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Targeted NGS meets expert clinical characterization: Efficient diagnosis of spastic paraplegia type 11

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ABSTRACT

Next generation sequencing (NGS) is transforming the diagnostic approach for neurological disorders, since it allows simultaneous analysis of hundreds of genes, even based on just a broad, syndromic patient categorization. However, such an approach bears a high risk of incidental and uncertain genetic findings. We report a patient with spastic paraplegia whose comprehensive neurological and imaging examination raised a high clinical suspicion of SPG11. Thus, although our NGS pipeline for this group of disorders includes gene panel and exome sequencing, in this sample only the spatacsin gene region was captured and subsequently searched for mutations. Two probably pathogenic variants were quickly and clearly identified, confirming the diagnosis of SPG11. This case illustrates how combination of expert clinical characterization with highly oriented NGS protocols leads to a fast, cost-efficient diagnosis, minimizing the risk of findings with unclear significance.

1. Introduction

Hereditary spastic paraplegia (HSP) is a syndromic designation for a group of inherited neurological disorders with predominant manifestations of lower extremity weakness and spasticity. HSPs are clinically and genetically heterogeneous with over 70 known genetic types named SPG followed by a cardinal number. The prevalence of HSP is estimated in a range from 1.3 to 9.6 per 100,000. They are classified into pure and complicated or complex HSPs, the latter presenting with additional neurological symptoms such as cognitive impairment, cerebellar signs, peripheral neuropathy, extrapyramidal manifestations and other features (Fink, 2000).

With next generation sequencing (NGS) technologies the simultaneous analysis of hundreds of genes can be carried out at an affordable price, allowing to apply this type of testing successfully even in less informative cases and pedigrees (Chae et al., 2015). Like other categories of disease with genetic heterogeneity and a high degree of clinical overlap, currently the preferred approach to the study of HSP patients is NGS — either through a panel of HSP related genes, whole exome or even whole genome sequencing (Kumar et al., 2013; Bettencourt et al., 2014). However, the more megabases of genomic sequence are included in the NGS pipeline, the higher post-sequencing effort is needed to filter the list of genetic variants observed in any individual. Interpreting the potential pathogenicity of the identified variants represents much of the current workload for laboratory and clinical geneticists (Quintáns et al., 2014).

Spastic paraplegia type 11 is one of the most frequent autosomal recessive HSPs. It is caused by mutations in the *SPG11* gene [MIM *610844], which has 100,982 coding nucleotides distributed in 40 exons and translated into spatacsin, a 2443 amino acid protein (Stevanin et al., 2007). Although SPG11 can present as a pure HSP, more often patients develop a complex HSP with cognitive impairment, peripheral neuropathy, cerebellar, lower motor neuron and/or extrapyramidal features. A recognized hallmark of SPG11 is the presence of a thin corpus callosum (TCC) in the brain MRI of patients with this disease (Stevanin et al., 2008). Over 160 pathogenic variants have been described in families with SPG11, most of them missense mutations

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and small truncating indels. This large number of variants without a mutational hotspot makes conventional, exon by exon Sanger sequencing a cumbersome diagnostic approach. Here we report the case of a patient who was diagnosed with SPG11 using NGS targeted exclusively to the spatacsin gene.

2. Case report

2.1. Clinical and genealogical data

A 25-year-old woman without any significant previous disease presented with a five year history of progressive gait difficulty. There were no sensitive disturbances, paroxysmal symptoms, cranial nerve involvement or Lhermitte's sign. The patient's father had suffered a cerebellar hemorrhage due to an arteriovenous malformation; family history was otherwise negative for neurological problems. There was no consanguinity. Her neurological examination showed a slightly asymmetric spastic paraplegia with brisk reflexes, bilateral ankle *clonus* and Babinski sign. She also presented dystonic posture in both feet. Sensory exam, as well as cognitive evaluation was normal. She had *pes cavus* and her gait was spastic. Full blood count, biochemical test with liver, renal and thyroid function, proteinogram, plasma level of vitamins B9, B12 and E, lactate and pyruvate were all within normal range. Autoantibodies and serologic tests for syphilis, Lyme disease, HIV, HTLV-I, as well as hepatitis B and C were negative. Cranial and spinal magnetic resonance imaging (MRI) showed moderate, diffuse cortical atrophy and a markedly thin corpus callosum (TCC), with some high signal lesions located in the periventricular white matter (Fig. 1). The electroneuromyography findings were indicative of a sensory–motor axonal polyneuropathy more pronounced in the lower limbs. Based on the clinical picture of a sporadic, young onset spastic paraplegia with axonal neuropathy and TCC, without a family history of the disease and without evidence of any other primary etiology, the possibility of an autosomal recessive HSP was raised, strongly suggestive of SPG11.

