Dima El-Khechen[1], Kelly Gonzalez[1], Layla Shahmirzadi[1], Sha Tang[1], Brigette Tippin[1], Wenqi Zeng[1], AHM Mahbubul Huq[2], Lindsey Murray[2]
1) Ambry Genetics Corporation, Aliso Viejo, CA
2) Departments of Pediatrics and Neurology, Wayne State University, MI

Diagnostic Exome Sequencing (DES) Unmasks Two Discrete Genetic Etiologies in a Patient With Ataxia and Epilepsy: Dual Molecular Diagnosis in a Patient Highlights the Utility of Diagnostic Exome Sequencing

BACKGROUND

- Whole exome sequencing has been quite successful at diagnosing individuals with single Mendelian disorders. Here we report a case where diagnostic exome sequencing (DES) identified two separate disorders in the patient, each accounting for part of his phenotype.
- Clinical history: The proband is a three-year old Caucasian boy referred for testing due to severe global delay, severe central hypotonia, infantile spasms, intractable seizures, movement disorder, dystonic episodes, microcephaly, and dysconjugate eye movements. He is non-dysmorphic. His family history was unremarkable.
- Prior tests/evaluations: A brain MRI had revealed supratentorial volume loss and subtle diffusion restriction and T2 signal abnormality in the region of the globus pallidus and posterior pons. Extensive previous unrevealing genetic and metabolic testing included a chromosome microarray, sequencing of *SCN1A*, *STXBP1*, *POLG*, and mitochondrial DNA (mtDNA), urine organic acids, plasma amino acids, lactate and acylcarnitine profile.

METHODS

- **Patients:** Genomic deoxyribonucleic acid (gDNA) was isolated from whole blood from the proband 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 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 on 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. Candidate alterations undergo Sanger sequencing confirmation and familial co-segregation analysis as needed for interpretation.
- Statistical analyses were computed by chi^2 goodness of fit tests and Fisher's Exact Probability.

Table 1: Number of Genes and Alterations Identified

NUMBER OF GENES & ALTERATIONS IDENTIFIED BASED ON BIOINFORMATICS & INTERPRETATION

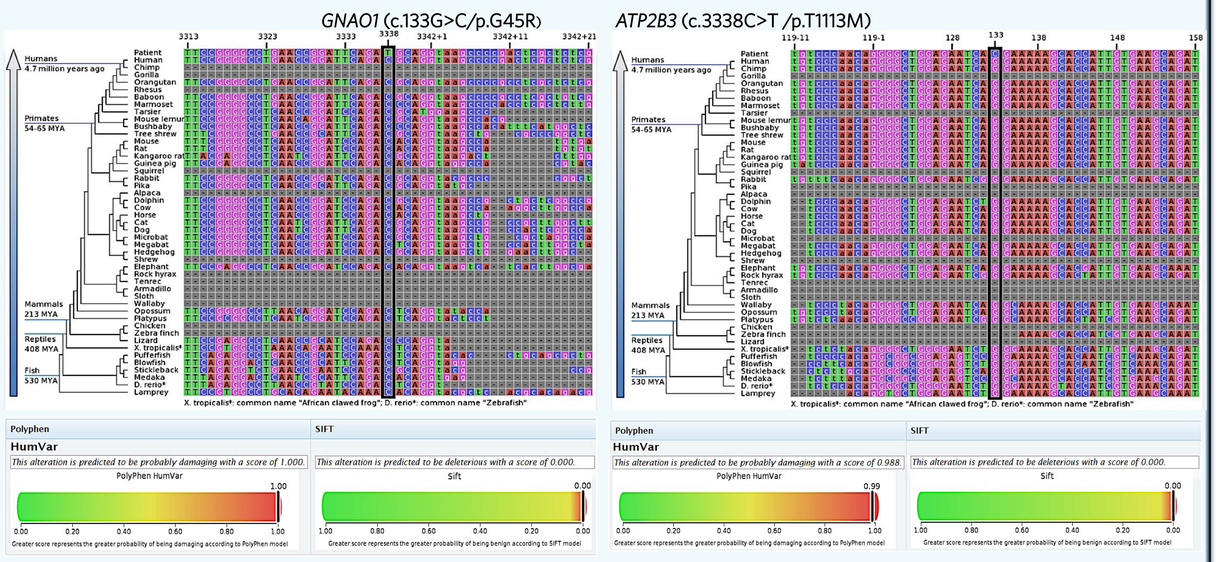
	Post-Inheritance Model Filtering	Post-Medical Review*				Notable Candidate Genes**
		Post-alteration review	Post-clinical association review			
		TOTAL	Characterized‡	Clinically novel‡	TOTAL	
Autosomal Dominant Genes (Alterations)	8 (8)	8 (8)	1(1)	5 (5)	6(6)	**1 (1)**
Autosomal Recessive Genes (Alterations)	7 (15)	4 (6)	0 (0)	4 (6)	4 (6)	**0 (0)**
X-linked Recessive Genes (Alterations)	8 (8)	8 (8)	2 (2)	6 (6)	8 (8)	**1 (1)**
X-linked Dominant Genes (Alterations)	0(0)	0(0)	0(0)	0(0)	0(0)	**0(0)**
Y-linked Genes (Alterations)	0(0)	0(0)	0(0)	0(0)	0(0)	**0(0)**
TOTAL GENES (Alterations)	**23 (31)**	**20 (22)**	**3(3)**	**15 (17)**	**18 (20)**	**2 (2)**

