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Molecular Case Studies

RESEARCH REPORT

SPG20 mutation in three siblings with familial hereditary spastic paraplegia

Leila Dardour,[1] Filip Roelens,[2] Valerie Race,[1] Erika Souche,[1] Maureen Holvoet,[1] and Koen Devriendt[1]

[1]Center for Human Genetics, KU Leuven, 3000 Leuven, Belgium; [2]AZ Delta, 8800 Roeselare, Belgium

Abstract Troyer syndrome (MIM#275900) is an autosomal recessive form of complicated hereditary spastic paraplegia. It is characterized by progressive lower extremity spasticity and weakness, dysarthria, distal amyotrophy, developmental delay, short stature, and subtle skeletal abnormalities. It is caused by deleterious mutations in the *SPG20* gene, encoding spartin, on Chromosome 13q13. Until now, six unrelated families with a genetically confirmed diagnosis have been reported. Here we report the clinical findings in three brothers of a consanguineous Moroccan family, aged 24, 17, and 7 yr old, with spastic paraplegia, short stature, motor and cognitive delay, and severe intellectual disability. Targeted exon capture and sequencing showed a homozygous nonsense mutation in the *SPG20* gene, c.1369C>T (p.Arg457*), in the three affected boys.

[Supplemental material is available for this article.]

INTRODUCTION

Hereditary spastic paraplegias (HSPs) are a group of clinically and etiologically heterogeneous neurodegenerative disorders characterized by progressive spasticity and weakness of lower extremities (Fink 2000). When associated with additional manifestations (e.g., intellectual disability or other organ anomalies), it is called complicated spastic paraplegia. Troyer syndrome (MIM#275900) is an autosomal recessive complicated HSP associated with dysarthria, pseudobulbar palsy, distal amyotrophy, short stature, and subtle skeletal abnormalities. It is caused by biallelic mutations in the *SPG20* gene on Chromosome 13q13, encoding spartin, of which the expression is maximal in the limbs, face, and forebrain primordial during the early stages of embryonic development (Manzini et al. 2010). Three pathogenic *SPG20* variants were identified to date: a homozygous single-nucleotide deletion c.1110delA, in an Amish population (Patel et al. 2002), which leads to loss of protein spartin (Bakowska et al. 2008), a homozygous 2-nucleotide deletion c.364_365delTA in Omani, Turkish, and Filipino families, and a homozygous 1-nucelotide substitution c.988A>G (p.Met330Val) in an Israeli–Arab family (Manzini et al. 2010; Tawamie et al. 2015; Butler et al. 2016; Spiegel et al. 2016).

Here we report three brothers of a consanguineous Moroccan family with clinical features of complicated spastic paraplegia. The diagnosis of Troyer syndrome was made after we identified, by targeted exon capture and sequencing , a novel homozygous pathogenic nonsense mutation in the *SPG20* gene: (NM_015087.4:c.1369C>T; NC_000013.10: g.36888478G>A), which resulted in the substitution of arginine by a codon stop in position 457 (p.Arg457*).

Corresponding author: koenraad.devriendt@uzleuven.be

Ontology terms: absent speech; intellectual disability, mild; progressive spastic paraplegia; spastic gait

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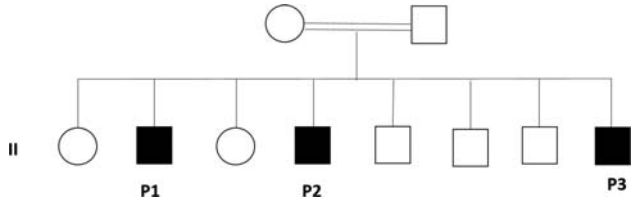

Figure 1. Family pedigree showing the three siblings (P1, P2, and P3).

RESULTS

Clinical Presentation and Family History

The three brothers were born to Moroccan first-cousin parents (Fig. 1). There is no known history of intellectual disability or genetic conditions in the family.

Patient 1

The oldest boy, now aged 24, was born at normal gestational age, with a birth weight of 2865 g (−1 SD), length 45 cm (below −2 SD), and head circumference 35 cm (0 SD). He learned to walk independently at the age of 3 yr, and lost this ability at the age of 16 yr. Clinical examination at current age shows severe intellectual disability, tetraspasticity with dyskinetic movements, and dysmetry of the upper limbs. He is wheelchair-bound for most of the time, walking only short distances with a walking frame. Speech is absent, but there is some language understanding. He has a short stature with height 145 cm (below −4 SD), weight 50 kg (below −2 SD), and macrocephaly (head circumference 59.9 cm, +2 SD). He has scoliosis. There are no evident dysmorphic features except for heavy eyebrows and malimplanted teeth.