2.2. Genetic analysis

A next generation sequencing study was carried out targeting the *SPG11* gene only. The assay was designed using the SureSelect® Target Enrichment System (Agilent Technologies, Santa Clara, CA). The captured region, including all coding exons, 10 bp exon–intron boundaries and UTRs was amplified using ligation-based sequencing with the SOLiD® platform (Life Technologies). The mean coverage of the target region was 921×, with 100% nucleotides covered ≥30×. The Lifescope® 2.5.1 software package (Life Technologies) was used for sequence alignment and variant calling, together with GATK 3.0.0 (McKenna et al., 2010). The BEDTools and PicardTools suites were used for coverage statistics and to remove duplicates (Quinlan and Hall, 2010). Variant annotation was carried out with ANNOVAR (2014Feb24 version) and ExomeDepth was used for deletion detection (Plagnol et al., 2012). The integrative genomics viewer (IGV) developed at the Broad Institute was used to visually explore NGS data (Robinson et al., 2011). The following features were recorded on each observed variant: population allele frequencies, conservation (GERP, Phylophen, SiPhy), and pathogenicity prediction with several algorithms (SIFT, Polyphen, Mutation Taster, LRT, CADD). Information on SPG11 genetic variants previously associated to disease was reviewed from the published literature as well as online genetics resources (OMIM®, GeneReviews®, ClinVar) and the HGMD™ mutation database. The criteria used for clinical interpretation of the variants were based on those previously published, which classify genetic variants in a five-tiered scheme (Quintáns et al., 2014., Richards et al., 2015). Only two variants fulfilled the criteria to be classified as "pathogenic": a single nucleotide missense substitution in exon 38 (NM_025137:c.6999G>C:p.Q2333H) and a partial gene deletion [NM_025137:c.(?_4907)_(5120_?)del] involving exon 29, predicted to lead to a frameshift and premature stop. The single nucleotide variant was validated by Sanger sequencing and the deletion was confirmed by multiplex ligation-dependent probe amplification analysis (MLPA®) (Fig. 2).

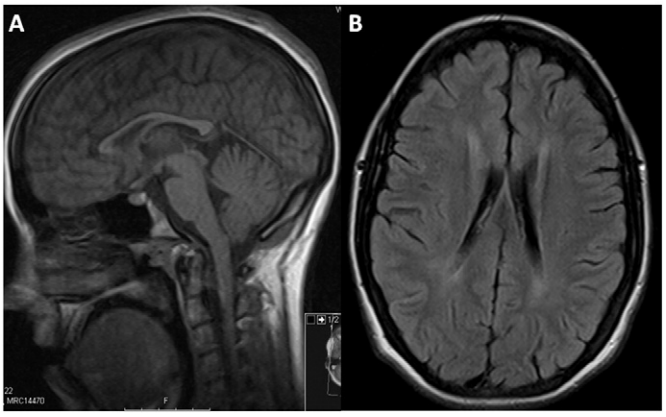

Fig. 1. Brain MRI study of the patient. (A) Saggittal T1WI revealed a thin corpus callosum; (B) axial FLAIR showed slight hyperintense lesions affecting the periventricular white matter.

