Julie Neidich[1], Megan Truitt Cho[2], Kelly D. Gonzalez[1], Sha Tang[1], Wendy K. Chung[3]
1) Ambry Genetics, Aliso Viejo, CA
2) New York Presbyterian Hospital, New York, NY
3) Columbia University Medical Center, New York, NY

Clinical exome sequencing identifies a novel gene, *SNAP25*, associated with seizures

BACKGROUND

- Over the last two years, clinical diagnostic exome sequencing has been instrumental in successfully providing a molecular diagnosis for families who had previously been unsuccessful in their pursuit of the underlying disease etiology.
- A 14 year-old female with intellectual disability, static encephalopathy, complex partial seizures, scoliosis, gastroesophageal reflux disease, and hypotonia was referred to Ambry Genetics for whole exome sequencing. The family history was negative for similar phenotypes.
- The patient evaded diagnosis through clinical evaluation and extensive genetic testing over many years including negative chromosome analysis, subtelomeric FISH, CGH/SNP microarray, Angelman/Prader Willi syndrome uniparental disomy analysis, *MECP2, CDKL5, FOXG1, ARX,* 90 gene X-linked intellectual disability gene panel, 53 gene seizure panel, 101 nuclear mitochondrial gene panel, as well as uninformative biochemical results.

METHODS

- Genomic deoxyribonucleic acid (gDNA) was isolated from whole blood from the patient and her parents. Samples were prepared using the SureSelect Target Enrichment System (Agilent Technologies, Santa Clara, CA). The enriched exome libraries were sequenced using paired-end, 100-cycle chemistry on the Illumina HiSeq 2000 (Illumina, San Diego, CA).
- Exome data undergoes alignment, base calling, and variant calling. Passing base calls have at least 7x coverage and quality scores of Q20 or higher, which translates to a base call error rate probability of 1:100, or a base call read accuracy of 99%. Exons plus at least 2 bases into the 5' and 3' ends of all the introns are analyzed and reported. Variants were filtered further based on family history and possible inheritance models. Data is annotated with the Ambry Variant Analyzer tool (AVA), including nucleotide and amino acid conservation, biochemical nature of amino acid substitutions, population frequency, predicted functional impact, and clinical disease associations (Human Gene Mutation Database (HGMD; Stenson, 2009), OMIM, and several other databases).
- A molecular geneticist performed interpretive filtering 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.
- Each candidate variant was analyzed by Sanger sequencing for mutation confirmation and co-segregation analysis.

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**		
	HGMD/ OMIM-Morbid‡	Clinically novel‡‡	TOTAL	HGMD/ OMIM-Morbid	Clinically novel	TOTAL	HGMD/ OMIM-Morbid	Clinically novel	TOTAL
Autosomal Dominant Genes (Alterations)	1 (1)	23 (25)	**24 (26)**	0 (0)	17 (17)	**17 (17)**	0 (0)	1 (1)	**1 (1)**
Autosomal Recessive Genes (Alterations)	2 (3)	14 (34)	**16 (37)**	1 (2)	11 (21)	**12 (23)**	0 (0)	0 (0)	**0 (0)**
X-linked Recessive Genes (Alterations)	0 (0)	0 (0)	**0 (0)**	0 (0)	0 (0)	**0 (0)**	0 (0)	0 (0)	**0 (0)**
X-linked Dominant Genes (Alterations)	0 (0)	1 (1)	**1 (1)**	0 (0)	1 (1)	**1 (1)**	0 (0)	0 (0)	**0 (0)**
Y-linked Genes (Alterations)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
TOTAL GENES (Alterations)	**3 (4)**	**38 (60)**	**41 (64)**	**1 (2)**	**29 (39)**	**30 (41)**	**0 (0)**	**1 (1)**	**1 (1)**

*Post-medical review filtering involves the manual removal of genes unrelated to the patient's evaluated phenotype and alterations considered benign
‡Characterized Genes: Genes known to be associated with a clinical phenotype based on the HGMD or OMIM-Morbid databases or the medical literature
‡‡A clinically novel gene is a gene which is not currently known to underlie a genetic condition
**Notable Candidate Genes: Gene alterations selected for co-segregation.

Figure 1. Next-gen sequence alignment

SNAP25: c.142G>T (p.V48F)

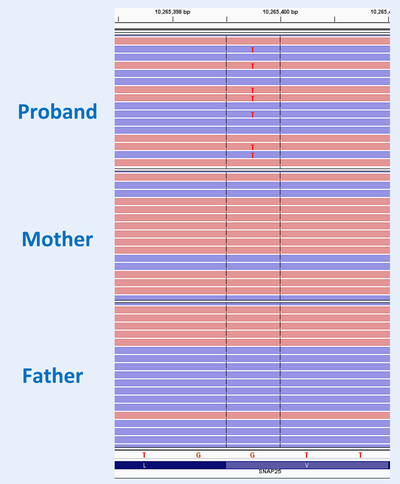
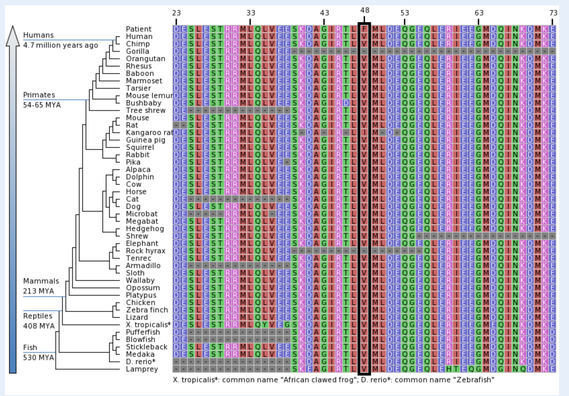