Magnetic resonance imaging (MRI) of the brain was performed at the age of 13 yr, showing hyperintense signal in the parieto-occipital regions of the periventricular white matter (Fig. 2A,B).

Patient 2

The second boy, now aged 17, is even more severely affected than his older brother.

He was born at normal gestational age, with a birth weight of 3 kg (−1 SD). He was never able to walk or to speak. Clinical examination at current age shows tetraspasticity (with lower limbs severely affected), dyskinetic movements, severe intellectual disability, staturo-ponderal retardation (weight 18 kg, −8.6 SD, height 117 cm, −7 SD), and mild microcephaly (head circumference 52 cm, −2.1 SD). He had epicanthic folds, low nasal bridge, protruding ears, malimplanted teeth, and drooling.

Brain MRI at the age of 6.5 yr revealed hyperintense periventricular white matter lesions especially in parieto-occipital and frontal regions (Fig. 2C,D).

Patient 3

The youngest boy, now aged 7, was born at the gestational age of 38 wk with a weight of 2.970 kg (−1 SD), length 45 cm (−2.5 SD), and head circumference 35.7 cm (+1 SD). There was respiratory distress due to pulmonary hypoplasia, presumably in the context of a small thorax. He was ventilated for 4 d. At the age of 2 wk, a subglottis web was diagnosed, treated by dilatation. He had a bicuspid aortic valve.

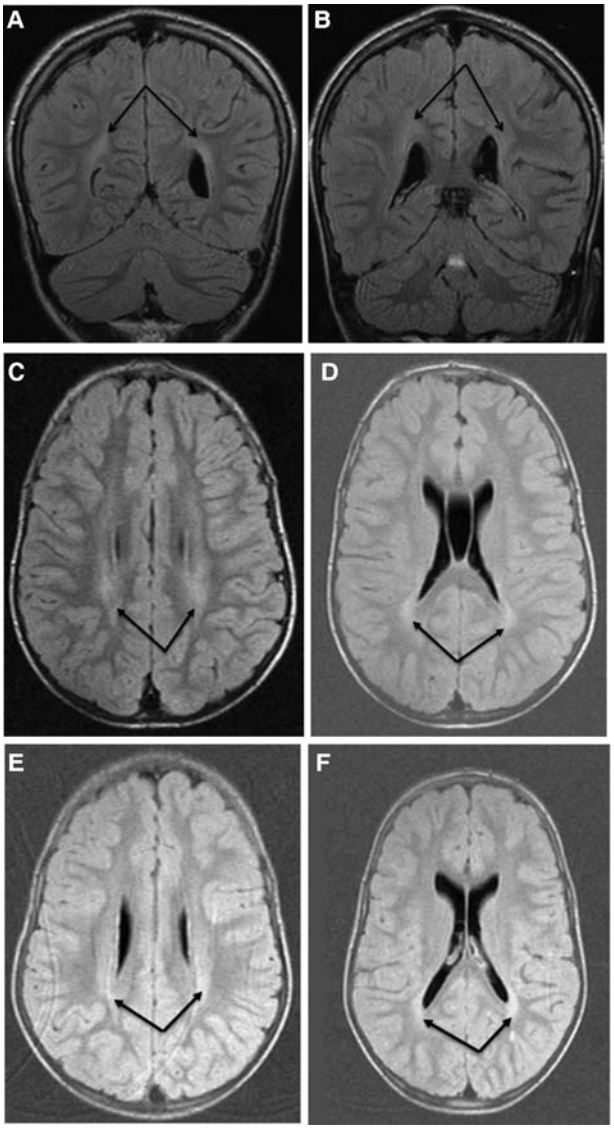

Figure 2. Coronal T2 fluid-attenuated inversion recovery (FLAIR) brain magnetic resonance imaging (MRI) images of Patient 1 (*A*,*B*) and axial T2 FLAIR brain MRI images of Patients 2 (*C*,*D*) and 3 (*E*,*F*) showing hyperintense periventricular white matter lesions, especially in parieto-occipital regions.

At the age of 4 mo, when first seen, his weight was 5.460 g (−2.2 SD), length 55 cm (−4.1 SD), and head circumference 43.7 cm (+1 SD). The thorax was short, with increased antero-posterior diameter (Fig. 3) . He had a short neck. There was rhizomelic shortening especially of the upper limbs. X-rays of the skeleton did not reveal anomalies. He had a sunken nose bridge and a glabellar nevus flammeus.

