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Genetic background of the hereditary spastic paraplegia phenotypes in Hungary — An analysis of 58 probands

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ABSTRACT

Background: Hereditary spastic paraplegias (HSPs) are a clinically and genetically heterogeneous group of neurodegenerative diseases with progressive lower limb spasticity and weakness. The aim of this study is to determine the frequency of different SPG mutations in Hungarian patients, and to provide further genotype-phenotype correlations for the known HSP causing genes.
Methods: We carried out genetic testing for 58 probands with clinical characteristics of HSP. For historical reasons, three different approaches were followed in different patients: 1) Sanger sequencing of *ATL1* and *SPAST* genes, 2) whole exome, and 3) targeted panel sequencing by next generation sequencing.
Results: Genetic diagnosis was established for 20 probands (34.5%). We detected nine previously unreported mutations with high confidence for pathogenicity. The most frequently affected gene was *SPAST* with pathogenic or likely pathogenic mutations in 10 probands. The most frequently detected variant in our cohort was the *SPG7* p.Leu78*, observed in four probands. Altogether five probands were diagnosed with SPG7. Additional mutations were detected in *SPG11*, *ATL1*, *NIPA1*, and *ABCD1*.
Conclusion: This is the first comprehensive genetic epidemiological study of patients with HSP in Hungary. Next generation sequencing improved the yield of genetic diagnostics in this disease group even when the phenotype was atypical. However, considering the frequency of the HSP-causing gene defects, SPG4, the most common form of the disease, should be tested first to be cost effective in this economic region.

1. Introduction

Hereditary spastic paraplegias (HSPs) are a clinically and genetically heterogeneous group of neurodegenerative diseases. Based on the presence or absence of additional neurological or psychiatric symptoms, HSPs are classified as complicated (cHSP) or uncomplicated, pure hereditary spastic paraplegias (pHSP) [1]. Altogether, there are more than 50 known spastic paraplegia (SPG) genes. The trait of inheritance may be autosomal dominant, recessive, X-linked, mitochondrial, or de novo [2]. Selection of a target gene based on the clinical presentation is not always straightforward due to complex genotype-phenotype correlations [3]. Some clinical findings, such as deafness, dementia, visual impairment, skeletal or skin abnormalities, epilepsy and MRI abnormalities may significantly narrow the list of the possible disease causing genes [4]. Nevertheless, testing all candidate genes by Sanger sequencing is a long and costly process. Sporadic HSP cases are not rare in the clinical practice. In these cases, autosomal dominant inheritance with incomplete penetrance, autosomal recessive inheritance and X-linked inheritance must be taken into consideration. Incorporation of existing knowledge concerning the relative frequencies of different SPG gene alterations into the diagnostic process could be helpful; however, there is limited data from Central and Eastern Europe. The aims of this study are to evaluate the usefulness of whole exome or targeted analyses by Next Generation Sequencing (NGS), to determine the frequencies of different SPG gene mutations, and to assess the genotype-phenotype correlations in Hungarian patients with HSP phenotypes.

2. Patients and methods

2.1. Patients

Our neurogenetic outpatient clinic and laboratory provide services for patients with suspected HSP from all over Hungary. All study patients were examined by a board certified specialist (ZG, EV, PK, BB, GT) in neurology. The criteria for the inclusion in the study were: slowly progressive spastic lower extremity weakness as the main presenting

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symptom, and the absence of a possible secondary etiology. Laboratory investigations were performed to rule out B12 or folate deficiency, and, where appropriate, infectious causes (syphilis or HIV) were excluded. Brain and spinal cord MRI was performed to rule out structural lesions and inflammatory diseases; however, mild white matter lesions were not regarded as exclusion criteria. Lumbar puncture was performed to rule out primary progressive multiple sclerosis where appropriate. Electromyography was performed to rule out amyotrophic lateralsclerosis. This manuscript is a retrospective case study, it has an institutional ethical committee approval. Informed consent for genetic testing and the use of the data for scientific publication was obtained from each individual. Altogether 58 probands (34 male, 24 female) were examined, as well as family members where possible. Age of onset varied between birth (neonatal onset) and 65 years (mean 28.6 ± 18.7 years), cHSP was present in 17 probands (29.3%).

2.2. Genetic investigations

Conventional Sanger sequencing of the *ATL1* and *SPAST* genes was performed in 15 selected probands at CENTOGENE AG, Rostock, Germany. In one selected HSP case, where muscle biopsy showed cytochrome oxydase (COX) negative fibers, Sanger sequencing and deletion duplication testing of the *SPG7* gene was also performed at CENTOGENE AG, Rostock, Germany. DNA specimens of nine probands, negative for mutations in the *ATL1* and *SPAST* genes, were further analyzed by whole exome sequencing (WES). WES was performed on the Illumina HiSeq platform using the capture chip Agilent – SureSelect Human All Exon V4 design at the Hussman Institute for Human Genomics, Miller School of Medicine, Miami, Florida, USA. Further, DNA specimens of 42 probands were investigated using an in-house compiled Agilent Haloplex capture panel for HSP on the Illumina MiSeq platform at the Semmelweis University, Budapest, Hungary. This panel covers a significant number of genes currently linked to HSP. See the full list of the examined genes in Supplementary Table 1.

