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A novel *AUTS2* Variant in a Patient with Global Developmental Delay and Intellectual Disability

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Abstract

AUTS2 haploinsufficiency causes a neurodevelopmental disorder known as AUTS2, which is characterized by global developmental delay, intellectual disability, autistic features, congenital brain anomalies, and other malformations. In this study, we report a case of AUST2 syndrome and describe the clinical manifestations and genetic etiology as well as provide a review of the literature. A 5-year-old girl presented with neurodevelopmental manifestations, skeletal features and dysmorphic features. Whole exome sequencing was carried out for the proband. A novel, heterozygous variant (c.1606C>T) in *AUTS2* gene was identified. Sanger sequencing confirmed the presence of this variant in the affected girl; however, it was not detected in all family members. The identified variant is predicted to cause premature termination of the corresponding AUTS2 protein (p.Gln536*), which will likely lack the C-terminal domain of the protein. This study revealed a novel *de novo* loss-of-function variant in the *AUTS2* gene and further expanded the phenotypic and genetic spectra of the AUTS2 syndrome. Moreover, this result might be helpful in genetic counseling for families with clinical phenotypes related to this syndrome. Further functional experiments are required to validate the impact of the identified variant.

Keywords: AUTS2, variant, Neurodevelopmental disorders, intellectual disability

1. Introduction

Neurodevelopmental disorders (NDD) are a heterogeneous group of disorders that affect the development and functions of the brain (Parenti *et al.*, 2020). NDD features, but is not limited to, developmental delay (DD), autism spectrum disorder (ASD), intellectual disability (ID), epilepsy, and other features (Pang *et al.*, 2021). Among these, NDD is the AUTS2 syndrome.

AUTS2 syndrome (OMIM #615834) is a combination of intellectual disability and developmental delay (reported in 80~100% of patients) in addition to autism (reported in 40% of patients) (Sultana *et al.*, 2002; Beunders *et al.*, 2013; Jolley *et al.*, 2013; Amarillo *et al.*, 2014; Liu *et al.*, 2015; Pang *et al.*, 2021). Nevertheless, other manifestations have also been reported such as low birth weight, short stature, craniofacial features microcephaly, epilepsy, and feeding difficulties, in addition to other variable neurological, brain, and skeletal abnormalities (Kalscheuer *et al.*, 2007; Beunders *et al.*, 2013, 2016; Jolley *et al.*, 2013).

AUTS2 syndrome is an autosomal dominant disorder resulting from disruption in *AUTS2* gene (OMIM 607270). By 2023, more than 60 AUTS2 patients have been reported, most of them carrying exonic deletions (Beunders *et al.*, 2013, 2015, 2016; Jolley *et al.*, 2013; Liu *et al.*, 2015; Fan *et al.*, 2016; Martinez-Granero *et al.*, 2021; Sanchez-Jimeno *et al.*, 2021), five patients with exonic or intragenic duplications (Ben-David *et al.*, 2011; Nagamani *et al.*, 2013; Martinez-Granero *et al.*, 2021), and three patients with balanced translocation that disrupt the *AUTS2* gene (Kalscheuer *et al.*, 2007), while a small number of sequencing variants such as missense, nonsense, and indels have been less frequently reported in the literature (Beunders *et al.*, 2015, 2016; Aldinger *et al.*, 2019; Saeki *et al.*, 2019; Stojanovic *et al.*, 2020; Zech *et al.*, 2020; Ziats *et al.*, 2020; Gieldon *et al.*, 2021; Martinez-Delgado *et al.*, 2021; Palumbo *et al.*, 2021; Sanchez-Jimeno *et al.*, 2021; Anikiej-Wiczenbach *et al.*, 2022; Fair *et al.*, 2023).

Activator of transcription and developmental regulator gene (AUTS2) previously named autism susceptibility candidate 2 was first reported as a candidate for autism in a monozygotic twin with ASD, epilepsy, and developmental delay because it was disrupted by a breakpoint of the t(7;20) (q11.2; p11.2) translocation in these patients (Sultana *et al.*, 2002).