Subsequently, there was failure to thrive, with feeding difficulties. There were recurrent respiratory infections. At the age of 5 yr, his weight was 11.2 kg (−5.3 SD), height 87 cm (−5.2 SD), and head circumference 51.3 cm (0.1 SD). His development was severely delayed. In the first years of life, he was rather hypotonic. He could sit at the age of 8 mo. He walked independently only at the age of 4.5 yr, but his gait was unstable, with frequent falling. Progressively, he developed spasticity, especially of the lower limbs, with clonus and positive

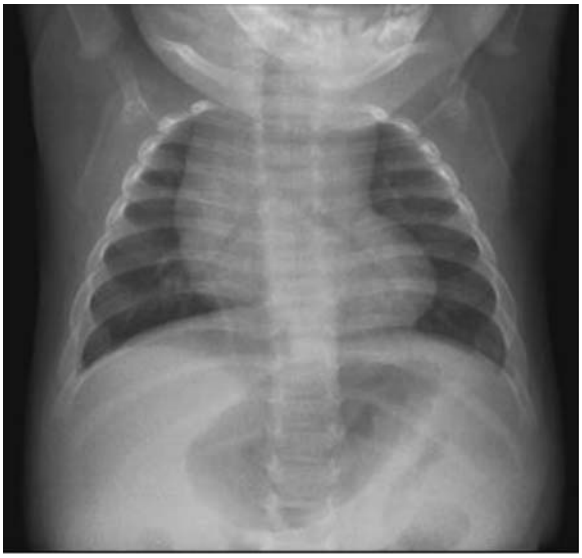

Figure 3. Neonatal chest radiograph of Patient 3 showing the shortening of the thorax.

Babinski response. There were intentional tremor and dysmetric movements of the upper limbs. At the age of 5 yr, he could speak only a few words.

MRI of the brain at the age of 6 yr showed hyperintense white matter on fluid-attenuated inversion recovery (FLAIR) and T2, especially in the parieto-occipital regions (Fig. 2E,F).

Extensive neurometabolic tests did not reveal a diagnosis in any of these three siblings. Molecular karyotyping by means of microarray-comparative genomic hybridization (CGH) (version Agilent 60k) was also normal in the three boys.

GENOMIC ANALYSES

The total number of variants on the TruSight One Panel (using paired-end reads of 151 bp, with a mean coverage of 146×) was 52,625, of which 10 were homozygous exonic variants (Table 1).

The only predicted deleterious mutation was a homozygous nonsense mutation found in the *SPG20* gene, c.1369C>T (p.Arg457*). The nomenclature of the mutation is based on transcript NM_015087.4. The mutation was classified as pathogenic, because it results in the substitution of an arginine for a stop codon in position 457 that is highly conserved among species (11/12 species Alamut V2.7) and leads to the interruption of the reading frame of the protein. This variant was detected in the Exome Aggregation Consortium (ExAC) Browser. The allele frequency was 0.000008244, with a probability of loss-of-function intolerance (pLI) value of zero for this gene. No homozygotes were detected.

The sequence variant was deposited in the Database of Chromosomal Imbalance and Phenotype in Humans (DECIPHER) (*SPG20* gene, DECIPHER ID 339774).

Subsequently homozygosity for this mutation was confirmed in his two affected brothers by Sanger sequencing (Supplemental Fig. S1), and both parents were found to be a carrier of this mutation. Three other siblings were tested by targeted Sanger sequencing. None was homozygous for the mutation.