3. Discussion

The patient described here presented a clinical picture of spastic paraplegia with axonal neuropathy, TCC and mild white matter involvement in the brain MRI. There was no consanguinity and no family history of similar neurological symptoms. These data were highly suggestive of one of the forms of HSP that associate TCC, among which SPG11 is one of the most frequent, accounting for 20–40% of autosomal recessive HSPs (Finsterer et al., 2012; Stevanin et al., 2008). The SPG11 phenotype may be mild and uncomplicated or variably associated with intellectual disability, dysarthria, nystagmus, upper extremity weakness and extrapyramidal features (Paisan-Ruiz et al., 2008; Stevanin et al., 2008). In some cases the clinical picture can be similar to a slowly progressive juvenile ALS and it may also present as Kjellin syndrome with childhood onset spastic paraplegia, retinopathy, dementia and distal muscular atrophy (Orlacchio et al., 2010; Puech et al., 2011). A TCC is a very characteristic finding in SPG11 and has been proposed to be the best phenotypic predictor of this form of HSP (Schüle et al., 2009). However, this finding is neither constant nor specific since this abnormality can be observed in other SPG genetic subtypes (Pensato et al., 2014).

Through highly selective, targeted NGS we have identified two mutations in spatacsin: an exonic deletion and a novel missense variant, confirmed through MLPA and Sanger sequencing, respectively. The in silico predictions and additional clinical and genetic criteria support the pathogenicity of the p.Q2333H variant, while partial gene deletions and other truncating mutations have previously been reported in SPG11 (Stevanin et al., 2008). Although we did not identify the exact breakpoints, the exon 29 deletion in our patient affects the same gene region as the deletion described by Pereira and collaborators in Portuguese families (Pereira et al., 2012), thus adding further support to the pathogenicity of this particular mutation.

This result had a major impact on the clinical handling of the patient. After five years into the disease course, the patient received a specific – and not just syndromic – diagnosis within a few weeks. The young disease onset, together with a frequent lack of family history in many patients with recessive HSPs, leads to an odyssey of workup tests in order to rule out non-genetic causes. Thus, it is important to reach a definitive diagnosis as soon as possible through a clinically-oriented, candidate gene approach. In this situation, a broader and unselective NGS strategy might lead to a long list of variants that will have to be scrutinized and interpreted before a final conclusion can be reported. Identifying the causal mutations and thus confirming that her HSP

