Original Article

Exome sequencing is a useful diagnostic tool for complicated forms of hereditary spastic paraplegia


Hereditary spastic paraplegias constitute a heterogeneous group of neurodegenerative diseases encompassing pure and complicated forms, for which at least 52 loci and 31 causative genes have been identified. Although mutations in the \textit{SPAST} gene explain approximately 40\% of the pure autosomal dominant forms, molecular diagnosis can be challenging for the sporadic and recessive forms, which are often complicated and clinically overlap with a broad number of movement disorders. The validity of exome sequencing as a routine diagnostic approach in the movement disorder clinic needs to be assessed. The main goal of this study was to explore the usefulness of an exome analysis for the diagnosis of a complicated form of spastic paraplegia. Whole-exome sequencing was performed in two Spanish siblings with a neurodegenerative syndrome including upper and lower motor neuron, ocular and cerebellar signs. Exome sequencing revealed that both patients carry a novel homozygous nonsense mutation in exon 15 of the \textit{SPG11} gene (c.2678G>A; p.W893X), which was not found in 584 Spanish control chromosomes. After many years of follow-up and multiple time-consuming genetic testing, we were able to diagnose these patients by making use of whole-exome sequencing, showing that this is a cost-efficient diagnostic tool for the movement disorder specialist.

Conflict of interest

The authors declare that they have no conflict of interests.

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Key words: genetic heterogeneity – molecular diagnosis – next-generation sequencing – spastic paraplegia – SPG11 – whole-exome sequencing

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Exome sequencing in SPG11

Subjects and methods

Subjects

We studied two Spanish siblings (Fig. 1: III-1 and III-3) with a complicated form of spastic paraplegia, and no family history of neurological disease. Thorough neurological examination and follow-up were carried out at the Department of Neurology of the Hospital Ramón y Cajal by some of the authors. The proband, who is currently 47 years old, is being followed up by us since 1991. Her elder brother was followed up between 1995, when he was 37, and 2011, when he died at the age of 53; no postmortem examination was performed. Their parents were born in the same small village but did not acknowledge consanguinity. After obtaining informed consent, we collected blood samples from both patients and extracted DNA using standard procedures. Mutations causing SPG4, SPG3A, SPG17, Friedreich ataxia, SCA1, 2, 3, 6, 7, 8, 12, 17, and DRPLA, were previously excluded as part of the routine genetic testing. DNA samples from 292 Spanish control subjects were also analyzed.

Whole-exome analysis

Genomic DNA samples were prepared following the ‘TruSeq™ DNA Sample Preparation v2 Guide, August 2011’ (Illumina, Inc., Eindhoven, The Netherlands). Briefly, DNA was fragmented into 150–200 base pairs (bp), end paired, adenylated, and ligated to adapters. Exonic sequences were enriched using the ‘SeqCap EZ Exome v2.0’, according to the instructions of the manufacturer (Roche/NimbleGen SeqCap EZ: Almere, The Netherlands, draft v1.5.4, March 2011). The captured fragments were purified and sequenced with the Illumina Hiseq2000 platform (Illumina, Inc.) using a 100-bp paired-end reads protocol. Bioinformatic analysis was performed using an in-house pipeline based on available tools. Sequence reads were aligned to the human reference genome (UCSC hg19) using burrows–wheeler aligner (7). Subsequently, single nucleotide polymorphisms (SNPs) and small indels were identified using samtools (8) and varscan (9). The raw lists of single nucleotide variants (SNVs) and indels were annotated with annovar (October 2011) (10), and filtered to include only exonic and donor/acceptor splicing variants. Additional filtering steps were then applied (Table 1). An in-house exome sequence (unrelated healthy Spanish subject) was used to define variants following a disease model (dominant or recessive). In accordance with the pedigree, priority was given to variants fitting a recessive model, and additionally to those not present in the dbSNP132 database, 1000 Genomes database or Exome Variant Server (http://evs.gs.washington.edu/EVS/), predicted as damaging by sift (11) and/or polyphen2 (12), and located in genes previously associated with HSP phenotypes (3).

Sanger sequencing validation

A fragment containing the novel SPG11 mutation was polymerase chain reaction (PCR) amplified for the two patients and 292 population-matched control subjects. The purified PCR products were sequenced in an ABI3730 sequencer (Applied Biosystems, Bleiswijk, The Netherlands). The following primers were used for both PCR and sequencing (detailed conditions are available upon request): SPG11-15F (5′-GCAACACAGCGAGATCCTG-3′) and SPG11-15R (5′-GCTCTTCCCCCTATTTCC-3′).