Table 1. Homozygous exonic variants

Chr	Start	Stop	Ref	Allele 1	Allele 2	Read depth (infoDP)	Gene (gene)	Variant type	Effect (coding effect)	HGVS cDNA-level nomenclature (fullCNomen)	HGVS genomic-level nomenclature (fullGNomen)	HGVS protein-level nomenclature (pNomen3LetterAA)	Allele frequency (ExAC Browser)
2	43,802,152	43,802,152	G	A	A	156	*THADA*	Substitution	NS	NM_001083953.1:c.1052C>T	NC_000002.11:g.43802152G>A	p.Thr351Met	0.0004575
2	44,040,347	44,040,347	T	C	C	123	*ABCG5*	Substitution	NS	NM_022436.2:c.1864A>G	NC_000002.11:g.44040347T>C	p.Met622Val	0.005412
12	7,053,672	7,053,672	C	T	T	34	*C12orf57*	Substitution	NS	NM_138425.2:c.86C>T	NC_000012.11:g.7053672C>T	p.Ala29Val	0.0006442
12	125,444,866	125,444,866	C	T	T	98	*DHX37*	Substitution	NS	NM_032656.3:c.2149G>A	NC_000012.11:g.125444866C>T	p.Val717Ile	0.002648
13	23,915,281	23,915,281	C	T	T	189	*SACS*	Substitution	NS	NM_014363.5:c.2734G>A	NC_000013.10:g.23915281C>T	p.Asp912Asn	0.000008268
13	**36,888,478**	**36,888,478**	**G**	**A**	**A**	**133**	***SPG20***	**Substitution**	**Stop gain**	**NM_015087.4:c.1369C>T**	**NC_000013.10:g.36888478G>A**	**p.Arg457***	**0.000008244**
14	21,811,213	21,811,213	A	G	G	82	*RPGRIP1*	Substitution	NS	NM_020366.3:c.3358A>G	NC_000014.8:g.21811213A>G	p.Ile1120Val	0.0003480
14	21,862,633	21,862,633	C	T	T	102	*CHD8*	Substitution	NS	NM_001170629.1:c.5402G>A	NC_000014.8:g.21862633C>T	p.Arg1801His	0.0003552
15	99,645,840	99,645,840	C	G	G	2	*SYNM*	Substitution	NS	NM_145728.2:c.435C>G	NC_000015.9:g.99645840C>G	p.Asp145Glu	NA
X	48,544,502	48,544,502	C	A	A	44	*WAS*	Substitution	NS	NM_000377.2:c.538C>A	NC_000023.10:g.48544502C>A	p.His180Asn	0.0008345

Boldface indicates the pathogenic mutation.
Chr, chromosome; Ref, reference; HGVS, Human Genome Variation Society; ExAC, Exome Aggregation Consortium; NA, not available; NS, nonsynonymous.

DISCUSSION

Troyer syndrome, named after the family in which the disorder was first identified, was reported in 1967 as one of the rare autosomal recessive conditions found in the Old Order Amish. Only a few patients with suspected Troyer syndrome were subsequently reported in other populations (Cross and McKusick 1967; Farah et al. 1997; Bertini et al. 1998; Auer-Grumbach et al. 1999).

The genetic cause of Troyer syndrome was identified in 2002 by Patel et al. (2002) in the Amish population. A homozygous 1-nt *SPG20* deletion predicted a loss of function of the spartin protein as the disease mechanism (Bakowska et al. 2008).

With the advent of molecular diagnostic testing, additional families are now being identified. The second mutation was first described in Omani families by Manzini et al. (2010), and then found in a Turkish family (Tawamie et al. 2015) and three Filipino patients (Butler et al. 2016). They carried the same mutation c.364_365delTA in exon 2 resulting in a stop codon in the first coding exon (p.Met122ValfsTer2).

A missense mutation was identified in an Israeli–Arab family (c.988A>G (p.Met330Val)) resulting in almost complete loss of spartin in skeletal muscle (Spiegel et al. 2016).

The three siblings presented here carry a new mutation in the *SPG20* gene. It is a homozygous transition in exon 6 leading to a nonsense mutation c.1369C>T that predicts the substitution of an arginine codon with a stop codon in position 457 (p.Arg457*).

Our patients share many features with the other described cases including short stature, spastic paraparesis, distal amyotrophy, motor, speech and cognitive delays, and gait abnormalities (Table 2). Microcephaly was noticed in Patient 2, in the Turkish family, and the Filipino patients. On the contrary, Patient 1 had macrocephaly. Affected Old Amish and Turkish patients presented emotional lability.

Skeletal abnormalities were present in all previous cases except the Filipino kindred. In our series, Patient 1 had scoliosis, and Patient 3 had skeletal anomalies involving the thorax.

Brain MRI in three patients of the Amish cohort showed abnormal T2 hyperintense signal in the periventricular white matter and the posterior limb of the internal capsule (Proukakis

Table 2. Cases previously reported and our series

Clinical features	Old Amish (21 patients)	Omani (six patients)	Turkish (two patients)	Filipino (three patients)	Israeli–Arab (one patient)	Moroccan (three patients)
Microcephaly	–	–	+	+	NA	+/–
Short stature	+	+	+	+	+	+
Intellectual disability, mild	+	+	+	+/–	+	+
Motor delay	+	+	+	+	+	+
Dysarthria	+	+	+	+	+	+
Delayed speech and language development	+	+	+	+	+	+
Gait ataxia	+	+	+	+	+	+
Spastic paraparesis	+	+	+	+/–	+	+
Distal amyotrophy	+	+	+	+	+	+
Abnormality of the skeletal system	+	+	+	–	+	+/–
Brain MRI abnormalities	+	+	–	+/–	–	+
SPG20 mutation	c.1110delA	c.364_365delAT	c.364_365delAT	c.364_365delAT	c.988A>G	c.1369C>T

