

[CASE REPORT]

Novel *VRK1* Mutations in a Patient with Childhood-onset Motor Neuron Disease

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Abstract:

A 24-year-old Japanese man exhibited slowly progressive gait disturbance from childhood to young adulthood. Physical and physiological examinations showed the involvement of both upper and lower motor neurons, fulfilling the diagnostic criteria for amyotrophic lateral sclerosis (ALS). Mild cognitive impairment and subclinical sensory involvement were also observed. A genetic analysis revealed novel compound heterozygous mutations, c.767C>T (p.Thr256Ile) and c.800A>G (p.Asp267Gly), in the vaccinia-related kinase 1 gene (*VRK1*). This is the first report of a Japanese patient with a motor neuron disease phenotype caused by *VRK1* mutations. This diagnosis should be considered in atypical cases of juvenile-onset and slowly progressive types of motor neuron disease.

Key words: motor neuron disease, amyotrophic lateral sclerosis, *VRK1*, childhood-onset

(Intern Med 58: 2715-2719, 2019)

(DOI: 10.2169/internalmedicine.2126-18)

Introduction

Motor neuron diseases (MNDs) are clinically and genetically heterogeneous disorders associated exclusively or predominantly with dysfunction of upper and/or lower motor neurons (1). Amyotrophic lateral sclerosis (ALS), a representative MND, is characterized by both upper and lower motor neuron involvement, and typically follows a rapidly progressive fatal clinical course (2). However, atypical forms of MNDs, including ALS, variably affect the upper and lower motor neurons and patients with these conditions exhibit extra-motor signs and/or slow progression (3).

Mutations in the vaccinia-related kinase 1 gene (*VRK1*) have been reported to cause an atypical, infantile-onset lower MND phenotype [i.e., spinal muscular atrophy (SMA)], which is associated with pontocerebellar hypoplasia as well as death in late childhood (4). Moreover, some pa-

tients have demonstrated clinical upper motor neuron signs mimicking ALS (5, 6). We herein present a 24-year-old Japanese man with childhood-onset mild MND that fulfilled the diagnostic criteria for ALS, and who had novel compound heterozygous mutations in *VRK1*. This study describes the first Japanese case of MND with *VRK1* mutations.

Case Report

The patient was a 24-year-old Japanese man who was born with a normal birthweight and whose motor developmental milestones were almost normal, including stable head control at 4 months and acquisition of walking at 14 months. He had no notable family history with the exception of a paternal cousin with intellectual disability and autism without motor involvement (Fig. 1a).

At around 3 years of age, his family suspected that he

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Received: September 13, 2018; Accepted: March 26, 2019; Advance Publication by J-STAGE: June 7, 2019

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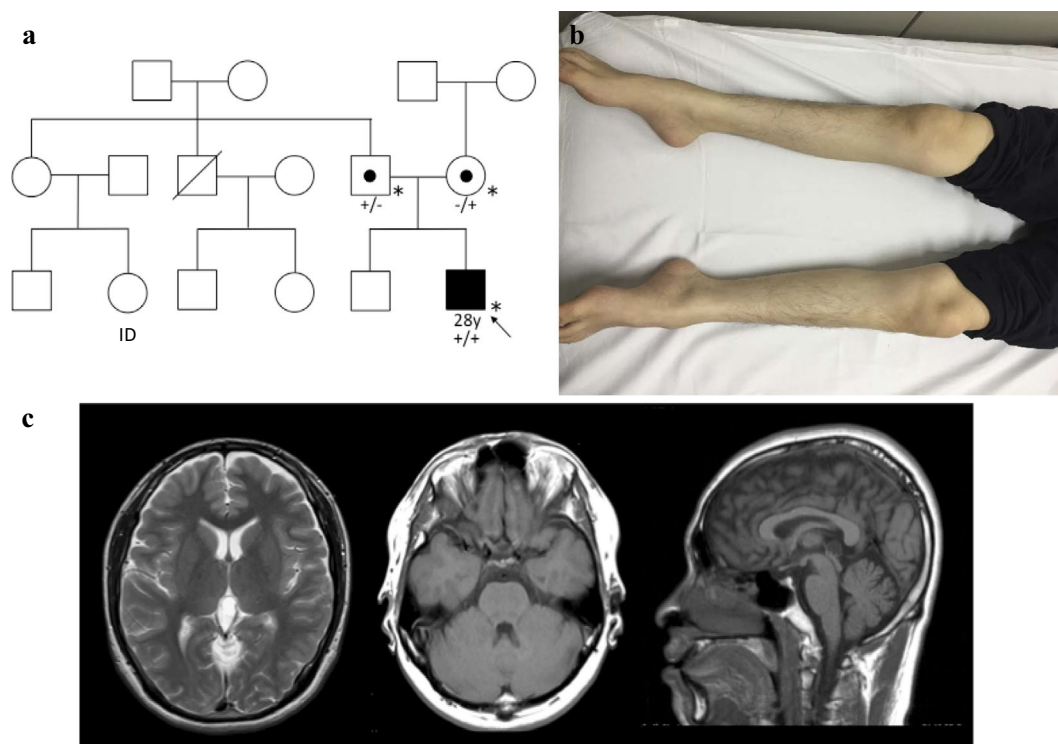


