

Case Report

Identification of Novel *UBE3A* Mutation Causing Angelman SyndromeTaghizadeh MA¹, Anvari SA², Eshaghkhani YE³, Golchehreh ZA³, Taheri SR⁴ and Keramatipour MO^{3*}¹Department of Medicine, University of California San Diego, USA²Department of Pediatrics, Milad Hospital, Iran³Department of Medical Genetics, Tehran University of Medical Sciences, Iran⁴Watson Genetic Laboratory, Iran***Corresponding author:** Keramatipour MO, Department of Medical Genetics, Tehran University of Medical Sciences, Poursina Ave., Keshavarz Blvd, Tehran, Iran**Received:** August 16, 2021; **Accepted:** September 11, 2021; **Published:** September 18, 2021**Abstract**

The Angelman Syndrome (AS) is neurodevelopmental disease associated with maternal disruption of the *UBE3A* gene and is mainly characterized by global developmental delay, severe mental retardation with absence of speech, seizures, dysmorphic facial features, and distinct behavioral profile. In this study a pedigree with one affected member with neurodevelopmental disease who was a result of an unconanguineous marriage were investigated by Whole Exome Sequencing (WES). DNA was extracted from whole blood and library was prepared using Agilent V6 capturing system. WES was performed on Illumina HiSeq 4000 platform. Genome Analysis Toolkit (GATK) was used for variant calling. Classification of selected variants was done based on ACMG guideline for variant interpretation 2015. WES revealed that the proband has previously unreported nonsense variant (c.2459T>G) in *UBE3A* gene that causes the substitution of Leu (TTA) with stop codon (TGA), confirming the diagnosis of Angelman syndrome. The patient had delayed motor development, speech impairment, an attention deficit, and an abnormal electroencephalogram (EEG), but no seizures by the age of 2 years. This study emphasizes the role of WES in the early diagnosis and better management for AS patient.

Keywords: Angelman syndrome; Whole Exome Sequencing; *UBE3A* Mutation**Case Presentation**

Angelman Syndrome (AS; MIM 105830) is a neurobehavioral disorder mainly characterized by global developmental delay, severe mental retardation with absence of speech, seizures, dysmorphic facial features, flat occiput, microcephaly, wide-based gait, tremor of limbs, paroxysmal laughter, and characteristic EEG discharges [1,2].

The incidence of AS is estimated to be about 1 out of 10000 to 1 out of 40000 live births [3]. Most cases are caused by disruption of an imprinted, maternally expression of region on chromosome 15q11.2-q13, containing E6AP ubiquitin-protein ligase (*UBE3A*; MIM 601623) [1].

Kishino and Wagstaff (1998) found that the *UBE3A* gene spans approximately 120kb and consists of 16 exons (NM_130838.1), and encodes the 865-amino E6-AP ubiquitin protein ligase, which acts as a cellular ubiquitin ligase enzyme [4]. Transferring the ubiquitin to the protein targeted for degradation occurs in a catalytic region of E6AP termed the homologous to the E6-AP carboxyl terminus; HECT; domain [5]. *UBE3A* has two pseudogenes, *UBE3AP1* and *UBE3AP2*, mapped to chromosomes 2 and 21 [4].

This gene can be disrupted through four molecular mechanisms: 1) Combination of three chromosomal breakpoints (proximal BP1, BP2, and a distal BP3), spanning about 5-7 Mb in the 15q11.2-q13 region, are involved in 65%-70% of the cases [6,7]. 2) Paternal UPD of chromosome 15 accounts for 3%-7% of the cases that revealed milder manifestation with low incidence of seizure [8,9]. 3) Defect in the imprinting center within the 15q11.2-q13 region, either due to genetic (microdeletion) or because of epigenetic (abnormal DNA

methylation pattern), change the DNA methylation and expression imprints, which are observed in 2%-5% of patients [10]. 4) Lastly, A few patients have complete or partial deletions of *UBE3A* [11]. Genetic analysis of patient with AS shows that the majority of *UBE3A* variants lead to protein truncation. Patient with milder mutations including missense and in frame deletions may show some of the clinical manifestations related to AS [12]. The investigation on the genetic aspect of diseases provides the opportunity to investigate the relationships between complex phenotypes and genomic variations [13].

Here, we report a patient with a novel nonsense mutation within the *UBE3A* gene.

Materials and Methods**Subjects**

A 2-year-old symptomatic girl with history of icterus, delayed motor development, inability to walk and stand, poor coordination, hand tremor during walking, bulbous nose, loss of speech, absence seizure, attention deficit, history of increased level of ammonia, VitB12 deficiency and mild increase of methylmalonic acid was referred to Watson Genetic Laboratory, Tehran, Iran for genetic counseling/analysis. Her parents are not consanguine. There is history of seizure and delayed speech in her pedigree.

Written informed consent was taken from the parents of the patients and peripheral blood samples were collected from the patients and their family (Figure 1 and 2).