The *AUTS2* gene is mapped to the long arm of chromosome 7 (7q11.22), spanning approximately 1.2 Mb of genomic DNA and comprising 19 coding exons that code for a 1,259 amino acid protein. These 19 exons are divided into 2 parts: Exons (1-6) at the 5' end have large introns, whereas exons (7-19) at the 3' end are separated by short introns. AUTS2 encodes the full-length (long) (1259 aa) isoform and two C-terminal (short) isoforms (produced by alternative transcription start sites in exons 8, and 9) that are differentially expressed during development

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(Beunders *et al.*, 2013; Hori *et al.*, 2014). In humans, *AUTS2* mRNA is expressed in different tissues and cells with the highest expression reported in the brain, kidney, and skeletal muscle (Biel *et al.*, 2022; Lepagnol-Bestel *et al.*, 2022).

The molecular function of AUTS2 is not fully understood; however, its neurodevelopmental functions have been well-studied in various model systems. Loss-of-function experiments in *zebrafish* and mouse models have displayed neurological developmental phenotypes and highlighted a crucial role for AUTS2 in RNA metabolism, activation of transcription, central nervous system cytoskeleton regulation, and neuronal differentiation and migration (Oksenberg *et al.*, 2013; Yamashiro *et al.*, 2020; Hori *et al.*, 2020; Monderer-Rothkoff *et al.*, 2021; Biel *et al.*, 2022).

In this study, we evaluated a patient with AUST2 syndrome who carries a *de novo* heterozygous nonsense variant in *AUTS2*, which was novel and classified as likely pathogenic according to the guidelines of the American College of Medical Genetics (ACMG) (Richards *et al.*, 2015). We also presented detailed clinical and genetic descriptions of AUTS2. To the best of our knowledge, this is the first AUTS2 case in our region with an *AUTS2* variant.

2. Materials and Methods

2.1. Samples and DNA extraction

This study was approved by the institutional review board /ethical committee of National Center for Diabetes, Endocrinology and Genetics (Protocol number IRB-1/2022). A signed informed consent was obtained from the family. The family pedigree is illustrated in Figure 1A. Genomic DNA (gDNA) was extracted from venous blood samples collected from the proband (II.2), parents (I.1 and I.2), and her healthy sisters (II.1 and II.3) following manufacturer's instructions (BioRobot EZ1; Qiagen, Solna, Sweden). The purity and concentration of DNA were evaluated using a spectrophotometer (Nanodrop 2000 C; Thermo Fisher Scientific, Waltham, MA, USA) and 1% agarose gel electrophoreses.

2.2. Whole exome sequencing (WES) and variant detection

Agilent's SureSelect Human All Exon V6 kit was used for exome capture following the manufacturer's protocol. The generated library was sequenced on an Illumina platform. Around 25,000 genes were sequenced, and ~97.75% of these genes were covered at least >10x. GRCh37/hg19 genome assembly was used for reads alignment. All pathogenic variants reported in ClinVar, in HGMD, and all variants with minor allele frequency (MAF) <1% in the gnomAD database were considered. We focused on nonsense and nonsynonymous, splice site variants (+/-10 intronic bases) as well as insertions and deletions (indels). Several *in-silico* prediction tools such as SIFT (https://sift.bii.a-star.edu.sg/), Polyphen2 (http://genetics.bwh.harvard.edu/pph2/), Mutation Taster (http://www.mutationtaster.org/),CADD (https://cadd.gs.washington.edu/), DANN (https://cbcl.ics.uci.edu/public_data/DANN/), PhyloP (http://compgen.cshl.edu/phast/),FATHMM (http://fathmm.biocompute.org.uk/index.html), and others were also used to predict the functional impact of identified variants. The classification of variants was based on ACMG guidelines. Partial AUTS2 amino acids sequences alignment was obtained from http://www.ncbi.nlm.nih.gov/protein/ website.

2.3. Confirmation of the identified variant

Sanger sequencing was carried out to validate the variant in *AUST2* (c.1606C>T). Genomic DNA samples were amplified using specific primers for Exon 9 (Forword-5'ggcagtccgatgtccttttc'3 and Rreverse-5'tccccattcgatctctggtg'3. The PCR condition was as follows: initial denaturation for 3 min at 95 ℃, followed by 30 cycles of denaturation for 15 sec at 95 ℃, annealing for 60 sec at 55 ℃, extension for 60 sec at 72 ℃ and final extension for 5 min at 72℃. PCR products were then purified and bi-directionally sequenced on a genetic analyzer (3500x; Applied Biosystems, Thermo Fisher Scientific) using BigDye Terminator Cycle Sequencing Kit v3.1 following the manufacturer protocol.