2.3. Bioinformatic analysis

Variant calling from the HiSeq reads was carried out consistently with the GATK Best Practices Guidelines [5]. Variant calling from the MiSeq reads was performed with the Agilent's Surecall software version 2.1, with default Haloplex method. Variant Call Format (VCF) files were annotated with the software KGGSeq [6] and ANNOVAR [7]. Variant filtration for exome sequencing data was performed with the software GEM.app [8], and the software VariantAnalyzer developed by the Budapest University of Technology and Economics.

The filtration process for possible disease causing variations contained:

1.) Selection of known disease causing variants and 2.) Selection of nonsynonymous variants with predicted significant effect on the protein function. Nonsense mutations and frameshift INDELS were accounted as pathogenic variants if they were consistent with the phenotype and inheritance pattern. Nonsynonymous mutations were evaluated based on the 12 protein prediction software results collected in the dbNSFP database [9], and the joint probability calculated by the KGGSeq [10]. Splice site mutation was analyzed with Human Splicing Finder (HSF3) [11] and Alamut software (Interactive Biosoftware). 3.) The minor allele frequencies of the variants were also taken into consideration, but this information was treated with precaution since large-scale single nucleotide polymorphisms (SNP) data is not available from Hungarians and thus a rare variant may be a population specific SNP. Minimum coverage depth of true variants were 20 reads, and all likely pathogenic variants from the NGS studies were confirmed by Sanger sequencing. In every case minimum 99% of the target bases were covered by minimum 20 reads, but additional Sanger sequencing was not performed. When a new mutation was detected, segregation analysis was performed in the patient's family (when available) to confirm the segregation of the candidate variant with the disease phenotype.

3. Results

3.1. Clinical characteristics of cases

Excluding patients with only mild neuropathy, cHSP was diagnosed in 17 of the 58 examined probands (29.3%). The most frequent complicating neurological symptoms included ataxia and intention tremor in six probands (10.3%), followed by cognitive deficit or dementia in five probands (8.6%). Hypacusis complicated the phenotype in three probands (5.2%), and severe psychiatric symptoms were present in two probands (3.4%). Epilepsy, severe polyneuropathy, and Parkinson-syndrome were the complicating symptoms in one proband (1.7%).

3.2. Genetic findings and the yields of different methods

Altogether, 58 probands were genetically tested. The *ATL1* and *SPAST* genes were sequenced by the Sanger method in the specimens of 15 probands. In this cohort, we detected 6 probands with *SPAST* mutations, and found no *ATL1* mutation. In one patient, who had COX negative fibers in her muscle specimen, a heterozygous, possibly autosomal dominant [12] *SPG7* p.Leu78* mutation was found. Altogether, the genetic diagnosis was established in 43.75% of patients in this subcohort by analyzing only these three genes. In nine probands, who were negative for *ATL1* and *SPAST* and who did not harbor COX negative muscle fibers, WES identified *SPG11* mutations in two cases, a *SPG7* mutation in one case, and adrenomyeloneuropathy - *ABCD1* mutation - in one case. This means that Sanger sequencing of *ATL1* and *SPAST*, followed by WES allowed us to make genetic diagnosis in 68.75% of our cohort. To establish the pathogenicity of the detected mutations, further investigations are needed in two cases by segregation analyses in the patients' families and mutation screening in Hungarian control patients. Panel sequencing (without prescreening the *ATL1* and *SPAST* genes) was performed in 42 HSP probands. The underlying genetic background of the disease was revealed in nine of these patients (21.4%).

Altogether, genetic diagnosis was made in 34.5% (20/58 probands) of the whole cohort.

Fig. 1 shows the distribution of the identified HSP genes in our cohort. We detected nine previously unreported mutations with high confidence for pathogenicity and two previously unreported variants, for which pathogenicity needs to be confirmed. We did not find any de novo mutation in our cohort.

Table 1 shows the detected mutations and associated phenotypes. Table 2 describes novel detected mutations. For more detailed

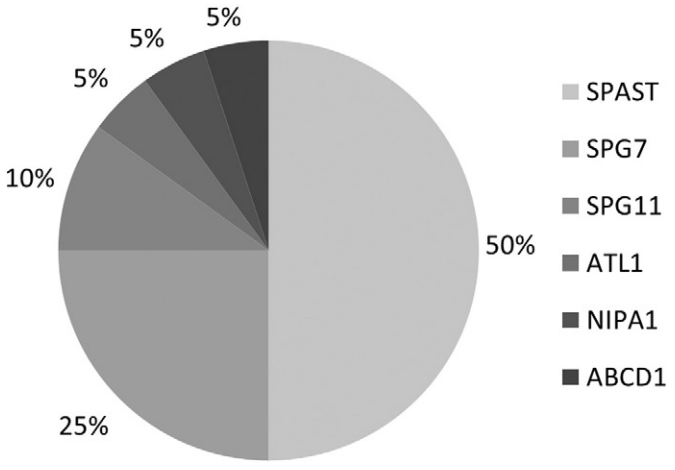

Fig. 1. Distribution of identified genetic causes of HSP in Hungary. Legend: Distribution of genetic causes of HSP in probands with genetic diagnosis.