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RESEARCH NOTE

A novel missense mutation (c.1006C>T) of *SPG20* gene associated with Troyer syndrome

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Abstract. The number of gene mutations involved in the hereditary spastic paraplegias is rapidly growing due to the expansion of the frontiers of genomic research by next-generation DNA sequencing platforms. Nevertheless, a comprehensive genetic diagnosis method remains yet unavailable for these diseases. In the current research, an 8-year-old boy with short stature and developmental delay impairment, from a nonconsanguineous family, was referred to our genetic lab. Firstly, based on the physician recommendation, the patient was evaluated by tandem mass spectrometry (MS/MS) for the quantitative examination of amino acids, and then the patient was genetically investigated by karyotype analysis and whole-exome sequencing (WES) technique. Subsequently, targeted Sanger sequencing was applied to confirm the presence of the candidate variant in all the members of the family and screening the other patients for Troyer syndrome. Analysis of inherited metabolic disorders by tandem MS/MS showed the state of all the family members as normal and also karyotyping indicated no chromosomal aberration in the patient. Further investigation by WES technique indicated a homozygous missense variant in the *SPG20* gene, c.1006C>T. Targeted sequencing result of the mutation confirmed homozygote state for the affected case and a heterozygote genotype for his parents. The mutation was classified as pathogenic. Detection of novel variants especially pathogenic variant in the *SPG20* gene was associated with Troyer syndrome, which encodes a multifunctional protein termed Spartin, assist in improving genotype–phenotype correlation of genetic variants and may facilitate initial diagnosis of Troyer syndrome.

Keywords. tandem mass spectrometry; karyotype analysis; whole-exome sequencing; hereditary spastic paraplegias; developmental delay.

Introduction

Troyer syndrome (MIM: 275900) is one of the complicated genetic disorders known as hereditary spastic paraplegias (HSP) characterized by distal amyotrophy, dysarthria (speech difficulties), mild developmental delay, short stature, weakness and skeletal abnormalities (Proukakis *et al.* 2004). Incomplete function of Spartin protein coded by the SPG20 gene located on 13q13 chromosome leads to Troyer syndrome (Patel *et al.* 2002). Former studies have shown that people with genetic variants in *SPG20*, *FARS2*, *C12ORF65*, *SPG7*, *HSPD1* and *IBA57* genes involved in mitochondrial function had variable manifestations of HSP (Bross *et al.* 2008; Shimazaki *et al.* 2012; Lossos *et al.* 2015; Shanmughapriya *et al.* 2015; Yang *et al.* 2016). The *SPG20* gene mutations are associated with changes in lipid droplets surface and the changes play key role in altering lipid-mediated signal transduction in HSP pathogenesis (Renvoise *et al.* 2012). Additionally, previous report revealed that a mutation in *SPG20* corresponded to altered neuronal growth and increasing neurite outgrowth (Diquigiovanni *et al.* 2018). Due to the absence of Spartin, a severe reduction in NADH-dehydrogenase activity of mitochondrial complex I leads to reduction in ATP synthesis and subsequent increase in extracellular pyruvate (Diquigiovanni *et al.* 2018). Further, increase in the rate of glycolysis is also observed due to the impaired complex I activity and excessive activation of signal transducer and activator of transcription 3 (STAT3) factor which is the main regulator of glycolysis (Diquigiovanni *et al.* 2018). Nowadays, identification of mutations in *SPG20* related to HSP incidence is growing due to the availability of next-generation DNA sequencing platforms

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such as WES. Nevertheless, the genetic diagnosis technique remains unavailable for many patients with Troyer syndrome. The present study attempts to inform a novel variant in *SPG20* gene associated with Troyer syndrome.

Results

Case presentation

The patient is an 8-year-old male born to Iranian parents referred to the medical genetic center after the indication of short stature, muscle weakness, developmental delay and speech difficulties (dysarthria). There were no known exposures to illnesses, infections, tobacco, alcohol, or drugs during pregnancy. After the birth, the proband was in NICU for 19 days. The subject's developmental delay was first evident at the age of 1.5 years. When he was 8-year old his weight was 15 kg and height was 110 cm. He felt fatigue after walking for long distances. He had no difficulties while learning at home but his social response was poor with his friends and teachers at school. All the other physical features were reported as normal. Molecular karyotype analysis showed normal 46, XY constitution. Brain MRI was normal and the patient's mother and father were 150 and 178 cm tall, respectively. History of various diseases including PKU, hearing and speech disorders, cardiovascular disease, favism and recurrent miscarriage are studied in their family history (figure 1). Both the parent's families are from the north of Iran and consanguinity was denied.

Biochemical investigations

The quantitative examination of amino acids in plasma was imperative for patient. The major types of inherited metabolic disorder include amino acid, organic acid and fatty acid. Currently, MS/MS is being used for the identification of several amino acid, organic acid and fatty acid disorders. Amino acid analysis revealed a normal state in initial concentrations of analytes using LC/MS-MS technique. GC-MS results of the patients' analytes confirmed the results of LC/MS-MS (table 1).

