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RESEARCH NOTE

Novel c.C2254T (p.Q752*) mutation in *ZFYVE26* (SPG15) gene in a patient with hereditary spastic paraparesis

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Abstract. Hereditary spastic paraplegias are clinically and genetically heterogeneous degenerative disorders, and pathological variants in the autosomal recessive *ZFYVE26* gene are considered as very rare causes. We describe a novel mutation in *ZFYVE26* gene found in a patient with autosomal recessive spastic paraplegias. The use of a 'target-gene' approach allowed us to expand the clinical spectrum associated with hereditary spastic paraplegias.

Keywords. *ZFYVE26* gene; hereditary spastic paraplegias; next-generation sequencing.

Introduction

In this study we describe a novel mutation in *ZFYVE26* gene found in a patient with autosomal recessive spastic paraplegias. This is one of the few studies using the 'target-gene' approach and our findings expand the clinical spectrum associated with hereditary spastic paraplegias. Hereditary spastic paraplegias (HSPs) are clinically and genetically heterogeneous disorders whose characteristic spastic paraparesis of limbs can be complicated by intellectual disability, optic atrophy, hearing loss, cerebellar ataxia, peripheral nerve involvement, or epilepsy (Depienne *et al.* 2007). HSPs, which may be transmitted as an autosomal dominant (AD-HSP), autosomal recessive (AR-HSP), or X-linked trait are neurodegenerative diseases with a prevalence of 0.1–9/100,000. About 80 genes or loci are known to cause HSPs but only a smaller number of genes are responsible for most cases (Burguez *et al.* 2017). Over 45 published genes and about 730 mutations (The Human Gene Mutation Database, HGMD professional 2017.4) (Stenson *et al.* 2014) are known to date as the main causative factor of spastic paraplegia. *SPAST* (SPG4) is the most common affected gene in AD-HSPs, followed by ATL1 *SPG3A*, while AR-HSPs are mostly due to mutations in *SPG11* and *ZFYVE26* (SPG15) genes (Ruano *et al.* 2014). *ZFYVE26* (SPG15) and primarily KIAA1840 (SPG11) have been identified as the most frequent genes causing spastic paraplegia with thin corpus callosum and intellectual disability (Casali *et al.* 2004; Denora *et al.* 2009). *ZFYVE26* (Zinc Finger Fyve Domain-containing Protein 26) gene, called spastizin (MIM: 270700), is located on chromosome 14q24.1 and contains 42 exons encoding a protein of 2539 amino acid residues including a zinc finger, a leucine zipper and a FYVE domains (Murmu *et al.* 2011). Itis primarily expressed in neurons and involved in lysosomal biogenesis. It appears to be particularly relevant in cortical motoneurons and Purkinje cells where ZFYVE26 mutations cause endolysosomal membrane trafficking defect, axonal degeneration, and cells loss (Khundadze *et al.* 2013). Traditional molecular diagnostic testing for HSPs is time-consuming and expensive, due to their high genetic heterogeneity and overlapping clinical symptoms. The advent of high throughput next-generation sequencing (NGS) has accelerated the identification of new genes and new genetic causative variations.

Materials and methods

Our NGS targeted panel approach analyses of 30 representative genes (*AP4B1*, *AP4E1*, *AP4M1*, *AP4S1*,