Figure 1. The clinical presentation of the patient. (a). The family pedigree. Individuals with available blood samples are indicated by an asterisk (*). The *VRK1* compound heterozygous mutation is indicated by +/+. The heterozygous mutation is indicated by +/- or -/+. ID: intellectual disability (b). Photograph of the lower limbs. There was atrophy of the distal limbs, especially the lower legs. (c). Brain MRI findings. T2- (left) and T1-weighted imaging (middle and right). There were no abnormal findings, with the exception of a pineal cyst. There was no pontocerebellar hypoplasia.

had mild intellectual disability, which was confirmed by intelligence testing at 6 years of age. At 7 years of age, he developed difficulty in running, and gait instability was noted approximately 2 years later. He was admitted to a nearby hospital and spasticity was observed in his legs. However, the cause of the symptoms remained unknown. After graduation from a regular high school class at 18 years of age, he became aware of weakness in his lower legs, leading to difficulty in walking. At 23 years of age, he developed difficulty in climbing stairs and fell occasionally. He also noticed weakness in his grip strength in the same year.

Neuromuscular diseases such as cerebral palsy, ALS, and SMA were suspected at that time, and genetic testing for SMA, including the survival of motor neuron 1 (*SMN1*) and NLR family apoptosis inhibitory protein (*NAIP*) mutations, was conducted, but no mutations were found in these genes.

At 24 years of age, he was admitted to our hospital. On physical examination, he had prominent muscular atrophy that particularly affected his lower legs, including the tibialis anterior and gastrocnemius muscles (Fig. 1b). Manual muscle testing showed symmetrical weakness predominantly in his distal limbs; the Medical Research Council (MRC) scores of the proximal muscles of the upper and lower limbs were 5/5 and 4/5, respectively, while those of the distal muscles were 3/5 and 1/5, respectively. The patellar tendon reflexes were brisk, and tendon reflexes in the upper limbs

were slightly increased. He showed steppage gait due to weakness of the tibialis anterior muscles. There were no fasciculations, pathological signs (e.g., extensor plantar reflexes), sensory symptoms, or cerebellar ataxia. His Mini-Mental State Examination score was 23/30, indicating mild cognitive impairment.

The patient's serum creatine kinase level was mildly elevated [488 U/L (normal range: 40-265 U/L)]. Needle electromyography (EMG) showed chronic neurogenic changes in the upper and lower limb muscles; the rectus femoris and extensor carpi radialis muscles showed muscle unit potentials (MUPs) with high amplitude and a long duration, as well as reduced recruitment of MUPs without spontaneous activity at rest. The mentalis and sternocleidomastoid muscles showed normal MUPs. In a nerve conduction study, compound muscle action potentials (CMAPs) exhibited low amplitudes in the median and peroneal nerves without any reduction in motor conduction velocities. The sensory nerve action potentials (SNAPs) of the ulnar and sural nerves showed low amplitudes (Table).

Muscle CT revealed mild atrophy in the thigh and severe atrophy in the lower legs. On brain MRI, a pineal cyst was incidentally detected, but cerebellar hypoplasia was not found (Fig. 1c).

The patient's respiratory function was normal (forced expiratory volume 1.0%=91.5%, %vital capacity=83.6%), and

electrocardiography and echocardiography revealed no evidence of cardiac involvement.

The patient's physiological findings suggested the involvement of both the upper and lower motor neurons in the cervical and lumbosacral segments of the spinal cord, fulfilling the revised El Escorial criteria for probable ALS (7). However, the slowly progressive course, mild intellectual disability beginning in childhood, and mild electrophysiological sensory nerve involvement were atypical for ALS.