Molecular analysis

Karyotype analysis: To evaluate the probable chromosome abnormalities in patient, karyotype analysis of 15 metaphases spread based on GTG technique and 500 bands resolution were implemented which exhibited 46 chromosomes for the patient (figure 2).

Whole-exome sequencing (WES): To explore the probable disease-causing variants, genomic DNA was isolated from blood samples and WES was conducted by Illumina platform. The only predicted deleterious mutation was a homozygous missense variant in the *SPG20* gene, c.1006C>T (table 2). The SPG20 variant c.1006C>T (p. Glu336*) changes amino acid sequence of the protein (from glutamine to stop codon) at position 336.

In the current study, one homozygous patient and two heterozygous individuals for c.1006C>T were identified in the *SPG20* gene which was nominated as pathogenic variant. There is no published data available on allele frequency in patients with Troyer syndrome but was detected in our laboratory in a homozygous person, and we were unable to find the second variant.

Amplification and Sanger sequencing

Polymerase chain reaction (PCR) amplification and targeted sequencing of the identified variant were used in genomic DNA of all family members to verify the mutation and segregation analysis. The DNA sequence of *SPG20* gene was obtained from the NCBI database. We designed locus-specific primers using the Oligo7 software. Then, the samples were sequenced by Sanger sequencing method on an Applied Biosystems 3130xl Genetic Analysers. Finally, the obtained sequences were assessed by BioEdit software. All reactions were performed with two replicates per sample besides a nontemplate control (NTC) for each test. Both parents were found to be the carrier of c.1006C>T mutation in the heterozygote state (figure 3). The mutation was classified as pathogenic, because the substitution of glutamine creates a new stop codon which stops the synthesis of the protein.

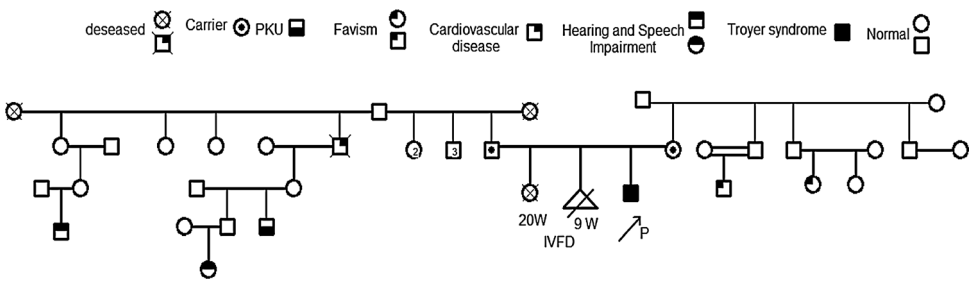

Figure 1. Pedigree chart shows known history of different diseases such as Troyer syndrome in the studied family.

Table 1. Analysis of inherited metabolic disorders diagnosis by tandem mass spectrometry (MS/MS).

Amino acid disorders	Result	Fatty acid oxidation disorders	Result	Organic acid disorder	Result
Phenylketonuria (PKU)	Normal	CACT deficiency	Normal	Methymalonic acidemia (MMA)	Normal
Maple syrup urine disease	Normal	CPT−1 deficiency	Normal	Isovaleric acidemia (IVA)	Normal
Homocystinuria (HCY)	Normal	CPT−2 deficiency	Normal	Multiple carboxylase deficiency (MCD)	Normal
Tyrosinaemia	Normal	TFP deficiency	Normal	MCC deficiency	Normal
Citrullinaemia (Cit)	Normal	SCHAD deficiency	Normal	Ketothiolase deficiency	Normal
Argininosuccinin acidaemia	Normal	MCAD deficiency	Normal	OH−3-methylglutaric acidemia	Normal
Argininaemia	Normal	VLCAD deficiency	Normal	Propionic acidemia (PA)	Normal
		Primary carnitine deficiency	Normal	Glutaric acidemia (GA)	Normal

Results that lie outside the laboratory reference ranges are considered to be abnormal otherwise it is regarded as normal.

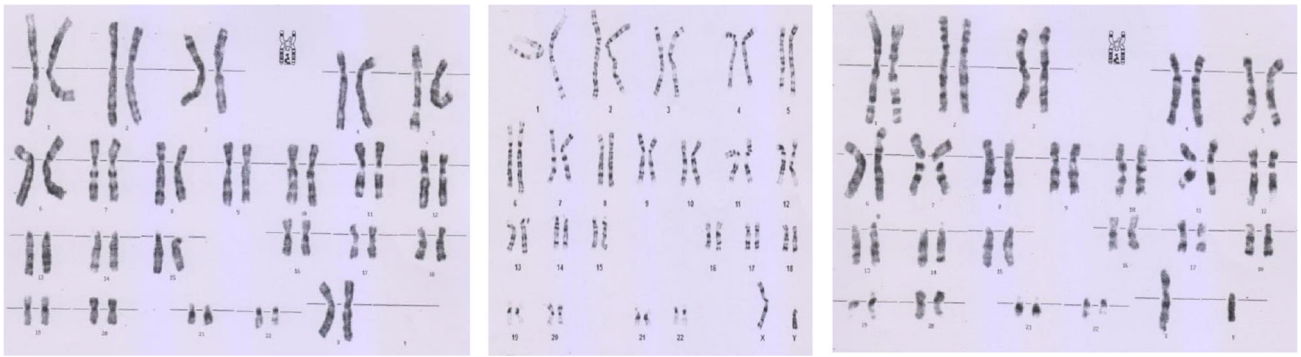

Figure 2. The results of karyotyping showed no chromosomal aberration detected in the patient and his parents (from left to right: father, patient and mother)

Table 2. The detected homozygous missense variant in *SPG20* gene using WES.

