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Roughly Half of Patients Presenting with Ataxia and/or Spasticity Receive a Definitive Diagnosis with Diagnostic Exome Sequencing (DES)

BACKGROUND

- Ataxia refers to the incoordination of muscle movements stemming from an underlying dysfunction of the cerebellum. Spasticity, or hyperreflexia, refers to muscle stiffness caused by an imbalance of excitatory and inhibitory of motor neurons.
- Hereditary spastic paraplegias (HSP) and spinocerebellar ataxia (SCA) are neurodegenerative disorders with overlapping clinical features . The clinical and genetic heterogeneity of HSP and SCA, as well as the ever growing number of new loci and causative genes, have historically made clinical differentiation between the two groups of disorders virtually impossible (Hedera, 2009).
- Diagnostic exome sequencing (DES) has enabled simultaneous interrogation of the entire coding regions of the genome and thus can overcome these obstacles.
- This study provides an analysis of the 59 cases with ataxia and/or spasticity of the first 500 DES cases at Ambry Genetics.

METHODS

- **Patients/study population:** Genomic deoxyribonucleic acid (gDNA) was isolated from whole blood from probands and relatives referred to Ambry Genetics (Aliso Viejo, CA) for diagnostic exome sequencing (DES).
- **Whole exome sequencing:** Samples were prepared using the SureSelect Target Enrichment System (Agilent Technologies, Santa Clara, CA) or or SeqCap EZ VCRome 2.0 (Roche NimbleGen, Madison, WI). The enriched exome libraries were sequenced using paired-end, 100-cycle chemistry on the Illumina HiSeq 2000 (Illumina, San Diego, CA).
- **Characterized and Disease-causing (ChaD) and Novel gene databases**: The Characterized and Disease-causing (ChAD) gene database was curated on a weekly basis to include genes currently known to be responsible for causing human disease. The ChAD database included genes which are associated with syndromes listed in the Human Gene Mutation Database (HGMD) (Stenson, 2009) and the Online Mendelian Inheritance in Man (OMIM) database. Novel genes were defined as those not known to underlie a Mendelian condition at the time of data analysis. Any RefSeq gene not included in the ChAD database was included in the novel gene database.
- **Bioinformatics annotation, filtering of variants, and Family history Inheritance-based Detection (FIND):** HGMD, OMIM, the Single Nucleotide Polymorphism database (dbSNP) (Sherry, 2001), 1000 genomes, HapMap data (International HapMap, 2003) and online search engines (e.g., PubMed) were used to search for previously described gene mutations and polymorphisms. Stepwise filtering included the removal of common SNPs, intergenic and 3′/5′ UTR variants, non-splice-related intronic variants, and lastly synonymous variants. Variants were then filtered further based family history and possible inheritance models using the informatics program "FIND" (Family history Inheritance-based Detection).
- **Personalized Medical Review with Enhanced and Comprehensive Assessment (PRECISE) of potentially causal variants:** Each candidate mutation was assessed by a molecular geneticist to identify the most likely causative mutation(s) using the "PRECISE" (Personalized Medical Review with Enhanced and Comprehensive Assessment) analysis method. In brief, interpretive filtering was based on the deleterious nature of the candidate alterations, literature search, and analysis of the relevance of the candidate genes' function in relation to the patient's phenotype. Most candidate alterations undergo Sanger sequencing confirmation and familial co-segregation analysis.
- Patient demographics (age, gender, referral indication) were collected from required testing documents supplied to the laboratory with the requisition form and the biospecimens. Patient identifiers were removed. Data curation included the primary exome test option ordered, patient age, diagnosis and/or clinical description, and exome sequencing results, including gene(s), alteration(s), gene category (novel, characterized), alteration interpretation (pathogenic, likely pathogenic, uncertain, likely benign), and clinical overlap of gene-association and patient phenotype (positive, uncertain, partial).
- Statistical analyses were computed by chi^2 goodness of fit tests and Fisher's Exact Probability.