Results

Clinical description

The proband was a female (Fig. 1: III-3), who first noticed problems at the age of 20. The neurological examination showed a mild cognitive impairment affecting her memory, calculation skills, and abstract thinking. She also showed bilateral horizontal nystagmus and dysarthric speech. The motor examination revealed a symmetrical atrophy of the hand muscles, generalized fasciculations (detected clinically and confirmed by electromyography), and paraparesis with severe spasticity and extensor plantar responses. All modalities of the sensory examination were normal. Brain magnetic resonance imaging (MRI) showed enlargement of the lateral ventricles, abnormal supratentorial white matter signal, suggesting demyelination and moderate atrophy of the anterior part of the corpus callosum. The third and fourth ventricles as well
as the peribrainstem cisterns were also dilated, suggesting brainstem and cerebellar atrophy. Electrodiagnostic studies were compatible with both upper and lower motor neuron disorder, with no evidence for polyneuropathy. Her cerebrospinal fluid had normal cells and proteins, and no oligoclonal bands. She had normal visual and brainstem evoked potentials. Biochemical studies were normal (GM1 and GM2 gangliosidoses and mucolipidosis types 2 and 3). At age 42, the patient suffered severe deterioration of her neurological status. She was unable to orally communicate, and her activities were restricted to passive transference from bed to wheelchair.

The proband’s brother (Fig. 1: III-1) had suffered from spastic gait since age 10. At age 15, he had hand-muscle weakness and amyotrophy. At age 18, widespread fasciculations could be observed and the electromyography revealed active denervation. At age 37, he was unable to walk unaided; he had scoliosis and spastic tetraparesis with prominent distal amyotrophy in all extremities, hyperreflexia in the upper limbs and triple withdrawal response in the lower limbs. He also had bilateral optic atrophy and irregular ocular saccades. The sensory examination was unremarkable. Enzymatic deficiencies were ruled out (hexosaminidase, glucosidase, glucosaminidase, glucuronidase, manosidase, mucolipidosis types 2 and 3, and GM1 and GM2 storage disease). Muscle enzymes were normal. A blood smear showed no acanthocytes or vacuolated lymphocytes. Thyroid hormones, folic acid, and vitamins E and B12 were normal, and tests for syphilis and Human T-cell leukemia virus type 1 (HTLV1) were negative. The disease worsened with progressive amyotrophy and vision loss. He eventually complained of dysphagia for liquids and involvement of speech, which became occasionally unintelligible with pseudobulbar features, while comprehension was relatively intact. Follow-up ophthalmological examination revealed progressive optic atrophy as well as retinal pigmentary degeneration, and by age 47 he had completely lost vision. Motor manifestations evolved to a more severe amyotrophy and fixed postures in the four extremities. Sensory function as well as control of sphincters were preserved. With the exception of vision, the cranial nerves were normal. Brain MRI at age 47 showed generalized supra and infratentorial atrophy, signs of a left basal ganglia stroke and leukoaraiosis. Atrophy of the corpus callosum was, at that time, pronounced. Spinal cord MRI showed a marked scoliosis of the spine, but no spinal cord compression or major damage. The patient died at the age of 53 because of aspiration pneumonia.

Table 1. Direct identification of the causative mutation for a complicated spastic paraplegia by whole-exome sequencing

<table>
<thead>
<tr>
<th>Filter</th>
<th>Number of SNVs</th>
</tr>
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<tbody>
<tr>
<td>Fit a recessive model</td>
<td>1992</td>
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</table>
| Excluding synonymous
dbSNP | 949 |
| Rare variants (≤1%) | 51 |
| Not in dbSNP/1000G/EVS | 9 |
| Predicted as damagingdbSNP | 4 |
| In known HSP genes | 1 |

DbSNP, dbSNP132 database; EVS, exome variant server; HSP, hereditary spastic paraplegias; SNV, single nucleotide variant; 1000G, 1000 Genomes database.

aOnly synonymous variants not affecting splicing signals.
bBy SIFT and/or POLYPHEN2.

dSequence analysis

About 12 Gb of sequence per sample was yielded. Median read depths of 77 and 78 were obtained for patients III-1 and III-3, respectively, with at least 87% of the targeted regions with a coverage of 30-fold. When comparing the three exomes (two patients and one healthy unrelated subject), a total of 31,491 exonic and splicing variants were detected (31,209 SNVs and 282 indels). None of the indels was located in known HSP genes. After data filtering (Table 1), only one.
SNV in the \textit{SPG11} gene, which had been previously associated with complicated autosomal recessive HSP, was retained. Different filtering strategies (data not shown), not restricted to a recessive model, also retrieved the same final result. The two siblings presented a novel homozygous nonsense mutation in exon 15 of the \textit{SPG11} gene (c.2678G\textgreater A; p.W893X). Both shared at least one allele in the whole extension of chromosome 15, and the novel mutation was included in a homozygous region flanked by rs56265533 and rs8023906, spanning approximately 4.5 Mb.