+, Present; –, absent; NA, not available ; MRI, magnetic resonance imaging.

et al. 2004). The two Omani patients had, on brain MRI, mild atrophy of the cerebellar vermis, mild white matter volume loss, and periventricular white matter hyperintensity on T2-weighted images (Manzini et al. 2010). The Turkish and Israeli–Arab patients had no brain MRI abnormalities (Tawamie et al. 2015; Spiegel et al. 2016). Only one Filipino patient had increased T2/FLAIR signal within the ventrolateral thalami and posterior limb of the internal capsule (Butler et al. 2016). In our cohort, all three patients had hyperintense periventricular white matter lesions on T2/FLAIR, especially in parieto-occipital and frontal regions. These white matter hyperintensities are not seen in the normal population but are nonspecific. For example, they can be present in several other forms of hereditary spastic paraplegia.

Despite these similarities, some specific differences were observed in the present family, including the severe intellectual disability.

In the patients presented in this study, targeted exon capture and sequencing revealed the diagnosis of Troyer syndrome years after their first consultation and several neurometabolic and other genetic investigations. This further highlights the value of diagnostic evaluation via targeted exon capture and sequencing of unresolved cases of HSP.

METHODS

Identification of the *SPG20* Mutation

Targeted exon capture and sequencing using TruSight One Sequencing Panel Kits (Illumina) was performed on the youngest child. It targets 4813 genes associated with known Mendelian phenotypes. Annotation of the variants was done using the Reference Sequencing Database (RefSeq) (release 64) and Cartagena (version 3). The pipeline analysis was used to extract homozygous variants from the sequence data of the TruSight One Panel (Tables 3 and 4).

Targeted Sanger sequencing was performed in the other family members, using, in addition to the M13 universal primers, these exon 6-specific primers:

SPG20 ex6 For TGT AAA ACG ACG GCC AGT CAT GGC ACA TTT AGC ATC TGA

SPG20 ex6 Rev CAG GAA ACA GCT ATG ACC AGG ACG ATG TGA TGT TGC TG

Table 3. Variant filtering

Clinical exome	52,626 variants
Total number of reads	29,912,898
Average read depth	146
Population frequency (<2%)	7905 variants
Exonic	500 variants
Exonic indel	54 variants
Exonic nonsynonymous, stop	243 variants
Exonic synonymous	203 variants
Splicing (±) 20 bp	533 variants
Coding ±20 bp	1033 variants
Hypothesis 1 = AR homozygous variants	51/1033 variants
Exonic indel	3 variants
Exonic nonsynonymous, stop	10 variants
Exonic synonymous	13 variants
Splicing	25 variants

AR, autosomal recessive.

Table 4. Proportion of targeted exons covered by 0, 2, 10, 20, and 30 reads

Clusters	14,956,449
Reads	29,912,898
Reads in binary alignment	29,739,094
Unique	26,924,175
Unique and aligned	25,915,934
Unique and aligned (%)	86.63799141
Selected bases	0.72
Mean target coverage	146.00
Target at 0×	0.01
Target at 2×	0.99
Target at 10×	0.98
Target at 20×	0.97
Target at 30×	0.95
CS1	0.952

ADDITIONAL INFORMATION:

Data Deposition and Access

The *SPG20* variant was deposited in DECIPHER (https://decipher.sanger.ac.uk/) under DECIPHER ID 339774. The targeted sequencing could not deposited because of a lack of patient consent.

Ethics Statement

Molecular genetic investigations for the three patients and their family were performed after parental informed consent in a clinical diagnostic setting. This study was approved by the KU Leuven university Hospitals Ethical committee (B322201010111—S52853). The patients' family consented to publication of the study.

Author Contributions

L.D. interpreted data and wrote the manuscript; F.R. followed and treated the patients, performed metabolic investigations and MRI imaging, and contributed to the manuscript writing; V.R. performed the targeted exon capture and sequencing; E.S. analyzed and interpreted data and contributed to the manuscript writing; M.H. ensured the follow-up and genetic counselling for the family and contributed to the manuscript writing; and K.D. followed the patients, performed the different genetic testings after clinical examination, collected blood samples, interpreted data and contributed to the manuscript writing.

Competing Interest Statement

The authors have declared no competing interest.

Referees

Gholson Lyon
Anonymous

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