ATL1, *BSCL2*, *C12orf65*, *CYP2U1*, *CYP7B1*, *DDHD2*, *ERLIN2*, *HSPD1*, *KIAA0196*, *KIAA0415*, *KIF5A*, *L1CAM*, *NIPA1*, *PLP1*, *PNPLA6*, *REEP1*, *RTN2*, *SLC33A1*, *SPAST*, *SPG11*, *SPG20*, *SPG21*, *SPG7*, *VPS37A*, *ZFYVE26*, *ZFYVE27*). This study was approved by the Ethical Committee of the our Institute, and informed consent was obtained by parents. A 27-year-old male born of consanguineous parents (grandparents were first cousins), was observed for a progressive worsening of motor skills up to the loss of ambulation. The course of pregnancy was uneventful and childbirth without evidence of perinatal diseases; development of motor skills were age-appropriate and he had no learning difficulties at school. At the age of 18, he started having difficulty in balance and ambulation. The severity of clinical features and medical tests are shown in table 1. Electromyography/nerve conduction velocity (EMG/NCV) showed axonal motor neuropathy, denervation with fasciculations. Neuroimaging showed hemispheric cerebral atrophy, mega cisterna magna, thinning of the corpus callosum, subtle alterations of the periventricular white matter. Biochemical blood test revealed elevated blood levels of liver enzymes. The patient has three younger siblings who do not have clinical signs of the disease yet. Genomic DNA (gDNA) was isolated from lymphocytes using the salt chloroform extraction method. Degradation was checked on agarose gel, and DNA was quantified by the Qubit 2.0 Fluorometer with the Qubit dsDNA HS assay kit (Thermo Fisher Scientific, Foster City, USA). The panel used included 30 genes (Thermo Fisher Scientific, IAD37592). This panel consisted of two primer pools with 847 different amplicons. The average coverage was 94%. Amplicon library, emulsion PCR, sequencing runs, data analysis of runs, and Sanger confirmation were performed as described in Calì and coworkers (Calì *et al.* 2017). NGS was performed using the Ion Torrent Personal Genome Machine (Ion-Torrent). We removed all the common variants (minor allele frequency, MAF >1%) reported in the following public databases: 1000 Genome Project and Exome Sequencing Project. We searched for all pathogenic variants in The Human Gene Mutation Database (HGMD Professional 2017). Pathogenic status of variant identified was classified using criteria shown in table 3 of ACMG guidelines (Richards *et al.* 2015).

Results and discussion

Pathological variants in the autosomal recessive *ZFYVE26* gene are considered to be a rare cause of HSPs, contributing in less than 5% of patients; about 35 variants (missense/nonsense/small deletions) have been described, 40% of which are nonsense (Stenson *et al.* 2014). We identified a homozygous variant c.C2254T (p.Q752*) in the *ZFYVE26* gene (NM_015346, exon12; reads total count: 136, T = 136, C = 0) not described before (figure 1). Average depth of NGS: 237 reads. The variant results in a point-nonsense codon which is predicted to result either in a defective messenger RNA (mRNA) degraded by nonsense-mediated mRNA decay or in a truncated, and nonfunctional protein product, removing all three recognizable domains: zinc finger (1563–1587 aa residues), FYVE domain (1818–1867 aa) and leucine zipper domain (2217–2238 aa). The impact of the mutation on the

Table 1. Clinical findings and medical tests in the patient.

Obesity	+++
Micropenis	+
Cognitive impairment	+++
Stereotyped movements	+++
Psychosis	+
Soliloquies	++
Spastic tetraplegia with prevalent lower limb impairment	+++
Axonal neuropathy	+
Distal amyotrophy	+++
Diffuse fasciculations	+++
Pes cavus with hammer toes	+++
Extensor plantar response	+
Wrist trochlea	+
Dysmetria with action tremor	+++
Ataxia	++
Urinary and fecal incontinence	+
Decreased visual acuity	+
Pigmentary retinal degeneration	–
Visual evoked potential (VEP) (Delayed P100)	+
Dysarthria	++
EEG pathological (slow)	+
Seizures	+

+++, severe; ++, mild; +, present; –, absent.