* Post-medical review filtering involves the manual removal of genes unrelated to the patient's evaluated phenotype and alterations considered benign.

** Gene alterations selected for co-segregation.

‡ Characterized genes: Genes known to be associated with a clinical phenotype based on the HGMD or OMIM-Morbid databates or the medical literature. A clinically novel gene is a gene which is not currently known to underlie a genetic condition.

Figure 1. Conservation and *in silico* Data



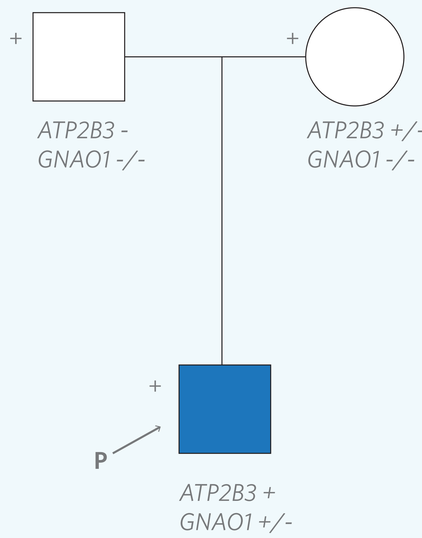
RESULTS

DES identified clinically significant alterations in two* characterized genes:

1. A *de novo* missense alteration in *GNAO1* (c.133G>C/p.G45R). *GNAO1* encodes the G α o subunit of the Go heterotrimer which is extremely abundant in brain tissue, and transduces a variety of signals from seven-transmembrane-type receptors to intracellular effectors. *GNAO1* mutations are associated with early infantile epileptic encephalopathy 17, which is associated with significant developmental delay, seizures, involuntary movements, dystonia, chorea, and brain MRI abnormalities including cerebral atrophy and delayed myelination (Nakamura, 2013). This alteration likely accounts for the patient's severe psychomotor delay, intractable seizures, movement disorder, dystonia, and cerebral atrophy.
2. A maternally-inherited missense alteration in *ATP2B3* (c.3338C>T/p.T1113M). *ATP2B3* encodes the plasma membrane calcium-transporting ATPase 3, which functions in cellular homeostasis of CA^{2+} and shows a highly restricted pattern of expression to human neuronal tissues (Brini, 2013 and Brown, 1996). *ATP2B3* mutations are associated with X-linked spinocerebellar ataxia-1, which is associated with congenital cerebellar atrophy, severe hypotonia, motor delay, slow eye movements, non-progressive cerebellar ataxia, tremor, dystonia, and atrophy on brain MRI (Bertini, 2000). This alteration likely accounts for the patient's hypotonia, dysconjugate eye movements, pons abnormality, and part of the developmental delay.

*The *ATP2B3* alteration was initially reported as a single finding in July of 2013, however after the report was issued the data was retrospectively re-analyzed based upon new publications on *GNAO1* and an amended report was issued in November 2013.

Figure 2: Co-Segregation Data



+ Indicates whole exome sequencing performed

CONCLUSION

- Our findings highlight the importance of diagnostic exome sequencing in the context of complex phenotypes resulting from two or more underlying pathogenic genetic alterations.
- Traditional models of single-gene testing would have likely proven unsuccessful in providing an accurate, comprehensive diagnosis in this case.
- While cases like this are likely to be uncommon, it may be helpful to consider digenic or oligogenic etiologies during a genetic workup, especially in cases with an atypical or unusually severe phenotype, even if one diagnostic gene mutation has already been identified.
- As genetics is a rapidly evolving field, this case also highlights the importance of periodic retrospective data mining and analysis based upon new data.

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