

Clinical diagnostic exome sequencing identifies a maternally inherited 89 base pair deletion in the *UBE3A* gene associated with Angelman syndrome

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BACKGROUND

- Diagnostic exome sequencing (DES) is a cost-effective, powerful tool for establishing molecular diagnoses
- The exome, while accounting for only 1.5% of the total genome, is thought to be responsible for 85% of the mutations leading to Mendelian disease
- The diagnostic rate for DES in unselected patients is reported to be ~25%-37%
- DES may prove particularly useful in a proband that has eluded molecular diagnosis due to an atypical or milder presentation, as reported in the present case

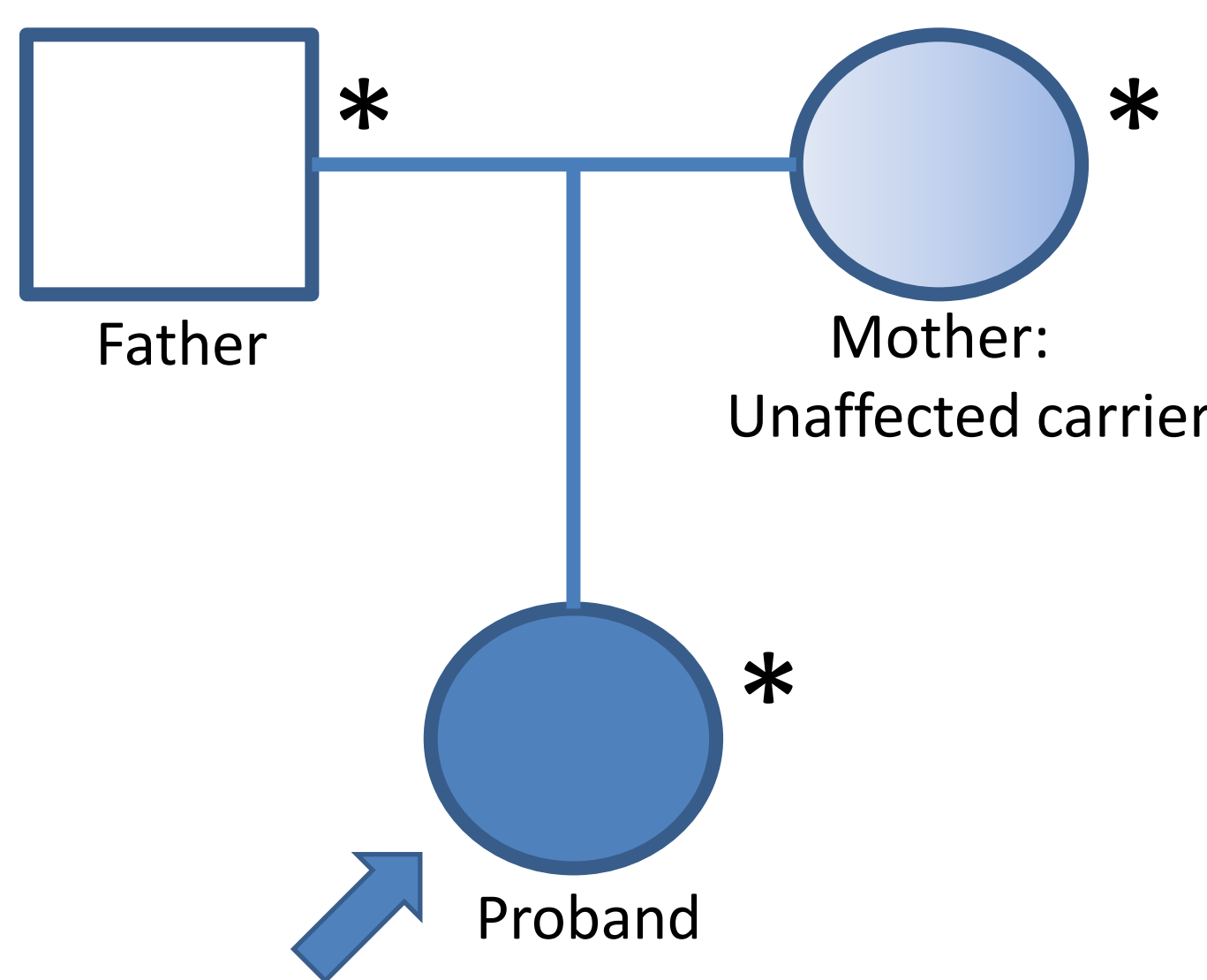
METHODS

- **Patient/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 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 database:** The Chad 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 analysis
- **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 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. Most candidate alterations undergo Sanger sequencing confirmation and familial co-segregation analysis

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FAMILIAL PEDIGREE

Shaded shapes indicate affected individuals. Asterisk (*) indicates whole-exome sequencing performed