To identify the causative genetic mutations, whole-exome sequencing was performed, as previously described (8). Genomic DNA was processed using a SureSelect Human All Exon Kit v5 (Agilent Technologies, Santa Clara, USA). Captured DNAs were sequenced using a HiSeq2500 (Illumina,

San Diego, USA). We checked whether the genes related to spastic paraplegia, ALS/frontotemporal lobar degeneration, hereditary motor neuronopathies (HMN), or SMA exhibited any rare variants (minor allele frequency <1% in our database of 575 normal Japanese exomes). Among these, we identified potentially pathogenic homozygous or compound heterozygous variants for autosomal recessive diseases and heterozygous variants for dominant diseases.

Only two variants of *VRK1* were detected as potential causative mutations for the disease. Sanger sequencing of the patient and his parents confirmed the existence of *VRK1* mutations in the patient (NM_003384) c.767C>T (p.Thr256Ile) and c.800A>G (p.Asp267Gly), and each parent had one of the mutations, suggesting autosomal recessive inheritance (Fig. 2a). Neither mutation was registered in the Exome Aggregation Consortium (ExAC) database (<http://exac.broadinstitute.org/>) or in our in-house database of 575 normal Japanese exomes. The mutations were predicted to be possibly damaging (score=0.726, sensitivity: 0.86, specificity: 0.92) and probably damaging (score=0.998, sensitivity: 0.27, specificity: 0.99) by PolyPhen-2 (<http://genetics.bwh.harvard.edu/pph2/>), respectively, and to be disease-causing by Mutation Taster (www.mutationtaster.org/), but neutral (score=-0.511) and deleterious (score=-5.046) by PROVEAN (<http://provean.jcvi.org/index.php>), respectively. The conservation and location of the mutated amino acids are shown in Fig. 2b and c, respectively. Amino acid Asp 267 was evolutionally highly conserved, while the degree of conservation was somewhat lower at amino acid Thr256 (Fig. 2b). The distribution of reported and currently identified *VRK1* mutations did not show a specific "hotspot" (Fig. 2c).

Although the mutations had not been reported previously,

Table. Nerve Conduction Study Results.

Motor nerve			
	DL (ms)	NCV (m/s)	CMAP (mV)
R. Median	4.0 (<4.2)	51 (>48)	1.2 (>3.5)
R. Ulnar	2.7 (<3.4)	64 (>49)	5.4 (>2.8)
R. Tibial	4.0 (<6.0)	50 (>41)	5.7 (>2.9)
R. Peroneal	N.E. (<5.5)	N.E. (>40)	N.E. (>2.5)
Sensory nerve			
	DL (ms)	NCV (m/s)	SNAP (μ V)
R. Median	2.2 (<3.5)	65 (>47)	26 (>20)
R. Ulnar	2.0 (<3.1)	65 (>44)	10 (>18)
R. Sural	3.4 (<3.4)	43 (>41)	2 (>10)

Median nerve stimulation shows low CMAP in the adductor pollicis brevis muscle, and low SNAP in the sural nerve. Normal limits are indicated in parentheses. DL: distal latency, NCV: nerve conduction velocity, CMAP: compound muscle action potential, N.E.: not evoked