3. Results

3.1. Clinical Description

The 5-year-old female patient (II.1) is the second child of a non-consanguineous Syrian family (Figure 1). Family history was unremarkable for ID/NDD or congenital anomalies.

The affected girl was born at full term by vaginal delivery without complications. Her birth weight was (2700 g; 5th-10th percentile), height (50 cm; 25th-50th percentile), and occipitofrontal head circumference (35 cm; 50th-75th percentile). In the first year, she complained of feeding problems and poor weight gain with mild developmental delay. At the age of 2 years, she started walking with a tendency to walk on her toes. Speech, social and motor development remained delayed. At the age of 3 years, she showed generalized hypotonia with high muscle tone, frequent seizures (3-5 times per day lasting for 1 min), and stereotypic movements. Brain MRI and electroencephalogram were normal.

Her latest examination was done at the age of 5 years. Her weight was (13,700 g; between the 10th and 15th percentiles), height (102 cm; between the 25th and 50th percentiles), and occipitofrontal head circumference (46 cm; between 0.1 and 1st percentile). She displayed minor facial anomalies including an open mouth, anteverted nares, highly arched eyebrows, upward slanting palpebral fissures, ptosis, hypertelorism, strabismus, and squint (Figure 1B). She had stereotypic actions with hyperactive behavior patterns, sensitivity to sounds, and sleeping difficulty. She had frequent salivation, ataxia, and involuntary movement as well as tip-toe walking. Her social and motor development remain delayed, IQ was not formally tested, but her intellectual disability can be described as severe. Scoliosis was also prominent.

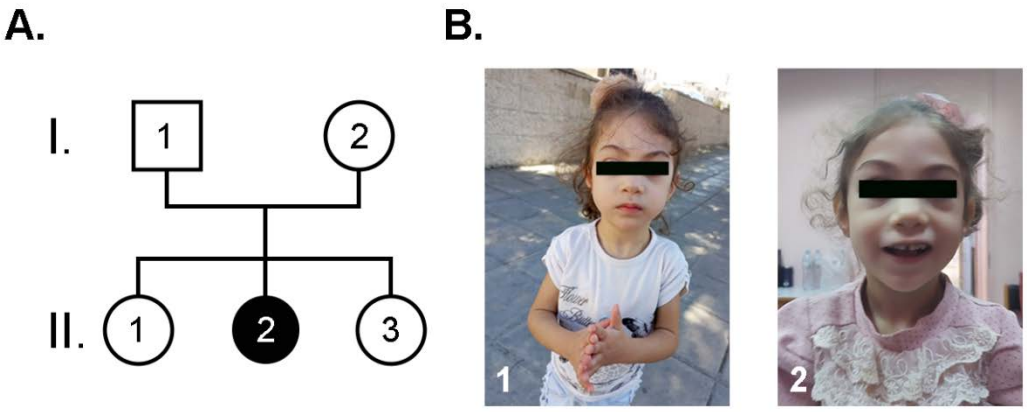

Figure 1. Family pedigree and patient characteristics. A. Pedigree of the family: Circle and square denote female and male, respectively. The filled circle represents the proband and unfilled symbols represent unaffected individuals, respectively. B. Clinical features of the affected patient (II.2) (Photos at the age of 3 (picture 1) and 5 years (picture 2)) showing dysmorphic features such as high-arched eyebrows, broad nasal bridge, and microcephaly.

3.2. Genetic findings

In this patient, around 25,000 genes have been sequenced, and ~97.75% of these genes are covered at least >10x. Out of 164,177 variants, 26,760 variants were detected across protein-coding exons (23,232 variants), and splice sites (3,528 variants). After filtration, we narrowed down the list of variants to 4 heterozygous variants. According to the clinical pictures and the pedigree, which indicates a dominant mode of inheritance, the nonsense variant in *AUTS2* gene c.1606C>T (p.Gln536*) was on the top of these variants and fits with diseases phenotype.

Segregation analysis revealed the absence of this variant in both parents and healthy siblings, implying a *de novo AUTS2* variant in the proband (Figure 2A). This variant lies in a conserved C-terminal domain of AUTS2 protein (Figure 2B). The identified variant is predicted to cause premature termination of the corresponding protein (p.Gln536*). This truncated protein will likely lack the C-terminal domain resulting in loss-of-function. The AUTS2 (p.Gln536*) variant was predicted as 'Disease Causing' as well as deleterious by various prediction tools (Table 1). The identified variant was absent from Genome Aggregation Database (gnomAD (https://gnomad.broadinstitute.org/),ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/, and HGMD (https://www.hgmd.cf.ac.uk/ac/index.php) databases. The identified variant was classified as likely pathogenic according to ACMG guidelines.

