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Recurrent null mutation in *SPG20* leads to Troyer syndrome

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ABSTRACT

Troyer syndrome is an autosomal recessive form of complex hereditary spastic paraplegia. To date, the disorder has only been described in the Amish and in kindred from Oman. In Amish, all affected individuals have a homozygous one nucleotide deletion; c.1110delA. In the Omani kindred, all affected have a homozygous two nucleotides deletion; c.364_365delTA (p.Met122ValfsTer2). Here we report the results of homozygosity mapping and whole exome sequencing in two siblings of a consanguineous Turkish family with mild intellectual disability, spastic paraplegia, and muscular dystrophy. We identified the same deletion that has been identified in the Omani kindred, but haplotype analysis suggests a recurrent event, and not a founder mutation. We summarize current knowledge of Troyer syndrome, and propose wider use of whole exome sequencing in routine diagnostics. This applies in particular to nonspecific phenotypes with high heterogeneity, such as spastic paraplegia, intellectual disability, and muscular dystrophy, since in such cases the assignment of a definite diagnosis is frequently delayed.

1. Introduction

Hereditary spastic paraplegias (HSP) are clinically heterogeneous neurodegenerative disorders characterized by progressive spasticity of the lower extremities. HSP is classified as pure or complicated, depending on the accompanying clinical features [7]. In pure HSP, hyperreflexia of the upper extremities, bladder disturbance, and decreased vibratory sense might be also present. In complicated HSP, additional neurological symptoms such as intellectual disability, extrapyramidal symptoms, optic neuropathy, or deafness, also occur [12].

One form of complicated HSP is Troyer syndrome (MIM#275900) that is characterized by the additional symptoms of distal amyotrophy, dysarthria, mild developmental delay, and skeletal abnormalities [12]. The syndrome was first described in an Old Order Amish population in Ohio, USA [3]. In 1994 the first non-Amish family has been described [5]. A subsequent investigation then identified the founder mutation, a single nucleotide deletion c.1110delA in the *SPG20* gene [11], which leads to loss of the protein spartin [2]. In 2010, Manzini et al. reported homozygous loss of function mutation c.364_365delTA (p.Met122ValfsTer2) with an absent expression of the gene *SPG20*, and the assignment of a diagnosis of Troyer syndrome, in an Omani kindred presenting with short stature, dysarthria, and motor- and cognitive developmental delay [9]. The present report describes the identification of two further cases of Troyer syndrome in a family of Turkish origin through homozygosity mapping and whole exome sequencing (WES).

1.1. Methods

Genotyping and homozygosity mapping were performed using the human 610-Quad DNA analysis Beadchips microarray (Illumina, San Diego, CA, USA) as described in details elsewhere [1]. WES was performed using DNA of one patient (P2, Fig. 1). Enrichment of DNA was performed using Agilent SureSelect whole Exome 50 Mb (Agilent Technologies, Santa Clara, CA, USA). Sequencing was performed using the SOLiD 5500 XL system (Life Technologies, Carlsbad, CA, USA) and about 160 million reads were obtained. Read mapping to the hg19 reference genome was performed with SOLiD

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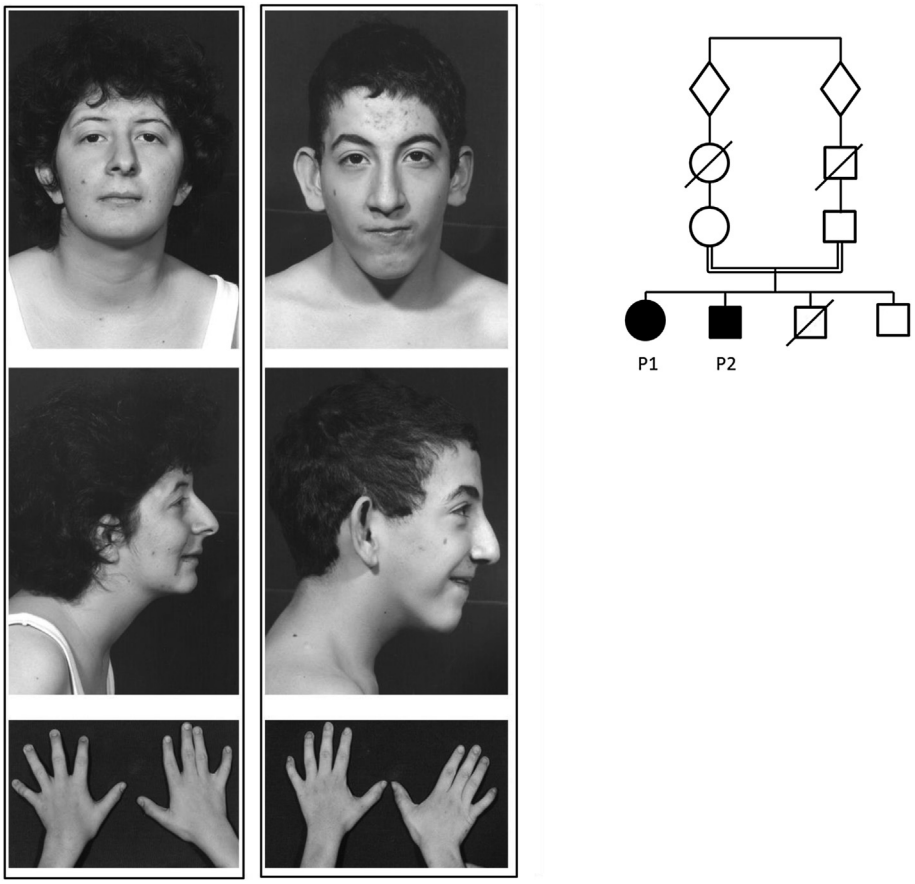