Figure 1: Percentage of Positives, Negatives, and Uncertain Results in 59 HSP/SCA Cases vs. Overall Rate

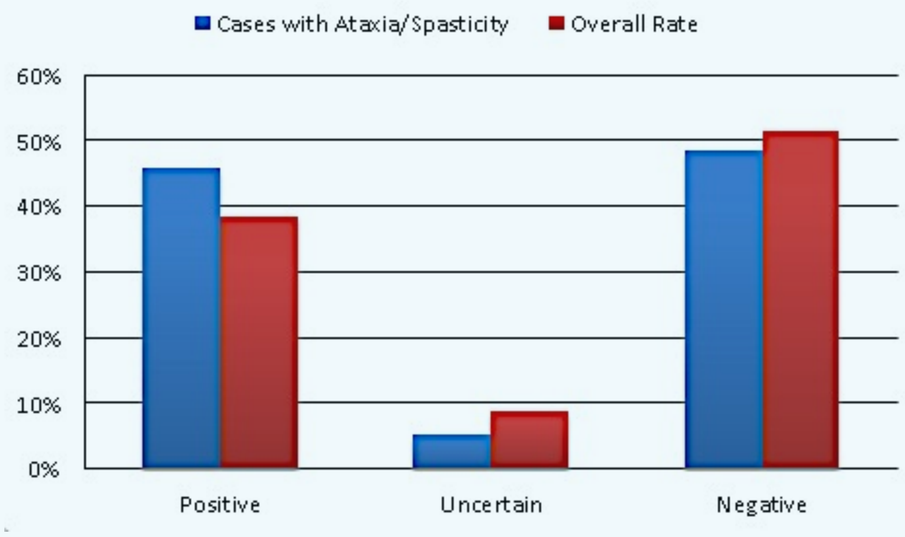

Table 1: Mutations Identified[1]

Gene	Mutation
ADCK3	c.1015G>A
	c.1665G>A
ALS2	c.4897C>T
AMPD2	c.1133G>C
	c.1232A>G
ANK2	c.8681delA
BRAT1	c.638_639dupA
	c.803+1G>C
CACNA1A	c.3043G>A
COL3A1	c.2114_2121+4del12insCT
COL4A1	c.2494G>A
CYP7B1	c.334C>T
DGUOK	c.211C>G
DNA2	c. 1808T>C
ELP2	c.812A>G
	c.1579C>T
FA2H	c.565C>T
GABRA1	c.1165A>G
GNAO1	c.736G>A
IQSEC2	c.804delC
ITPR1	c.800C>T
	c.7640_7642delAGA
KCNC3	c.1268G>A
KCNQ2	c.710A>G
MPZ	c.380G>C
NID1[2]	c.1640C>T
NUBPL	c.311T>C
	c.815-27T>C
PANK2	c.1561G>A
PKD1	c.9564_9566del
PRNP	c.314C>T
SPAST	c.1634C>G
TGM6	c.115A>T
TPP1	c.1266G>C
	c.509-1G>C
TUBB4A	c.467G>T
ZNF335	c.3378_3379delCA
	c.3332G>T

[1]Mutations were gathered from positive cases only. Any uknown mutations were elimated.
[2]Novel Gene

Figure 2: Types of Mutations Found

RESULTS/DISCUSSION

- Of the first unselected 500 DES cases with a wide range of clinical conditions evaluated at Ambry Genetics (Aliso Viejo CA):
  - 59 (12%) presented with symptoms of ataxia and/or spasticity. Each of these patients previously had numerous uninformative diagnostic tests (e.g. ataxia panels, arrays, metabolic and mitochondrial studies).
    - 27 of these cases had a positive result (46%) (**Figure 1**), as compared to the overall positive rate for patients with all presenting features of 39% positive result overall.
    - Three patients (5.3%) were found to have an uncertain result, less than the overall uncertain rate of 9%. One result (1.8%) was found in a novel gene, less than the overall novel gene rate of 8%.
- Among the 30 patients with positive and uncertain results, a total of 38 pathogenic mutations were found (**Table 1**).
  - Of these 38 alterations, 63% were found to be Missense, 13% were Frameshift, 11% Splice, 8% Nonsense, and 5% In-Frame mutations (**Figure 2**).
  - Of the 30 positive and uncertain results, 53% were inherited in an autosomal dominant pattern, 43% were inherited in an autosomal recessive pattern and 3% were found to be X-linked.
  - Of note, only nine individuals (33% of positive cases) were found with a mutation in traditional HSP or SCA genes (*ALS2* , *CACNA1A, CYP7B1, FA2H* , *ITPR1 (2 cases), KCNC3, SPAST,* and *TGM6*).

TAKE-HOME POINTS

- Diagnostic exome sequencing establishes a molecular diagnosis in almost half of patients with symptoms of ataxia and/or spastic paraplegia in whom traditional testing methods is uninformative.
- Missense alterations are the most commonly-observed mutations type, suggesting that ataxia and spasticity more often result from gain-of-function or dominant-negative mutational mechanisms.
- Two-thirds of the mutated genes are not within genes traditionally associated with *HSP/SCA*.
- The high ratio of positive findings in unconventional *HSP/SCA* genes in our cohort also indicates that DES should be considered as a first tier test in patients presenting with ataxia and/or paraplegia.

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