Sample	Affected	SNP	Call copies	Genotype
Affected case	Yes	SPG20: c.1006C>T	2	TT
Father	No	SPG20: c.1006C>T	1	CT
Mother	No	SPG20: c.1006C>T	1	CT

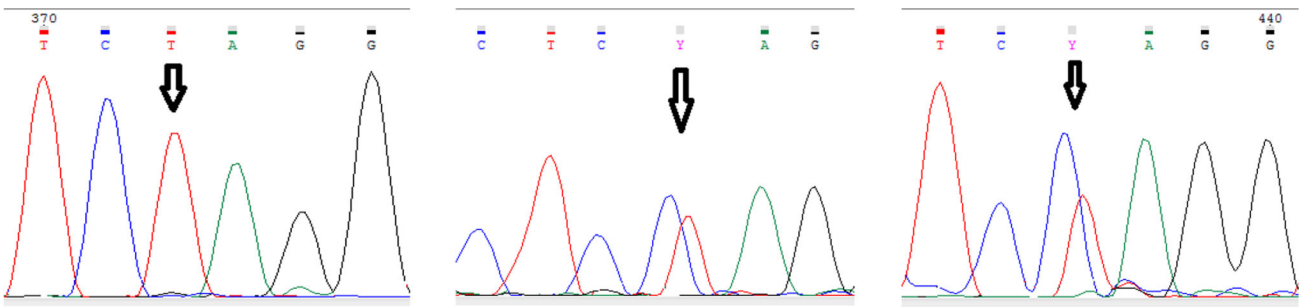

Figure 3. Targeted sequencing result of c.1006C>T mutation in *SPG20* gene indicates a homozygote state for affected case and a heterozygote genotype for his parents, respectively (from left to right).

Discussion

In this study, the patient presented with clinical features was associated with Troyer syndrome which was due to deleterious mutations in *SPG20* gene leading to loss of Spartin protein function (Bakowska *et al.* 2008). Spartin is a multifunctional protein with an eminent role in the dendritic aggresome-like induced structures (DALIS) formation (Karlsson *et al.* 2014), lipid droplet formation (Eastman *et al.* 2009) and cytokinesis (Yang *et al.* 2008; Renvoise

et al. 2010). We detected a homozygous missense variant located in coding region of *SPG20* gene (c.1006C>T). Our result is consistent with the previous studies that identified missense mutations and frameshift changes resulting in disruption of the Spartin protein (Patel *et al.* 2002; Manzini *et al.* 2010; Alazami *et al.* 2015; Butler *et al.* 2016). To date, five pathogenic variants of *SPG20* gene associated with Troyer syndrome have been reported. These variants including c.1369C>T (p.Arg457Ter), c.1110del (p.Lys370Asn), c.696delT (p.Phe232Leu), c.685C>T (p.Gln229Ter), and c.364_365delAT (p.Met122Val) revealed variable clinical features (Tawamie *et al.* 2015; Spiegel *et al.* 2016; Dardour *et al.* 2017). The present study attempts to inform about a novel variant (c.1006C>T) in *SPG20* gene causing Troyer syndrome. This variant has not been reported in related databases and is identified in an Iranian male patient for the first time. Comparison of this patient's clinical features with Troyer syndrome patients from former reports indicated a phenotypic overlap, including delayed milestones, dysarthria, skeletal abnormalities and small stature. In spite of these similarities, some specific differences such as severe intellectual disability were observed among reported findings (Tawamie *et al.* 2015). In contrast with the brain MRI scans of the Amish patients that revealed a deep white matter abnormalities, the MRI scan results for this presented case was normal, which are in consistent with Turkish (Tawamie *et al.* 2015) and Israeli-Arab patients (Spiegel *et al.* 2016). The pathogenesis of the disease is not fully elucidated, however, recent evidences reveled that mitochondrial dysfunction plays a major role in Troyer syndrome clinical manifestations (Spiegel *et al.* 2016).

In conclusion, Troyer syndrome is a complicated genetic disorder with various clinical manifestations caused by lack of Spartin in different tissues. Pathogenic variant in the *SPG20* gene associated with Troyer syndrome, which encodes a multifunctional protein termed Spartin, assist in improving genotype–phenotype correlation of genetic variants and may facilitate initial diagnosis of Troyer syndrome.

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