Diagnostic Exome Sequencing (DES) Provides Diagnoses Among Patients with Abnormal Brain MRI Findings

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BACKGROUND

- The high diagnostic rate, the generation of clinically actionable data for patient care guidance, and dramatic cost-savings are just some of the features that make diagnostic exome sequencing (DES) ideally suited to be the standard of care for diagnostic medicine in the 21st century.
- Reaching a diagnosis results in an end to the expensive, time consuming, and potentially invasive diagnostic odyssey which poses a heavy burden both for families and on the healthcare system (Beaulieu, 2014; Farwell, 2014; Iglesias, 2014; Srivastava, 2014).
- Magnetic resonance imaging (MRI) is an effective tool in the evaluation of patients with neurological diseases, often leading to the identification of findings pathognomonic of a particular syndrome.

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). Informed consent was obtained from all family members involved in the testing process.
- **Whole exome sequencing:** Samples were prepared using the SureSelect Target Enrichment System (Agilent Technologies, Santa Clara, CA) 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 ChAD gene database (Ambry Genetics, Aliso Viejo, CA) 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 on family history and possible inheritance models using the informatics program "FIND" (Family history Inheritance-based Detection) (Ambry Genetics, Aliso Viejo, CA).
- **Personalized Medical Review with Enhanced and Comprehensive Assessment (PRECISE) of potentially causal variants:** Each candidate alteration 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 (Ambry Genetics, Aliso Viejo, CA). 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.
- **Data curation:** Data regarding the primary exome test ordered, patient age, diagnosis and/or clinical description, and exome sequencing results were also curated. Data on results included 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. DES Detection Rates Among Patients with Abnormal Brain MRI Findings Do Not Differ from Overall Detection Rates

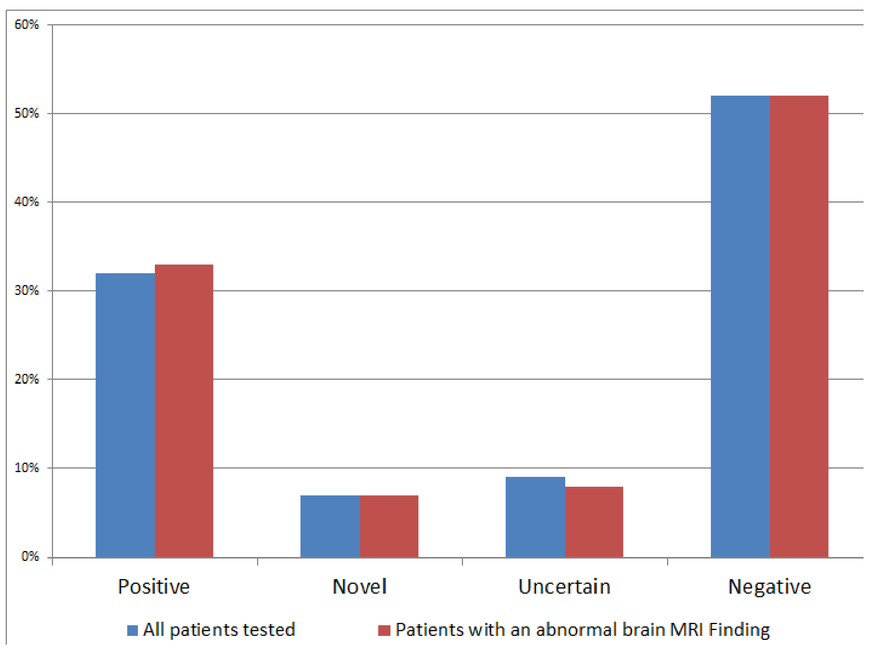
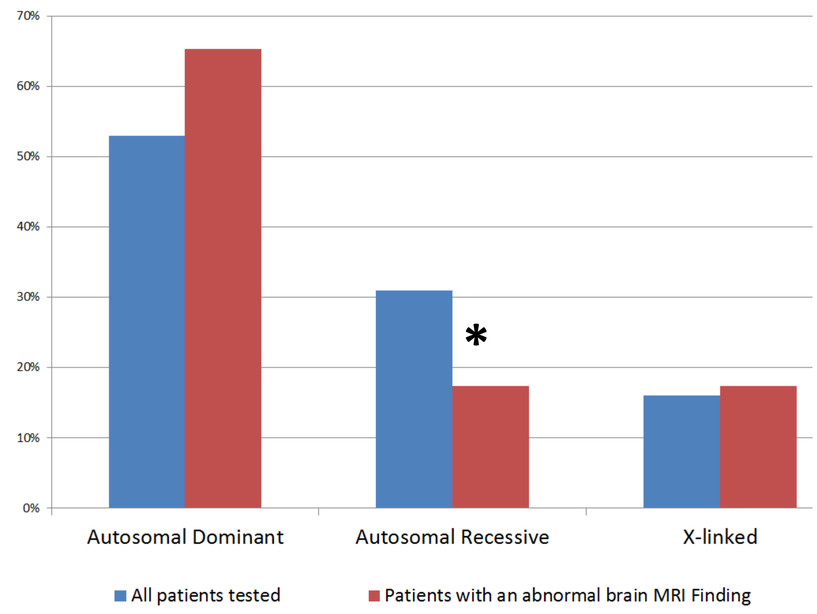