Sanger sequencing confirmed that the two patients were homozygous for the \textit{SPG11} mutation (Fig. 1). Besides not being present in public databases (dbSNP132, 1000 Genomes, and Exome Variant Server), this mutation was not found in 584 Spanish control chromosomes.

**Discussion**

Reaching a definitive molecular diagnosis is often difficult and costly in the movement disorder clinic, because phenotypic complexity, inter- and intra-familial variability, and genetic heterogeneity are the rule. The new genomic analysis tools – especially next-generation sequencing methodologies – will bring a transformation to this diagnostic problem, but still need to be validated in the clinical setting.

We present here the case of two siblings with a complex movement disorder affecting the upper and lower motor neurons as well as the brainstem, and possibly the cerebellum–cerebellar function being difficult to assess because of the severity of pyramidal damage. The clinical picture also included cognitive impairment, optic atrophy and retinal degeneration. No other relatives were known to be affected and both parents were from the same village, suggesting a recessive inheritance. These clinical and neuroimaging findings led to a thorough and intensive work during many years of follow-up in the movement disorder clinic. Brain MRI, electrophysiological studies as well as cerebrospinal fluid examination, biochemical and genetic testing for HSPs, SCAs and other movement disorders were performed without getting to a definitive diagnosis. Recently, the possibility of carrying out an exome sequencing analysis was available to us, and allowed the identification, in both siblings, of a novel homozygous mutation (c.2678G\textgreater A; p.W893X) in the \textit{SPG11} gene (OMIM #610844). Mutations in this gene are known to cause spastic paraplegia type 11 – \textit{SPG11} (OMIM #604360), an autosomal recessive HSP, and therefore a definitive diagnosis could now be established.

The \textit{SPG11} gene is large, comprising 40 exons and spanning 101 kb (13), and its spectrum of mutations is wide (14). More than 120 \textit{SPG11} mutations, spread all over the gene, have already been described (available at ‘The Human Gene Mutation Database – HGMD’), including missense, nonsense, splicing mutations in addition to small indels, and gross deletions. This gene encodes for a 2443 amino acid protein, spatacsin, of yet unclear function (suggested as a membrane-associated receptor or transporter) (13, 14). The new mutation described here causes a very pre-mature stop codon (c.2678G\textgreater A; p.W893X). Even if the protein is produced, it is predicted to result in a truncation of spatacsin, which would lack more than 1500 amino acids, supporting a possible loss-of-function mechanism. Previously, Denora et al. (15) described another truncating mutation (c.2697G\textgreater A; p.W899X) just 19 nucleotides downstream of the mutation described here. Similarly, other \textit{SPG11} mutations are also thought to alter the correct formation of a complete protein product and to lead to loss of function (14, 15).

Besides early-onset progressive spasticity and weakness of the lower limbs, \textit{SPG11} is usually complicated by additional features, namely cognitive deficits, peripheral neuropathy, mild cerebellar ataxia, and other deficits. Neuroimaging hallmarks of \textit{SPG11} include thin corpus callosum and white matter abnormalities (2), which are compatible with what we observed in both siblings. Both patients presented with a complex phenotype, and although \textit{SPG11} was among the diagnostic possibilities based on the clinical findings and MRI pattern (2, 16, 17), such combination is not unique to this specific HSP subtype, and a molecular testing would still be demanded.

Similar to what has been previously described (3, 17–19), clinical heterogeneity within a given HSP kindred was evident in our patients, making their diagnosis more difficult. We described two siblings harboring the same mutation, but with distinct clinical presentations, from which we can highlight the mental impairment in the proband (Fig. 1: III-3) and an earlier age at onset and severe ophthalmologic involvement in her brother (Fig. 1: III-1). While cognitive deficits have been largely reported in patients with \textit{SPG11} (15, 19), and ophthalmologic symptoms have also been previously described (18, 20), to our knowledge this is the first report of complete loss of vision.

As a result of both clinical and genetic heterogeneity, and the ever growing number of new loci and causative genes, many families with HSP and other movement disorders remain undiagnosed. Molecular diagnoses by conventional techniques, which usually imply expensive and time-consuming gene-by-gene screenings, sometimes unavailable in small laboratories, are becoming unpractical (3). Amplicon-based high-throughput sequencing has already been shown to be useful in detecting \textit{SPG5} and \textit{SPG7} cases in large patient cohorts with complex phenotypes (21). With the successful study of a kindred with a complicated form of HSP, our data support the recent reports stating the usefulness of exome sequencing for the identification of causal variants in genetically heterogeneous neurodegenerative diseases (22, 23).

In summary, using whole-exome sequencing, we were able to reach a molecular diagnosis for two siblings with a spastic paraplegia-plus syndrome, who have been previously studied for a long time in our clinic. Furthermore, this approach allowed us to identify a truncating mutation in \textit{SPG11} not previously
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described. This strategy is cost-effective and avoids a time-consuming diagnostic screening of heterogeneous and complicated movement disorders.

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