Journal of the Neurological Sciences 383 (2017) 18–25

Contents lists available at ScienceDirect

Journal of the Neurological Sciences

journal homepage: www.elsevier.com/locate/jns

Clinical and molecular characterization of hereditary spastic paraplegias: A next-generation sequencing panel approach

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ARTICLE INFO

Keywords:
Cerebrotendinous xanthomatosis
Diagnosis
Hereditary spastic paraplegia
HSP
Next-generation sequencing
SPG

ABSTRACT

Background: Molecular diagnosis of hereditary spastic paraplegias (HSP) is a difficult task due to great clinical and genetic heterogeneity. We aimed to characterize clinical and molecular findings of HSP families from Rio Grande do Sul, Brazil; and to evaluate the diagnostic yield of a next-generation sequencing (NGS) panel with twelve HSP-related genes.

Methods: A consecutive series of HSP index cases with familial recurrence of spasticity, consanguinity or thin corpus callosum (TCC) were included in this cross-sectional study.

Results: Among the 29 index cases, 51.7% (15/29) received at least a likely molecular diagnosis, and 48.3% (14/29) a defined diagnosis. NGS panel diagnostic yield was 60% for autosomal dominant HSP (6/10, all SPG4), 47.4% for autosomal recessive HSP (9/19: 5 SPG11, 2 SPG7, 1 SPG5 and 1 cerebrotendinous xanthomatosis), and 50% for patients with TCC (3/6, all SPG11). Remarkably, 2/6 SPG11 patients presented keratoconus, and tendon xanthomas were absent in the patient with cerebrotendinous xanthomatosis.

Conclusion: A likely molecular diagnosis was obtained for more than half of families with the NGS panel, indicating that this approach could be employed as a first-line investigation for HSP. SPG4 is the most frequent form of autosomal dominant and SPG11 of autosomal recessive HSP in Southern Brazil.

1. Introduction

Hereditary spastic paraplegias (HSP) are a group of heterogeneous genetic disorders caused mainly by degeneration of the corticospinal tract longest axons [1,2]. HSP are clinically classified as pure or complicated forms, with ages at onset varying from early childhood to late adulthood [1–4]. An isolated pyramidal syndrome with predominance at lower limbs with or without vibration sense impairment and urinary urgency defines pure HSP; whereas complicated HSP presents a more complex clinical picture with additional neurological findings, such as ataxia, epilepsy, and cognitive decline [3].

All forms of inheritance are known to cause HSP with > 80 published genes or loci [5,6]. However, a smaller number of genes are responsible for most cases [1,5,7,8]. Most HSP-related proteins will affect axon and vesicle transport, control of endoplasmic reticulum morphology, mitochondrial quality control, myelination, protein folding/degradation, or lipid and purine nucleotide metabolism [1,4].

HSP are rare diseases that represent a significant burden to affected

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http://dx.doi.org/10.1016/j.jns.2017.10.010
Received 3 August 2017; Received in revised form 22 September 2017; Accepted 6 October 2017
Available online 10 October 2017
0022-510X/

individuals and families. A recent systematic review of studies from European, Northern African and Japanese populations reported different prevalence estimations ranging from 0.1 to 9.6 per 100.000 individuals [7]. There is no prevalence study of HSP in Latin America.

Due to the great clinical and genetic heterogeneity of HSP, achieving a genetic diagnosis can be a difficult task. Overall, > 50% of autosomal dominant HSP (AD-HSP) cases and 70% of autosomal recessive HSP (AR-HSP) cases never receive a genetic diagnosis by conventional sequencing methods [7,8]. With the advent of next generation sequencing (NGS), simultaneous sequencing of even thousands genes is now possible, faster and at lower cost [8,9], decreasing the diagnostic odyssey and the need for additional tests.

Our aim was to characterize clinical and molecular findings of HSP families from Rio Grande do Sul, Brazil, and to evaluate the diagnostic yield of a NGS panel with twelve HSP-related genes.

2. Materials and methods

2.1. Design and subjects

Index cases from consecutive families with clinical suspicion of HSP were recruited from April 2011 to November 2014 at Neurogenetics outpatients' clinics, Hospital de Clinicas de Porto Alegre, in a cross-sectional study. Eligibility was suspicion of HSP according to clinical diagnosis criteria [10] and presence of at least one of the following criteria: familial recurrence, consanguinity or thin corpus callosum (TCC) on magnetic resonance imaging (MRI). Age was not an exclusion criterion. The study was approved by the Ethics in Research Committee of our institution (GPPG-HCPA/14-0695), which follows the Declaration of Helsinki. Informed written consent was obtained from all individuals' prior participation.

2.2. Neurological and genetic evaluation

Severity of disease was evaluated with the Spastic Paraplegia Rating Scale (SPRS, range: 0–52, crescent in severity) [10]. Peripheral polyneuropathy was defined on clinical grounds by abnormal tactile-algesic and/or thermal discrimination, and decreased distal deep tendon reflexes and, when available, by nerve conduction studies and electromyography. The mode of inheritance was classified as apparently autosomal dominant when HSP was reported in > 1 generation. Families with several affected members in only one generation, those from consanguineous marriages, and simplex cases with TCC were classified as apparently autosomal recessive.

