Wenqi Zeng[1], Sha Tang[1], Kelly D. Gonzalez[1], Heather Feenstra[2], Kwame Anyane-Yeboa[3]
1) Ambry Genetics, Aliso Viejo, CA
2) St. Luke's Hospital, New York, NY
3) Columbia University Medical Center, New York, NY

Nonsense mutations identified in *UBE3B* in a patient with Blepharophimosis-Ptosis-Intellectual Disability Syndrome by clinical diagnostic exome

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 33 year-old Hispanic male with a clinical history consistent with Blepharophimosis-Ptosis-Intellectual Disability was referred to Ambry Genetics for whole exome sequencing.
- The family history was notable for consanguinity (parents are second cousins once removed) and a brother with Sudden Infant Death Syndrome (SIDS). The patient presented at birth with muscular hypotonia and hearing impairment, is severely delayed in motor and cognitive development, is non-verbal with profound intellectual disability, and has facial dysmorphism including severe blepharophimosis, ptosis, depressed nasal bridge, upward slanting palpebral fissures, and hyperterolism.

METHODS

- Genomic deoxyribonucleic acid (gDNA) was isolated from whole blood from the patient, his mother and brother. 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[1]		Notable Candidate Genes[‡]
		Post review of alterations	Post review of clinical association	
Autosomal Dominant Genes (Alterations)	50 (52)	49 (51)	2 (2)	**2 (2)**
Autosomal Recessive Genes (Alterations)	9 (22)	8 (21)	3 (4)	**2 (2)**
X-linked Recessive Genes (Alterations)	0 (0)	0 (0)	0 (0)	**0 (0)**
X-linked Dominant Genes (Alterations)	0 (0)	0 (0)	0 (0)	**0 (0)**
Y-linked Genes (Alterations)	0 (0)	0 (0)	0 (0)	**0 (0)**
TOTAL GENES (Alterations)	**59 (74)**	**57 (72)**	**5 (6)**	**4 (4)**

[1]Post-medical review filtering involves the manual removal of genes unrelated to the patient's evaluated phenotype and alterations considered benign
[2]Notable Candidate Genes: Gene alterations selected for co-segregation.

Figure 1: Photographs of Proband

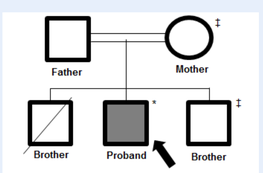
RESULTS/ DISCUSSION

- Full exome sequencing, bioinformatics analysis, and filtering based on autosomal and X-linked dominant and recessive inheritance models revealed 59 genes (74 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 5 genes (6 unique alterations) (**Table 1**).
- Four genes (4 alteration)s with likely clinical relevance ("Notable Candidate Genes") were further investigated via co-segregation analysis/ Sanger sequencing: *UBE3B, KIAA1033, EHMT1,* and *KIRREL3* (**Table 2**).
- The patient had significant clinical overlap with reported patients with *UBE3B* alterations, which present with autosomal recessive (AR) Blepharophimosis-Ptosis-Intellectual Disability (BMR) presents with developmental delay, growth retardation, microcephaly, facial dysmorphisms, and low cholesterol levels (Basel-Vanagaite, 2012) (**Figure 1**).
- The *KIRREL3* alteration was eliminated based on co-segregation analysis (Table 2). The *UBE3B* homozygous c.61G>T (p.E21X) was confirmed via Sanger sequencing and the mother and brother were heterozygous carriers (**Figure 2**).
- However, some clinical findings are not consistent with *UBE3B* alterations including the occipitofrontal circumference (OFC), abnormal sleep patterns, and occasional violent behaviors. Alterations in two other genes were identified which may provide explanation for additional and inconsistent findings including a rare, highly conserved homozygous rare missense alteration, c.878G>A (p.R293Q) in the *KIAA1033* gene and a highly conserved heterozygous missense alteration, c.1307C>T (p.P436L), in the *EHMT1* gene.
- Reported patients with *KIAA1033* alterations present with moderate to severe non-syndromic intellectual disability/developmental delay (ID/DD) with severe learning impairment, poor language and adaptive skills, delayed fine motor development, and short stature (3rd %ile) (Ropers et al., 2011).
- Heterozygous alterations in *EHMT1* are associated with a mental retardation syndrome known as Kleefstra syndrome which is characterized by severe mental retardation, hypotonia, brachycephaly, flat face with hypertelorism, upslanted palpebral fissures, and a thick everted lower lip (Kleefstra et al., 2006; Kleefstra et al., 2012; Kleefstra et al., 2005; Kleefstra et al., 2009; Willemsen et al., 2012).