Fig. 1. Family pedigree and photographs of the two affected siblings.

LifeScope software v2.5 and yielded approximately 143.5 million mappable reads [10]. The mean target coverage of the target sequences was 83.94 (71%, minimum depth of 20×; 83%, minimum depth of 5×). The mean target coverage of the targeted sequences in the candidate regions was even higher (93.3×) and 93.6% of the targeted sequences were covered 5× or more, which is often enough for homozygous variants. A total of 43,715 single nucleotide variants (SNV) and 2858 indels were identified. Only variants meeting the following criteria were considered for further analyse: (i) a minor allele frequency of <1% in the Exome Variant Server (ESP, version ESP6500, access December 2014), in the 1000 Genomes project (1000G, version 1000G2012, access July 2014), or in house exomes (>600 exomes); (ii) exonic variants (non-synonymous and InDels) or located at splice sites; and (iii) minimum 5× coverage. All inheritance patterns were considered. The variants were then prioritized according to *in silico* parameters (annotation using Annovar [15]). PCR and Sanger sequencing were performed using standard protocols for the exclusion of technical artifacts and for segregation testing. Details of the sequencing and filtering procedures are provided elsewhere [6].

2. Results

2.1. Clinical report

The study was approved by the Ethics Committees of the Universities of Bonn and of Erlangen-Nuremberg, and appropriate written informed consent was obtained for all participants. The family was referred for genetic counseling at the Institute of Human Genetics in Bonn with a history of an unclarified intellectual disability in two of the four children. The parents were second degree cousins and of Turkish origin.

The eldest daughter P1 (Fig. 1) was born in the 8th month of pregnancy, and required incubation for the first 23 days of life. She was unable to sit unaided until 12 months of age, and first walked at the age of 20 months. At the age of 5 years, clinical examination revealed growth retardation (all growth parameters <3rd percentile), general muscular hypotonia, joint hypermobility, genu valgum, a prominent nose, raised nostrils, epicanthus, down-slanting palpebral fissures, and low-set ears. All laboratory investigations were unremarkable. On follow up examination at the age of 6 years atrophy of the limb musculature was noted, but not confirmed by muscular biopsy. At the age of 14 years, clinical examination revealed muscular weakness of the upper extremities. At the age of 16 years, also a hoarse voice, high arched feet, atrophy of the lower arm muscles and increased proprioceptive muscular reflexes were noticed. Electromyography and investigations of nerve conduction velocity were not suggestive of muscle disease, implying a neurological nature of the muscular atrophy. Later that year, she was admitted to a child and adolescent psychiatry ward with a suspicion of an acute decompensating psychosis. At the age of 19 years, she was readmitted to the psychiatric ward with sleep disturbance, general anxiety and panic attacks with hallucinations (e. g. she is guilty for the Balkan war), and suicidal ideation. All laboratory investigations were unremarkable as well as brain magnetic imaging and electroencephalogram. Assessment using the Wechsler Adult Intelligence Scale (HSWI) revealed a total intelligence quotient (IQ) of 46. On presentation for genetic counseling, she was 26 years of age. Her head circumference was 51 cm (1 cm below the 3rd percentile), her height was 144 cm (4.7 cm below the 3rd

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