JJ Wei[1], KD Gonzalez[1], A Fatemi[2], Julie Cohen[2], W Zeng[1]
1) Ambry Genetics, Aliso Viejo, CA
2) Department of Neurogenetics, Kennedy Krieger Institute, Johns Hopkins Medical Institutions, Baltimore, MD

Exome sequencing in a patient with syndromic intellectual disability identifies *ZNF238*, a novel gene which lies within the 1q43q44 microdeletion syndrome

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.
- 4 year-old male with syndromic intellectual disability was referred to Ambry Genetics for whole exome sequencing. The patient had cerebral palsy, global developmental delay, dysmorphic features, microcephaly, cortical visual impairment, bilateral single palmar creases, bifid uvula as well as brain MRI showing thin corpus callosum and cerebellar vermis hypoplasia. The family history was negative for similar phenotypes.
- The patient evaded diagnosis through clinical evaluation and extensive genetic and biochemical testing over many years including negative chromosome analysis, fragile X, CGH/SNP microarray, TSH, T4, very long chain fatty acids, phytanic acid, pristanic acid, creatine kinase, arylsulfatase A enzyme testing, plasma amino acids, acylcarnitine profile, CBC, CMP, urine organic acids, carnitine total and free, ammonia, and an 81 gene X-linked intellectual disability sequencing panel.

METHODS

- Genomic deoxyribonucleic acid (gDNA) was isolated from whole blood from the patient and his 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

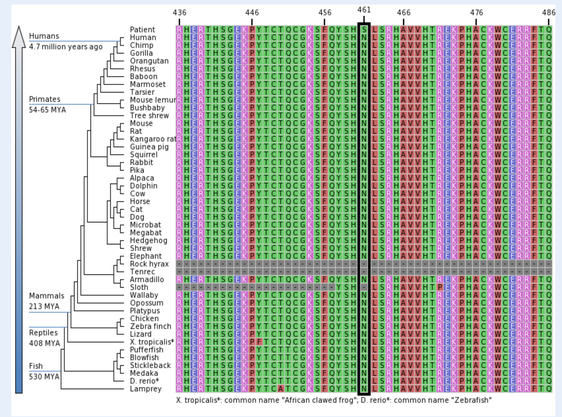
NUMBER OF GENES & ALTERATIONS IDENTIFIED BASED ON BIOINFORMATICS & INTERPRETATION*										
	Post-Inheritance Model Filtering			Post-Medical Review				Notable Candidate Genes		
				HGMD/ OMIM-Morbid						
	HGMD/ OMIM-Morbid‡	Clinically novel**	TOTAL	Post-alteration review	Post- clinical association review	Clinically novel	TOTAL	HGMD/ OMIM-Morbid‡	Clinically novel**	TOTAL
Autosomal Dominant Genes (Alterations)	4 (4)	16 (17)	**20 (21)**	3 (3)	0 (0)	5 (5)	**5 (5)**	0 (0)	1 (1)	**1 (1)**
Autosomal Recessive Genes (Alterations)	4 (8)	13 (31)	**17 (39)**	4 (8)	0 (0)	6 (9)	**6 (9)**	0 (0)	0 (0)	**0 (0)**
X-linked Recessive Genes (Alterations)	3 (3)	2 (2)	**5 (5)**	3 (3)	0 (0)	2 (2)	**2 (2)**	0 (0)	0 (0)	**0 (0)**
X-linked Dominant Genes (Alterations)	0 (0)	0 (0)	**0 (0)**	0 (0)	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)	**0 (0)**	0 (0)	0 (0)	**0 (0)**
TOTAL GENES (Alterations)	**11 (15)**	**31 (50)**	**42 (65)**	**10 (14)**	**0 (0)**	**13 (16)**	**13 (16)**	**0 (0)**	**1 (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
[2]Characterized Genes: Genes known to be associated with a clinical phenotype based on the HGMD or OMIM-Morbid databases or the medical literature
[3]A clinically novel gene is a gene which is not currently known to underlie a genetic condition
[4]Notable Candidate Genes: Gene alterations selected for co-segregation.