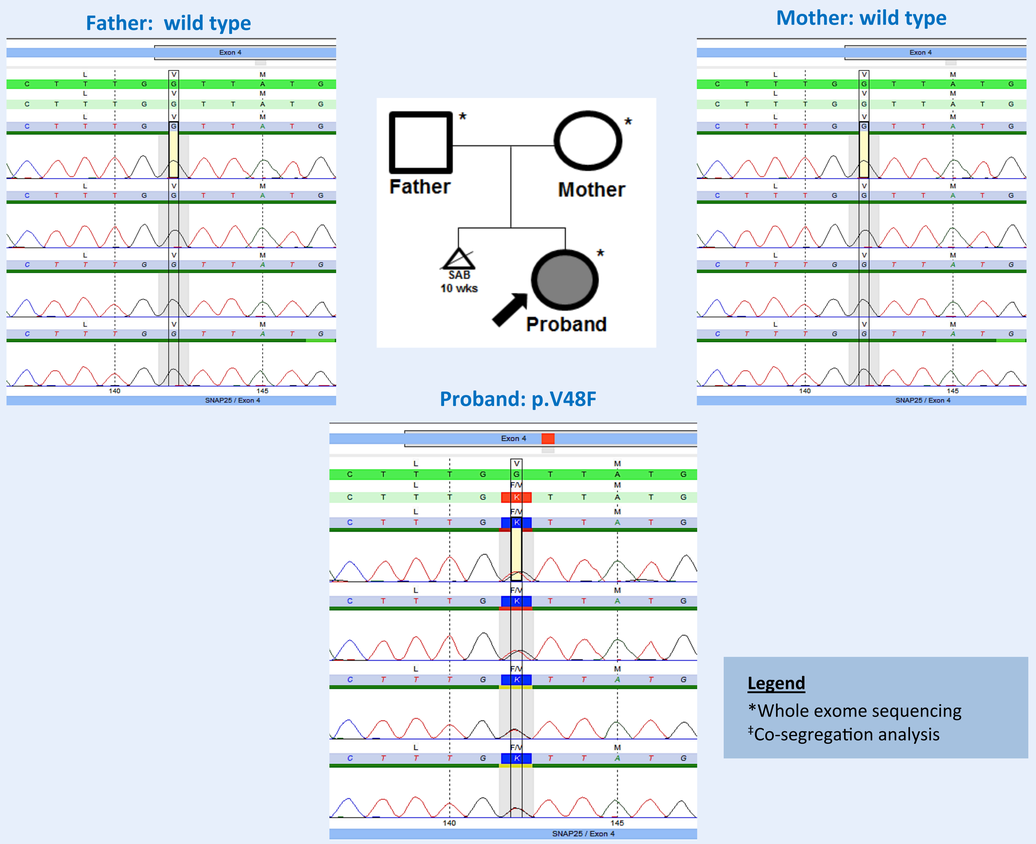
Figure 2. Amino acid V48 is conserved throughout evolution



RESULTS/ DISCUSSION

- Full exome sequencing, bioinformatics analysis, and filtering based on autosomal and X-linked dominant and recessive inheritance models of the proband, mother, and father revealed 64 unique alterations in 41 genes . Manual review to remove sequencing artifacts and polymorphisms along with medical interpretation to remove alterations in genes lacking clinical overlap with the patient's evaluated phenotype resulted in 41 unique alterations in 30 genes (**Table 1**).
- One gene with one alteration that had likely clinical relevance ("Notable Candidate Gene") was further investigated via co-segregation analysis and Sanger sequencing confirmation: a heterozygous *de novo* alteration, c.142G>T (p.V48F), in the *SNAP25* gene (**Figure 1**).
- The amino acid is highly conserved throughout evolution (**Figure 2**) and the alterations are predicted to be probably damaging and deleterious by PolyPhen and SIFT *in silico* analyses (Adzhubei, 2010; Ng, 2006).
- Sanger sequencing confirmation and co-segregation analysis revealed that both the unaffected mother and father did not carry the variant, indicating a *de novo* occurrence of the alteration (**Figure 3**).
- The SNAP25 (Soluable NSF Attachment Protein-25) protein plays a specific role in the exocytotic release of neurotransmitters (Sudhof, 2011).
- Analysis of postmortem brains of patients with schizophrenia show decreased levels of the SNAP25 protein (reviewed in Corradini, 2009). Electroretinogram recordings of *Snap25* null mutant drosophila show a complete loss of synaptic transmission (Vilinsky, 2002).
- Mice with *Snap25* gene alterations exhibit anxiety-related behavior, ataxia, and seizures (Kataoka, 2011; Wilson, 1996).
- It was discovered that blind-drunk mice (Bdr), which display ataxia and impaired sensorimotor gating, harbor a heterozygous single amino acid substitution (I67T) in the *Snap25* gene. (Jeans, 2007).

Figure 3. Sanger sequencing confirmation and co-segregation



CONCLUSIONS

- Clinical diagnostic exome identified a *de novo* mutation in the clinically novel *SNAP25* gene and established a molecular diagnosis for a patient in whom traditional testing methods were uninformative.
- The *SNAP25* gene alteration is likely to provide an explanation for the patient's seizures and no other likely candidate gene alterations were identified to explain the remainder of the patient's clinical symptoms.
- The identified mutation is highly consistent with the patient's clinical symptoms which provides an opportunity for clinical intervention and surveillance as well as recurrence risks estimation and family planning.
- Additionally, the findings establish the novel *SNAP25* gene as a new gene associated with seizures and may allow for targeted mutation sequencing for patients with similar clinical presentations.
- Functional and *in vitro* studies are ongoing to further define the impact of *SNAP25* gene alterations.

References

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