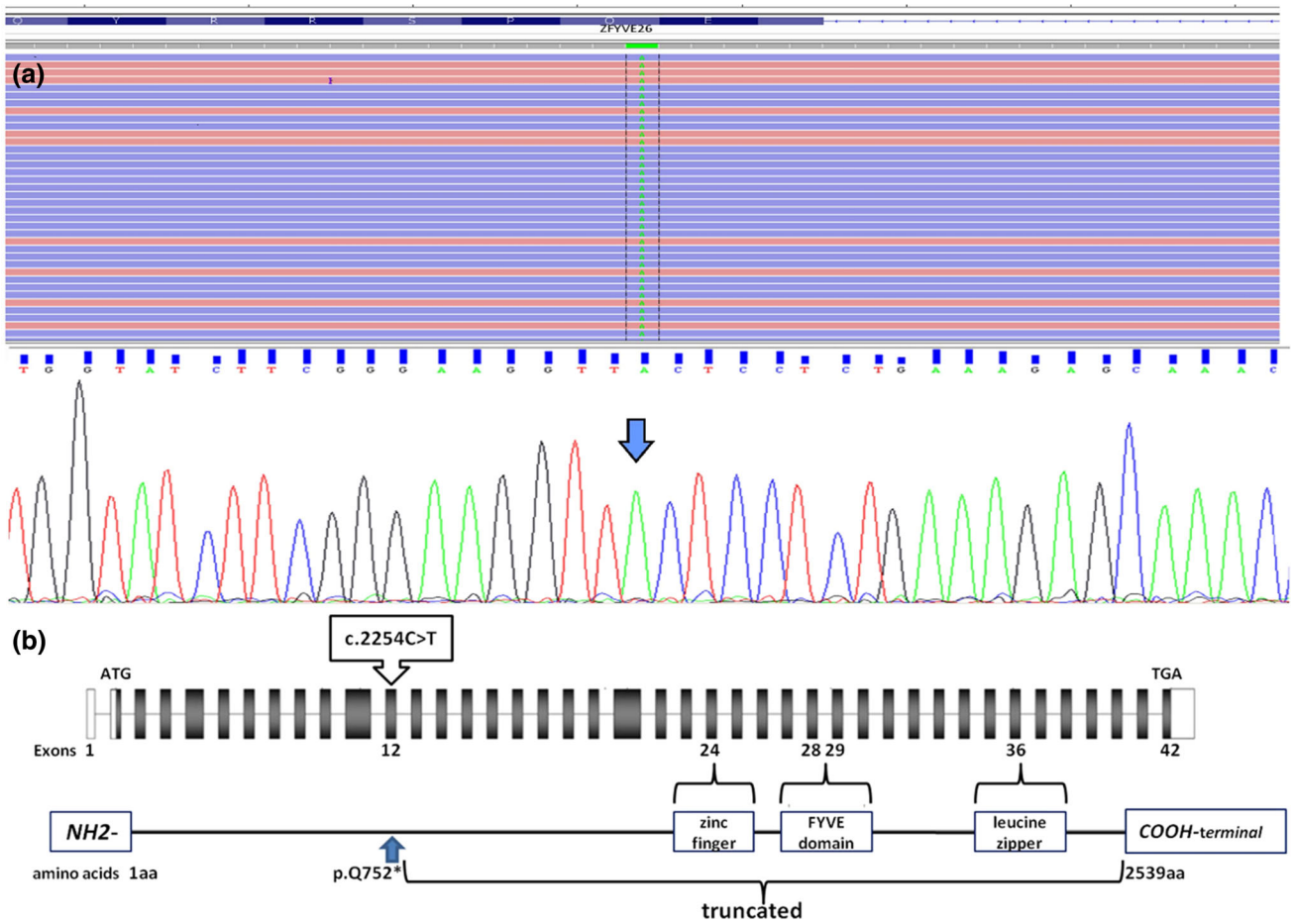

Figure 1. (a) Integrative genomics viewer visualization (up) and DNA sequencing electropherogram (down) showing homozygous novel c.C2254T (p.Q752*) variation in *ZFYVE26* gene. (b) c.C2254T (p.Q752*) in *ZFYVE26* gene. The variant results a truncated protein product, removing all three recognizable domains: zinc finger, FYVE and leucine zipper domains.

structure of protein was predicted to be 'very strong' according to ACMG standard guidelines. The analysis did not identified any other clinically significant variants in the rest of the HSP-genes targeted by our assay, additionally supporting the causative role of the *ZFYVE26* gene mutation.

Analysis of genotype–phenotype correlations is complicated by low number of mutations identified and by the phenotypic heterogeneity. Clinical overlap between patients with different mutations indeed greatly complicates genetic diagnosis, suggesting the important role of new sequencing technologies in genetic investigations. This is one of the few studies using the 'target-gene' performed on HSPs patients that has allowed to increase the number of HSP-related mutations and extend the clinical features associated with variant in *ZFYVE26* gene.

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