Figure 1. Next-gen sequence alignment

***ZNF238*: c.1382A>G (p.N461S)**

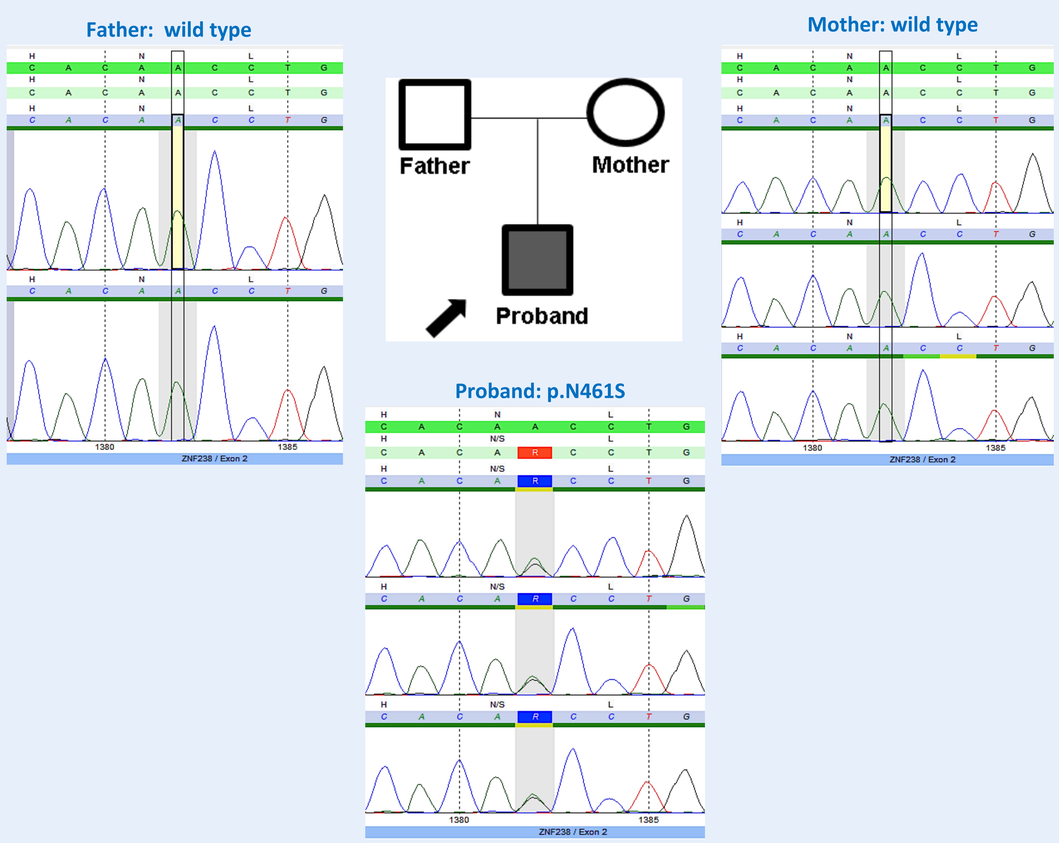
Figure 2. Amino acid N461 is conserved throughout evolution



RESULTS / DISCUSSION

- Full exome sequencing, bioinformatics analysis, and filtering based on autosomal and X-linked dominant and recessive and Y-linked inheritance models of the proband, mother, and father revealed 42 genes (65 unique alterations). Manual review to rule out sequencing artifacts and polymorphisms along with medical interpretation to rule out genes lacking clinical overlap with the patient's evaluated phenotype resulted in 13 genes (16 unique alterations) (**Table 1**).
- One gene (1 alteration) with likely clinical relevance ("Notable Candidate Gene") was further investigated via co-segregation analysis/ Sanger sequencing confirmation: heterozygous *de novo* alteration, p.N461S (c. 1382A>G) in the *ZNF238* 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 unaffected mother and father did not carry the mutation, indicating a *de novo* mutation occurrence (**Figure 3**).
- *ZNF238* is among the genes deleted in patients with 1q43q44 microdeletions who present with intellectual disability, microcephaly, agenesis of the corpus callosum, and seizures (Ballif, 2012; Boland, 2007; Caliebe, 2010; Nagamani, 2012; Thierry, 2012).
- CNS-specific *ZNF238* knock-out in mice leads to post-natal microcephaly with loss of the corpus callosum and cerebellar vermis hypoplasia, a phenotype which closely resembles the human chromosome 1q43q44 deletion syndrome (Xiang, 2012).

Figure 3. Sanger sequencing confirmation and co-segregation



CONCLUSIONS

- Clinical diagnostic exome identified a *de novo* mutation in the *ZNF238* gene and established a molecular diagnosis for a patient in whom traditional testing methods were uninformative.
- Based on critical region analysis and knowledge of gene function and expression among patients with 1q43q44 microdeletions, the *ZNF238* has widely been suspected as the candidate gene for abnormalities of the corpus callosum (ACC) and the *AKT3* gene for microcephaly (Ballif, 2012; Boland, 2007; Caliebe, 2010; Nagamani, 2012; Thierry, 2012).
- We report, to our knowledge, the first example of a patient with microcephaly with alteration in *ZNF238* alone, providing evidence against the suggestion that *AKT3* acts as the critical gene for microcephaly while providing supportive evidence for *ZNF238* as the critical gene for ACC.
- Additionally, the findings further delineate the phenotypic spectrum of patients with mutations in the *ZNF238* gene and may allow for targeted mutation sequencing for patients with similar clinical presentations.
- Since missense alteration of *ZNF238* has not been previously implicated in intellectual disability, functional and *in vitro* studies are required to further define the impact of *ZNF238* gene alterations.

References

- Ballif BC, *et al.* (2012) *Hum Genet* **131**:145-156.
- Boland E, *et al.* (2007) *Am J Hum Genet* **81**:292-303.
- Caliebe A, *et al.* (2010) *Eur J Med Genet* **53**:179-185.
- HGMD® [Internet]: Stenson PD, *et al.* (2009) *Genome Med* **1**(1):13.
- Nagamini SC, *et al.* (2012) *Eur J Hum Genet.***20**(2):176-179.
- PolyPhen [Internet]: Adzhubei IA, *et al.* (2010) *Nat Methods* **7**(4):248-249.
- SIFT [Internet]: Ng PC & Henikoff S (2006) *Hum Genet.* **7**:61-80
- Thierry G, *et al.* (2012) *Am J Med Genet A* **158A**(7):1633-40
- Xiang C, *et al.* (2012) *Cell Death Differ* **19**(4):692-702.