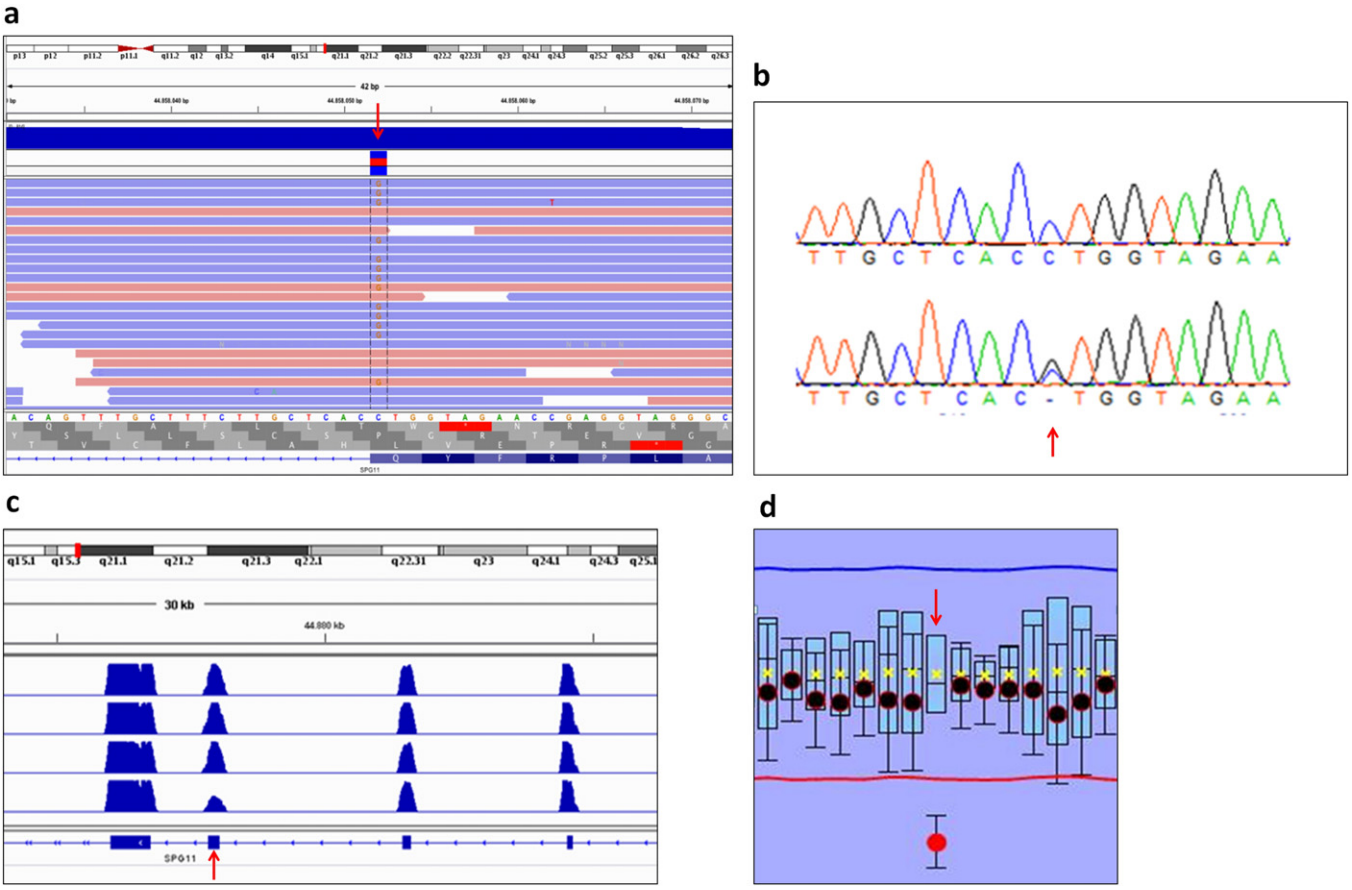

Fig. 2. Pathogenic variants identified in the *SPG11* gene. The heterozygous transversion NM_025137:c.6999G>C:p.Q2333H in the complementary strand visualized with the IGV (a) and in the electropherogram (b). The heterozygous exon 29 deletion c.(?_4907)_(5120_?)del as observed in the NGS output with the IGV (c) and through MLPA (d).

was recessive also had profound implications for genetic counseling of the patient and her family.

Even to screen a few or just one gene, like in this case, the NGS approach offers significant advantages over conventional techniques. It allows a faster analysis of genes with many exons, which would require longer hands-on laboratory time. Furthermore, whereas exon by exon Sanger sequencing only detects single nucleotide changes and small deletions, both point mutations and exonic deletions can be adequately detected in the NGS data with currently available software tools, as illustrated here. A single gene targeted approach – versus a gene panel or whole exome sequencing – was chosen given the high a priori probability of SPG11, in order to minimize the possibility of uncertain findings leading to difficult interpretation and decision-making.

Lessons learned

- In a patient with spastic paraplegia, TCC and axonal neuropathy, a partial gene deletion and a novel missense variants were identified in the *SPG11* gene, both of them probably pathogenic.
- Sanger Sequencing still is the gold standard technique to detect SNPs and small DNA variants, however NGS may be a more efficient approach even when targeting just one candidate gene. The strategy described here covered the complete *SPG11* coding region and led to identify both a point mutation and a deletion simultaneously.
- Knowledge of the phenotypic spectrum of each genetic subtype within a given syndromic category of disorders, a high degree of clinical expertise and careful observations allow to design a very selective, quick and cost-efficient genetic analysis strategy.
- When pathogenic variants are identified (probability of pathogenicity ≥98%) that are consistent with the phenotype and inheritance pattern, no further genetic screening is indicated (e.g. whole exome, gene panel). This minimizes the risk of genetic findings that are incidental or difficult to interpret.

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