CASE

- A five-year old female was referred for genetics evaluation given a history of developmental delay, including delayed walking (achieved at 2.5 years of age) and the absence of appropriate speech. She also exhibited some features within the autism spectrum, including hand flapping, and had been receiving physical, occupational and speech therapy. Prior brain MRI was reported to be essentially normal, and prior EEGs were also reported to be normal.
- Extensive clinical evaluation, biochemical work-up and genetic analysis had failed to provide a diagnosis for this patient prior to whole-exome sequencing. Specifically, prior metabolic work-up demonstrated normal urine organic acids, normal plasma amino acids and essentially normal acylcarnitines. Creatinine kinase was measured at 122 IU/L. Prior genetic testing results were all normal, including fragile X trinucleotide repeat analysis, DNA methylation analysis for *SNRPN*, routine chromosome analysis, BAC microarray (resolution of one megabase), SNP microarray (~2.67 million probe targets) and molecular genetic testing for Rett syndrome (*MECP2* sequence analysis).
- Proband-parents clinical diagnostic exome sequencing identified a frameshift mutation in the *UBE3A* gene (c.1254_1342del89, p.K418Nfs*26).

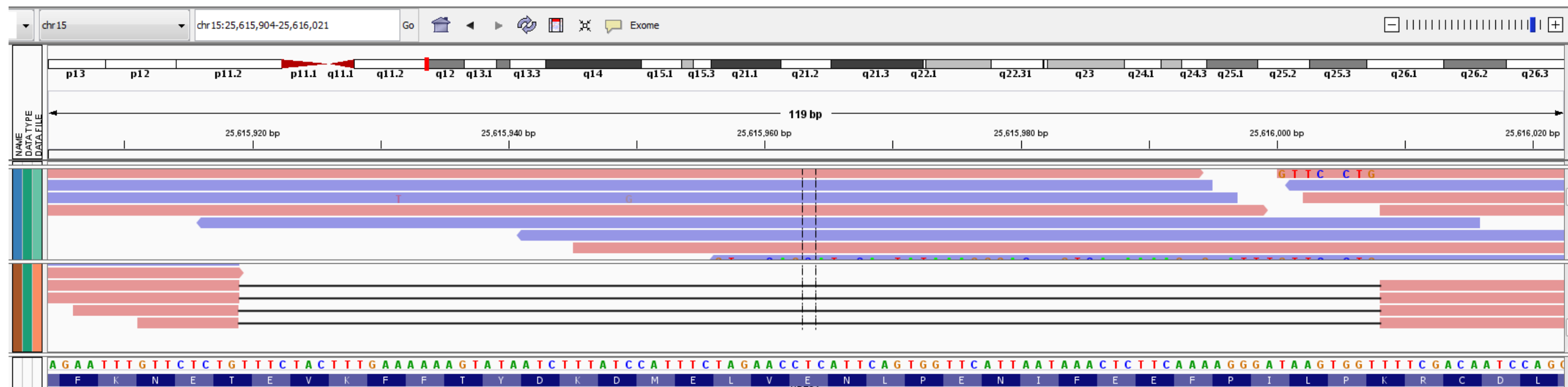
ADDITIONAL MOLECULAR AND CLINICAL INFORMATION

- The ubiquitin-protein ligase (*UBE3A*) gene encodes an E3 ubiquitin-protein ligase, part of the ubiquitin protein degradation system. Alterations in this gene are generally inherited in an autosomal dominant fashion associated with Angelman syndrome (AS). The *UBE3A* gene is also subject to genomic imprinting, with preferential maternal-specific expression in neurons located in the brain. Maternally inherited deletions are a rare cause of Angelman syndrome and account for approximately 10% of patients with AS. In the present case, it is presumed that the mother's deletion occurred on the paternally inherited allele and thus is silenced in the mother's neurons.
- As previously noted, the present proband had a normal EEG and no evidence of seizure activity, cardinal features present in more than 80% of patients with AS before the age of three years. The absence of cardinal features in this proband, the nonspecific presentation of AS and the cost of *UBE3A* Sanger sequencing all contributed to the delayed genetic diagnosis in the present case.
- For this family, the molecular diagnosis established by DES enabled accurate estimation of recurrence risk. Since the mother is a carrier, the risk of recurrence is 50%. Prenatal diagnosis and preimplantation genetic testing (PGT) are also available.

PROBAND



DES IDENTIFIED AN 89-bp DELETION



Nucleotides		Proband	Mother	Father
Reference	Reference	88.89%(32/36)	62.75%(64/102)	100.00%(6/6)
Mutation	c.1254_1342del89	11.11%(4/36)	37.25%(38/102)	0.00%(0/6)
Q Score		105	1765	0

The identified 89 base pair deletion, located in the middle of the large coding exon 3 (c.302_c.1548, 1,247 base pairs in size) represents the largest intraexonic deletion reported to date, and could have been missed by Sanger sequencing depending on the location of the primers.

SUMMARY

- DES may prove particularly useful in a proband that has eluded molecular diagnosis due to an atypical or milder presentation
- Molecular diagnosis provided by DES may result in a greater understanding of the phenotypic variability associated with previously described genetic syndromes

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