DNA extraction

Genomic DNA was extracted from whole peripheral blood using



Figure 1: The patient at the age of 2 years. She has hypertelorism, mild synophrys, and brachydactyly.

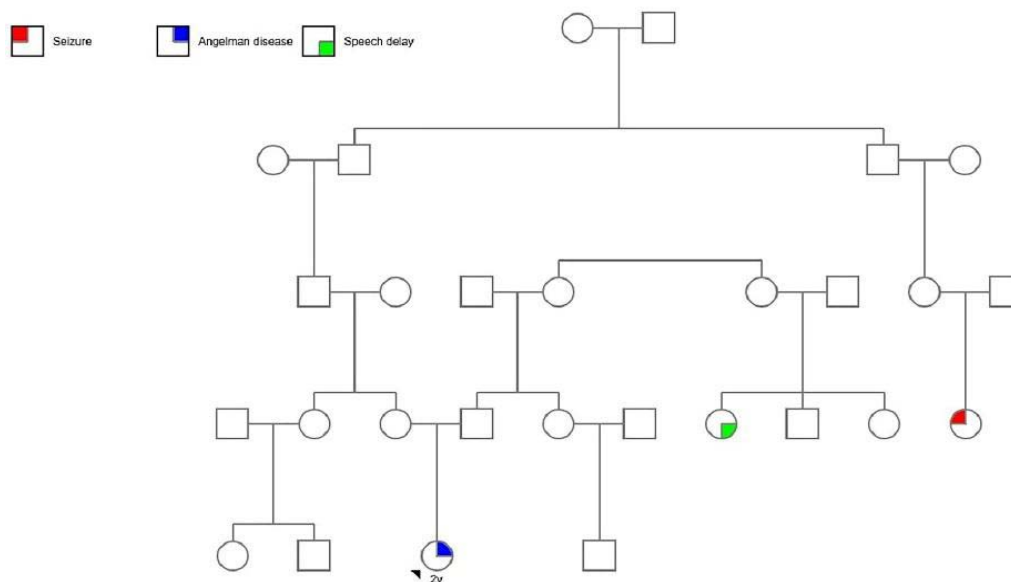


Figure 2: The investigated pedigree. The proband is indicated by an arrow.

the GeneAll Exgene™ kit (GeneAll Biotechnology Co., LTD, Seoul, Korea), according to the manufacturer’s instructions [14].

Whole exome sequencing

Human whole exome enrichment was performed using Twist Human Core Exome Kit and the library was sequenced on Illumina HiSeq 4000 platform with a raw coverage of 330X and mean on-target coverage of 105X, performed by CeGaT GmbH, Germany. Nearly all exons and flanking 10bp were detected and analyzed. Detected variations include single point mutations and small indels (within 20bp). Furthermore, CNV detection was performed for patient with breakpoint analysis. GATK was used for variant calling. Classification of selected variants (after filtering benign variants) was done based on ACMG guideline for variant interpretation 2015.

In addition, bioinformatic investigation was conducted by multiple in-silico predictive tools including Mutation Taster (<http://www.mutationtaster.org>) and CADD; Combined Annotation Dependent Depletion; (<http://cadd.gs.washington.edu/>) to determine

the pathogenicity of the novel variant which was not previously reported in any of the population/disease databases such as ExAC, 1000G, dbSNP, ClinVar, and HGMD.

Validation of the detected missense variant

In order to validate the detected nonsense variant in-patient and her parents, polymerase chain reaction followed by sanger sequencing was used. The sequences of designed primers are as follows; Forward primer: GAGCTGTTTCAGGATAAAATTTGCTTG and Reverse primer: GCTGGGAATCAAAAAAGTAAATGAATC. PCR reaction was performed in 20µl total volume containing 10µl Taq DNA Polymerase 2x Master Mix (Ampliqon A/S, Odense, Denmark), 7µl DH₂O, 1µl of each 5pM primers, and 1µl of 50ng/µl DNA. The PCR condition were as follows: Initial denaturation at 95°C for 5 min; 32 cycles of denaturation at 95°C for 30s, annealing step at 58°C for 45s, elongation at 72°C for 30s, and a final extension of 72°C for 5min. The PCR products were purified using Expin™ Combo GPM mini purification kit (GeneAll Biotechnology, Seoul, South Korea) and sequenced by ABI 3500 automated sequencer (Pishgam Biotech