For simplex cases irrespective of consanguinity we excluded structural/inflammatory lesions by MRI of brain/spinal cord and examined vitamin B12, cooper, lipid profile and lactate blood levels; thyroid and hepatic function and HIV-1/2, HTLV-1/2 and Lues serology. For simplex cases or patients with suspected AR inheritance, we systematically screened for deficiency of lysosomal enzymes (arylsulfatase A, β-galactosidase, hexosaminidase A/B and galactocerebrosidase) and performed urine sulfatides chromatography, plasma amino acid analysis by tandem mass spectrometry and GC/MS urinary organic acid analysis. For simplex cases and patients with possible X-linked inheritance we screened for elevated levels of very long chain fatty acids. Patient's with AD inheritance were screened for SCA1, SCA2, SCA3, SCA6 and SCA7 by PCR using specific fluorescent primers followed by capillary electrophoresis of respective genes.

2.3. Genetic analysis

A customized AmpliSeq™ panel (Thermo-Fisher-Scientific) was designed using Ion AmpliSeq™ designer software in order to target all coding DNA sequences and flanking regions of the 11 HSP genes *ATL1, BSCL2, CYP7B1, KIAA0196, KIF5A, NIPA1, REEP1, SPAST, SPG7, SPG11* and *ZFYVE26* plus *CYP27A1,* related to cerebrotendinous xanthomatosis (CTX). This panel consisted of two primer pools with 257 different amplicons. NGS was performed using the Ion Torrent Personal Genome Machine (Ion-Torrent™). For detailed NGS procedures see Appendix 1. The raw data generated from NGS run was processed by Torrent Suite Software v5.0 (Thermo-Fisher-Scientific). After sequencing, reads were mapped to hg19 using Torrent Mapping Alignment Program (TMAP). Coverage assessment was performed using the Coverage Analysis plugin available in the Torrent Browser. Variants were annotated using Ion Reporter (Thermo-Fisher-Scientific), and Enlis Genome Research software (Enlis-LLC). Integrative Genomics Viewer was used for variant visualization. All genes were considered for analysis, regardless inheritance pattern classification.

2.4. Variant analysis

Sequences were searched for using the National Center for Biotechnology Information (NCBI) protein database, and variants are described with reference to the following transcripts: *ATL1* (NM_015915.4), *BSCL2* (NM_001122955.3), *CYP7B1* (NM_004820.3), *CYP27A1* (NM_000784.3), *KIAA0196* (NM_014846.3), *KIF5A* (NM_004984.2), *NIPA1* (NM_144599.4), *REEP1* (NM_022912.2), *SPAST* (NM_014946.3), *SPG7* (NM_003119.3), *SPG11* (NM_025137.3) and *ZFYVE26* (NM_015346.3). Sequence variations were compared to data available in the Human Gene Mutation Database (HGMD®). Mutalyzer 2.0 [11] was used for checking variants' nomenclature.

PolyPhen-2 [12], SIFT [13], M-CAP [14], Mutation Taster [15], Human Splicing Finder v3.0 (HSF3.0) [16] and ESEfinder v.3.0 (ESE3.0) [17] were used for *in silico* analysis. Phylogenetic conservation was estimated with Genomic Evolutionary Rate Profiling (GERP + +) [18]. Allele frequencies were searched on ExAC [19], gnomAD [20] and 1000 genomes browser [21]. Variants were classified according to American College of Medical Genetics and Genomics criteria [22].

2.5. Confirmation of disease-causing variants

Sanger sequencing was used to confirm known or likely disease-causing variants found by NGS, for affected relatives of index cases and for segregation analysis. Polymerase chain reaction (PCR) was used to selective exon amplification. Annealing temperatures and primer sequences are given in Supplemental Table 1 and sequencing details in Appendix 1.

2.6. Statistical analysis

All variables in the study showed normal distribution on one-sample Kolmogorov-Smirnov test. Quantitative features are reported as mean and standard deviation (SD). Age at onset, disease duration, and SPRS were compared between patients with pure and complicated HSP and between patients with and without a probable molecular diagnosis using two-tailed unpaired Student's *t*-test. Statistical significance was defined as $p < 0.05$.

3. Results

3.1. Clinical and genetic classifications

Twenty-nine unrelated index cases were analyzed by the NGS-HSP panel (19 females, mean [SD] age at onset, 27.1 [14.7] years; disease duration, 17 [10.3] years). Twelve (41.4%) index cases presented with pure and 17 (58.6%) with complicated HSP. Pedigrees suggested AD-HSP in 10/29 (34.5%) and AR-HSP in 19/29 (65.5%) index cases, including two isolated cases with TCC and complicated HSP. Six (20.6%) index cases had TCC on MRI.

Pure-HSP: 8/12 (66.7%) index cases were classified as AD-HSP; mean (SD) age at onset was 29.7 (17.2) years, disease duration 14.5 (9.3) years and SPRS 20.9 (6.7) points. Complicated-HSP: 15/17