and possibly a deleterious effect (PVS1); (2) it was absent from the control databases (gnomAD, 1000 Genomes Project, ClinVar) (PM2); (3) the pedigree is consistent with an autosomal recessive mode of inheritance with segregation of the p.W375X mutation with the disease (PP1); and (4) in silico tools support a deleterious effect of this variant (PolyPhen2 score 0.999, deleterious; SIFT score 0.001, deleterious) (PP3).

The *VRK1* gene was initially associated with an infantile onset spinal muscular atrophy (SMA) with pontocerebellar hypoplasia (PCH) in an Ashkenazi Jewish family carrying a homozygous p.R358X mutation.[10] Four siblings with PCH from an Iranian pedigree had a *VRK1* homozygous p.R133C mutation.[12] Recently, two siblings from an American family with childhood-onset motor and sensory axonal neuropathy plus microcephaly were found to carry *VRK1* compound heterozygous (p.V236M and p.R89Q) mutations, and a third unrelated patient had a homozygous p.R358X mutation.[9] Compound heterozygous mutations (p.H119R and p.R321C, p.G135R and p.L195V) in *VRK1* were identified in one Hispanic man with sporadic ALS and an Australian with juvenile ALS, respectively.[8,11] Very recently, *VRK1* compound heterozygous mutations (p.H119R and p.R358X) were detected in two siblings of Ashkenazi Jewish origin with adult onset dSMA,[8] whose phenotype is similar to that of the proband in this study, who has a *VRK1* homozygous p.W375X mutation. *VRK1* mutations have previously been reported in Ashkenazi Jewish, American, Hispanic, Australian, and Iranian populations;[8–12] our patient is the first *VRK1* mutation carrier identified in a Chinese population, suggesting that *VRK1* mutation is pan-ethnic. The phenotypic spectrum of *VRK1* mutations include PCH, SMA-PCH, motor and sensory axonal neuropathy plus microcephaly, dSMA/dHMN, and ALS.

Most of the *VRK1* mutations reported are missense mutations and localize in the protein kinase domain of the *VRK1* gene (Fig. 1E).[8,9,11,12] These mutations may alter the charge, size, and hydrophobicity of the protein, resulting in disturbances of local structure and protein misfolding.[8] The only reported nonsense *VRK1* mutation, p.R358X, is within a highly conserved KKRKK nuclear localization signal (NLS) (Fig. 1E), resulting in an absence of functional VRK1 protein caused by significantly lower mRNA levels as a result of nonsense-mediated decay.[10] The homozygous *VRK1* c.1124G>A mutation identified in our proband created a stop codon (p.W375X) near the extreme C terminus (Fig. 1E), and therefore possibly encodes a partially functional, amino terminal truncated VRK1 protein. This may partly explain why the phenotype of our patient, with a homozygous p.W375X mutation, is less severe compared with infantile onset SMA

with PCH in the patient with a homozygous p.R358X mutation.[10]

Our finding extends the ethnic distribution of *VRK1* mutations, indicating that *VRK1* mutation is not restricted to selected populations. Because *VRK1* mutations can demonstrate various motor phenotypes, *VRK1* mutations should be included in panel-based genetic diagnostic testing for recessive dHMN.

Acknowledgments

Author Contributions

S-Y. F., L-Y. L., S-M. F., and Z-Y. Z. contributed to the conception and design of the study. S-Y. F., L-Y. L., S-M. F., and Z-Y. Z. contributed to data acquisition and/or data analysis. S-Y. F. and Z-Y. Z. contributed to drafting the text and preparing the figure.

Conflicts of Interest

The authors have no financial conflicts of interest.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Genes screened for the proband.