TABLE 1: Novel Gene Findings Among Patients with Abnormal Brain MRI Findings

Novel Gene	Inher-itance	Alteration Type	Genotype	Inherited or *de novo*
AIMP2	AR	Frameshift	Heterozygous	Inherited
		Splice	Heterozygous	Inherited
ARHGAP11A	AD	Frameshift	Heterozygous	Inherited
CDC42	AD	Missense	Heterozygous	*de novo*
DPYSL2	AD	Missense	Heterozygous	*de novo*
ETV6	AD	Splice	Heterozygous	Inherited
ITGA11	AD	Missense	Heterozygous	Inherited
		Missense	Heterozygous	Inherited
ITSN1	AD	Missense	Heterozygous	*de novo*
LRFN2	AD	Frameshift	Heterozygous	*de novo*
LRFN5	AR	Missense	Heterozygous	Inherited
		Missense	Heterozygous	Inherited
MAPK1	AD	Missense	Heterozygous	*de novo*
MYH10	AD	Nonsense	Heterozygous	*de novo*
NID1	AD	Missense	Heterozygous	Inherited
SNAP25	AD	Missense	Heterozygous	*de novo*
ZNF302		Frameshift	Heterozygous	Likely Inherited

FIGURE 2. Patients with Abnormal MRI Findings Are Significantly Less Likely to Have an Autosomal Recessive Finding as Compared to All Patients Tested



***p < 0.05**

RESULTS

- Among the first 500 reported DES families, 168 (34%) had a previous abnormal brain MRI.
- Positive findings were uncovered in 66 of these families (39%). Positive and likely positive alterations within clinically characterized genes were identified in 55 of the 168 families, for a molecular diagnostic rate of 33% among characterized genes. The diagnostic rates among patients in this cohort are similar to the overall detection rates among all 500 referred patients (**FIGURE 1**).
- A total of 75 gene findings were discovered among the 66 patients with positive findings (9 patients had a positive finding in two genes). Among the 75 gene findings, 49 (65%) were autosomal dominant, 13 (17%) were autosomal recessive, and 13 (17%) were X-linked molecular defects. The 66 positive patients with abnormal MRI findings were significantly less likely to have an autosomal recessive finding (18%) as compared to the entire positive cohort (51/163; 31%) ($p < 0.05$) (**FIGURE 2**).
- Among 49 patients with abnormal MRI findings and an autosomal dominant positive finding, 31 (63%) were confirmed *de novo*, statistically lower than the overall rate of *de novo* findings among all 500 patients (68 of 86; 79%) ($p<0.05$).
- Fourteen novel genes were uncovered among 11 families with abnormal MRI findings (7%) spanning a range of mutation types, inheritance, and mutation origin (*de novo* vs inherited) (**TABLE 1**).

TAKE-HOME POINTS

- The majority of patients with abnormal brain MRI findings have a dominant finding on DES, likely a reflection of the level of severity.
- These data highlight the utility of DES in providing the most comprehensive molecular diagnosis given the diversity of genetic findings and provide an opportunity for novel gene discovery as seen with 7% of patients with abnormal brain MRI findings.

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