Table 2. Familial Co-segregation Analysis

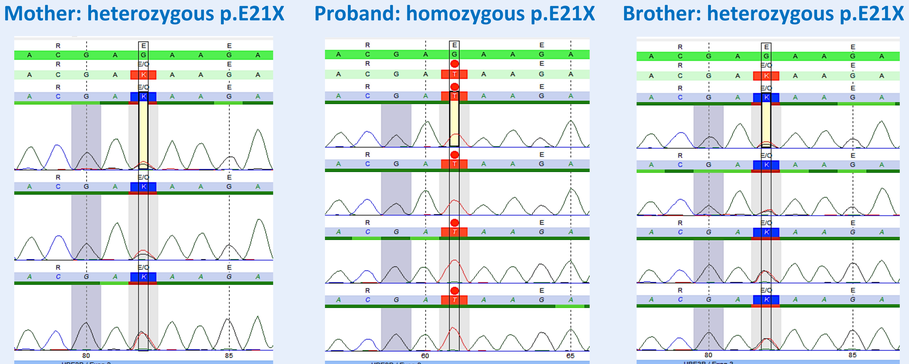
GENE(S)	EXON	ALTERATION	PROBAND	BROTHER	MOTHER
UBE3B	Exon 3	c.61G>T (p.E21X)	+/+	+/-	+/-
KIAA1033	Exon 11	c.878G>A (p.R293Q)	+/+	+/-	+/-
EHMT1	Exon 8	c.1307C>T (p.P436L)	+/-	-/-	-/-
KIRREL3	Exon 6	c.613C>T (p.R205W)	+/-	+/-	+/-

Figure 1. Sanger sequencing confirmation and co-segregation



Legend
*Whole exome sequencing
‡Co-segregation analysis

Mother: heterozygous p.E21X | **Proband: homozygous p.E21X** | **Brother: heterozygous p.E21X**



CONCLUSIONS

- Clinical diagnostic exome identified a homozygous nonsense alteration in the *UBE3B* gene and established a molecular diagnosis for a patient in whom traditional testing methods were uninformative.
- The *UBE3B* nonsense mutation is likely to be deleterious and clinical associations show significant overlap with the patient's described symptoms which provides an opportunity for clinical intervention and surveillance as well as recurrence risks estimation and family planning
- However, the *KIAA1033* and *EHMT1* genes also show partial clinical overlap and the likelihood that the three altered genes exhibit an oligogenic effect to produce a phenotype cannot be completely ruled out.
- In summary, this case reinforced that biallelic loss-of-function *UBE3B* mutations underlie AR BMR Syndrome and expanded the phenotypic spectrum associated with UBE3B deficiency while highlighting the utility of CDE in the identification of oligoneic contributions to atypical clinical presentations.

References

- Basel-Vanagaite LD, *et al.* (2012) *Am J Hum Genet* **91**:1–13.
- Kleefstra T, *et al.* (2005) *Am J Hum Genet* **42**:299-306.
- Kleefstra T, *et al.* (2006) *Am J Hum Genet* **79**:370-377.
- Kleefstra T, *et al.* (2009) *J Med Genet* **46**:598-606,
- Kleefstra T, *et al.* (2012) *Am J Hum Genet* **91**:73-82.
- Ropers F, *et al.* (2011) *Hum Mol Genet* **20**:2585-2590.
- Willemsen, MH, *et al.* (2012) *Molec syndromology* **2**:202-212.