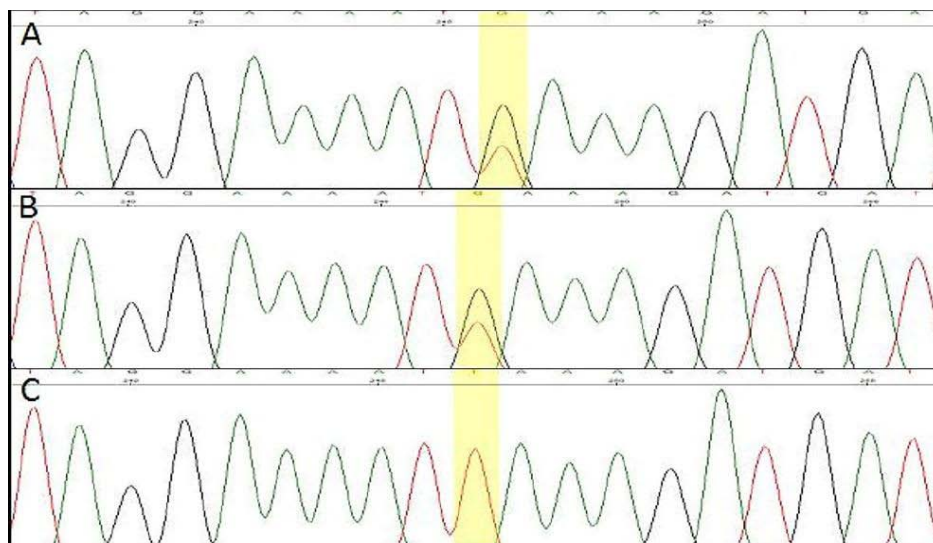


Figure 3: Results of DNA sequencing show heterozygosity in-patient and mother for variant c.2459T>G in UBE3A gene. (A, B), and normal homozygosity at the analyzed position for father (C).

Company, Tehran, Iran).

Results

Whole exome sequencing revealed heterozygous nonsense variant in the proband. The variant was a T-to-G transition at the second base of codon 820 in exon 12 of the *UBE3A* (ENST00000232165.3; NM_130839) that causes the substitution of Leu (TTA) with stop codon (TGA) (Figure 3A).

According to our survey, this nonsense variant (c.2459T>G) in *UBE3A* gene, has not been previously reported for its pathogenicity. However, null variants (including nonsense variants) in *UBE3A* gene is a known mechanism of disease [15]. UniProt database has reported this variant in mutational hot spot and/or critical and well-established functional domain of related protein. The bioinformatic investigation was performed using online tools including Mutation Taster and CADD to predict the possible effect of the variant on the function of the protein. The variant was predicted with high confidence to be “disease causing” by Mutation Taster and with CADD PHRED Score of 47. In addition, this variant has a frequency of zero in the largest available local database of genomic variations in Iranian population (Pishgam Biotech Company, Tehran, Iran). Based on ACMG guideline, this variant can be classified as a pathogenic variant.

Follow-up studies identified the nonsense variant in the patient’s unaffected mother, but not in her unaffected father (Figure 3B and 3C).

Discussion

Approximately 80% of patients suffering from AS have a defect due to a large deletion, uniparental disomy; UPD; or imprinting effect, and 10% of those have linked to a maternal pathogenic *UBE3A* gene mutation, that approximately 10% of patients are not included with any detectable mutations. Although there is no effective treatment for patient suffering from this disease, determination of an exact molecular cause of genetic defect is significantly important for recurrence risk assessment [16].

Mutations in *UBE3A* described to date are variable, nonsense and frameshifts mutations are most reported changes that resulting in premature stop codons and truncated proteins [17,18].

We report a patient with novel nonsense mutation in one copy of the *UBE3A* gene who had inherited it from her unaffected mother. This mutation results in a premature termination codon; that is predicted to produce a truncated protein product.

The greater part of mutations describe within *UBE3A* gene are located at the region of hect domain, that encompass the 3’ region of the exon 9, expand across the exon-16 and when occur on the allele with maternal inheritance, that is majority expressed in the neuronal tissues, can result in pathogenesis. The mutation found in the current study is placed at the of exon 12 causing a stop codon mutation changing the hect domain [19]. The main role of this protein is estimated to be the monoubiquitination of substrates that are predetermine for ubiquitin proteasome recycling or are tagged protein for trafficking pathway of different cellular compartments. In addition, E6-AP identified as co-activator of steroid hormone receptor [20].

Horsthemke (2008) reported that there are correlations between genotype-phenotype in Angelman syndrome. Patients with frameshifts and/or premature truncations are more severely affected, whereas patients with missense mutations or short inframe deletions are usually mildly affected [19]. In addition, De Molfetta et al. (2012) hypothesized that the stop codon mutation causes both severe and milder phenotypes [21]. The index of our patient had milder phenotypic manifestations of classical phenotype of AS. She did exhibit delayed motor development, inability to walk and stand, poor coordination, hand tremor during walking, bulbous nose, loss of speech, attention deficit, did not have a history of seizures, sleep disturbance by the age of 2 years.

Conclusion

In conclusion, a patient with a mild AS phenotype and a maternally

inherited intragenic nonsense *UBE3A* mutation is described. The recurrence risk of 50% was given to the proband's mother.

Pathogenic variants in any of the coding exons (7-16) are thought to disrupt these functions, and when present on the maternally inherited allele, which is exclusively expressed in the neuronal tissues, can lead to pathogenesis.

The case is aimed to sensitize clinicians about Angelman syndrome and to highlight the role of WES in the diagnosis, medical care and management for AS.

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