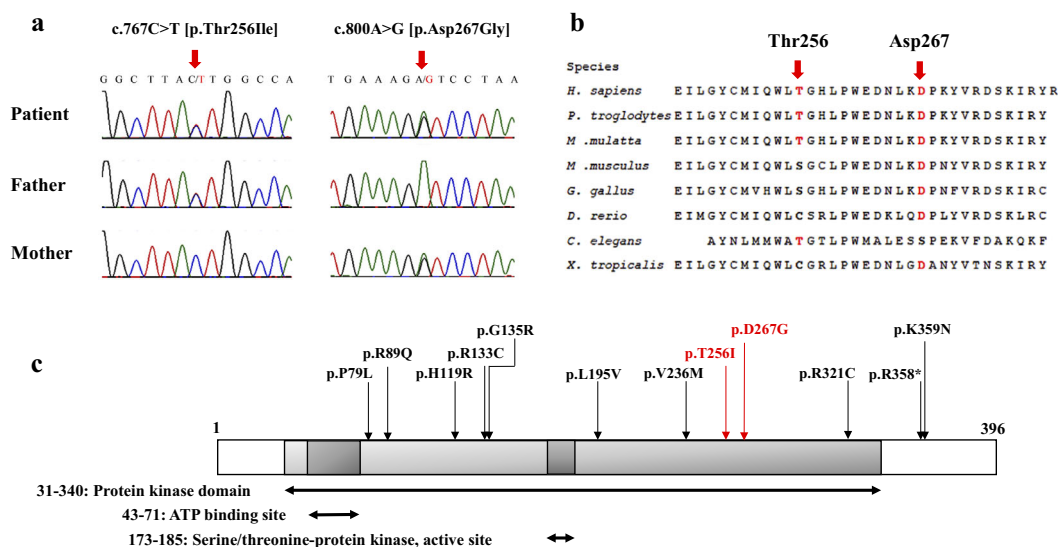


Figure 2. The *VRK1* mutations of the patient. (a) An electropherogram of the genomic *VRK1* sequence. Two mutations were detected in *VRK1*. Arrows indicate *VRK1* c.767C>T (p.Thr256Ile) and c.800A>G (p.Asp267Gly) mutations. (b) Conservation of amino acids Thr256 and Asp267. (c) A schematic illustration of the structure and mutations of the *VRK1* protein. Black and red arrows indicate the locations of known mutations and the mutations identified in this case, respectively.

we considered them to be causative based on the results of amino acid-level prediction algorithms, the conservation of amino acids, and the fact that the patient's phenotype was consistent with previous cases of *VRK1* mutations.

Discussion

We reported the case of a 24-year-old Japanese man who presented with a mild ALS-like phenotype of MND during childhood with novel compound heterozygous mutations in *VRK1*. This is the first Japanese case of an MND phenotype caused by *VRK1* mutations. *VRK1* was identified as a causative gene for infantile-onset SMA with pontocerebellar hypoplasia in a consanguineous family of Ashkenazi Jewish origin (c.1072C>T; p.Arg358*) (4). However, recent studies have shown that mutations in this gene could cause a broader spectrum of MND, including ALS and motor neuropathy (6). *VRK1* mutations were reported by Nguyen et al. in a case of slowly progressive adult-onset ALS (c.356A>G; p.His119Arg and c.961C>T; p.ArgR321Cys) (5), and Stoll et al. reported two families: one with childhood-onset ALS with short stature and microcephaly (c.403G>A; p.Gly135Arg and c.583T>G; p.Leu195Val); the other with childhood-onset SMA with mild to moderate generalized brain atrophy (c.356A>G; p.His119Arg and c.1072C>T; p.Arg358*) (6). *VRK1* mutations do not necessarily cause MND, and patients with only moderate to severe intellectual disability and pontocerebellar hypoplasia (c.397C>T; p.Arg133Cys) have been reported (9). Our patient presented with upper motor neuron signs and physiological findings corresponding to lower motor neuron degeneration, which met the diagnostic criteria for ALS; however, he also showed extra-motor signs such as intellectual disability and subclinical sensory nerve involvement, which was evidenced by low-amplitude SNAPs. Although ALS and SMA (excluding type I SMA) do not affect sensory nerves (10), a patient with complex motor and sensory axonal neuropathy with *VRK1* mutations (c.706G>A, p.V236M; and c.266G>A, p.R89Q) was described (11). Mutations of *VRK1* can affect both the motor and sensory systems, but it remains unclear whether the reduction of SNAPs observed in our patient was due to axonal neuropathy or neuronopathy.

VRK1 is a ubiquitously expressed serine-threonine kinase that is known to play multiple roles, for instance in cellular proliferation, cell cycle regulation, histone modification, DNA repair, disruption of RNA processing, and carcinogenesis (12). As a kinase, *VRK1* phosphorylates several proteins such as p53, barrier-to-autointegration factor 1 (BANF1), histone H3, and CREB, all of which are involved in cell cycle regulation (12). A recent report on partial *VRK1* knockout mice clarified the associated motor phenotype and identified significant changes of expression in the gene sets that predict novel roles for *VRK1*, such as neurotrophin signaling, axon guidance, proteasome-related genes, and oxidative phosphorylation, all of which have been implicated in the pathogenesis of ALS (13). However, the specific path-

way of *VRK1* dysfunction that contributes to motor neuron degeneration remains to be elucidated.

In conclusion, we described the case of a Japanese patient harboring novel *VRK1* mutations associated with mild, and slowly progressive form of ALS-like MND with subclinical sensory involvement, the onset of which occurred in childhood. Our patient demonstrated the broad phenotypic spectrum of *VRK1* mutations, and further studies should be performed to investigate the role of this gene in the differential diagnosis of atypical MNDs with juvenile onset and slow progression.

The authors state that they have no Conflict of Interest (COI).

Hiroshi Doi and Fumiaki Tanaka contributed equally to this work.

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Intern Med 58: 2715-2719, 2019