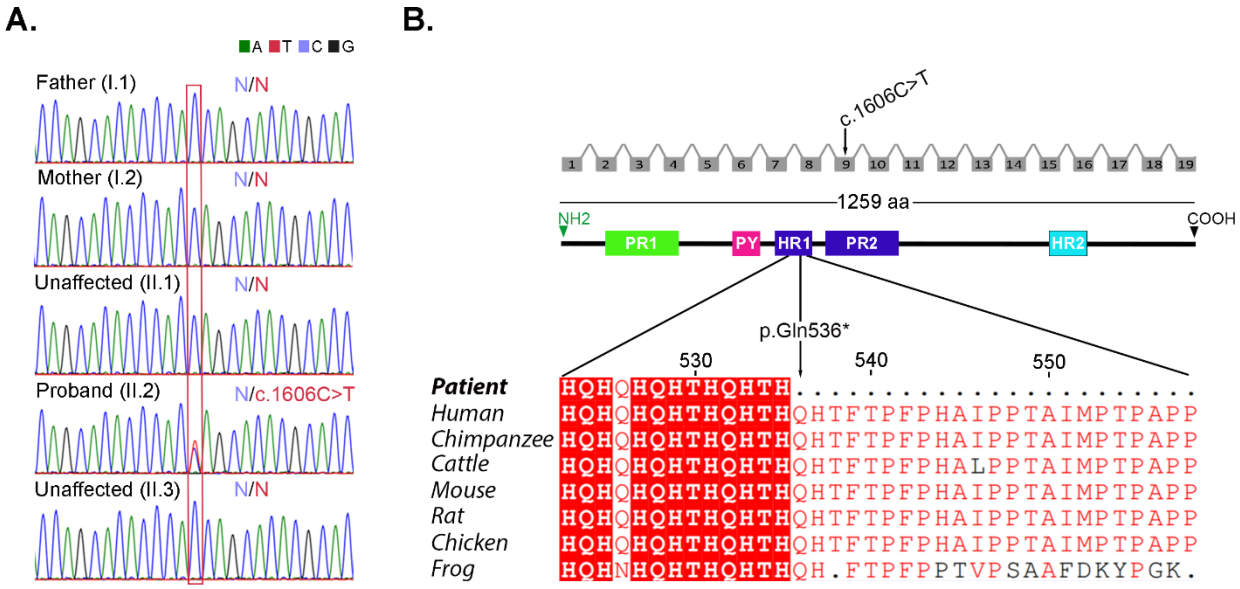

Figure 2. Segregation analysis and partial AUTS2 alignment. A. Sanger sequencing validated the *AUTS2* (c.1606C>T) variant in the affected girl and healthy parents and sisters (N: wild-type allele). B. Schematic illustrating the position of the variant in exon 9 and in the HR1 domain (PR: proline-rich domain; PY: PY domain; HR: histidine-rich domains). C. Partial amnio acid sequence alignment of AUTS2 illustrates the C-terminus region that is missing from the p.Gln536 amino acid residue in the patient, the position of the truncated variant is located in the HR1 domain (Arrow). The sequence of amino acids was obtained from http://www.ncbi.nlm.nih.gov/protein/ website: *Homo sapiens* (Human; NP_056385.1), *Pan troglodytes* (Chimpanzee; XP_009441212.2), *Bos taurus* (Cattle; XP_024841016.1), Mus musculus (Mouse; NP_001350409.1), Rattus norvegicus (Rat; UniProt# F1M388), *Gallus gallus* (Chicken; XP_015151429.1), and *Xenopus tropicalis* (Frog; XP_031752079.1). Alignment was carried out using http://multalin.toulouse.inra.fr/multalin/.

Table 1. Characteristics of the identified *AUTS2* variant.

Chromosome	chr7	
Start	70231237	
End	70231237	
Gene	*AUTS2* (NM_015570)	
Coding	Exon 9	
Variant	7:70231237 C>T c.1606C>T p.Q536*	
Read depth	140	
In-silico tool	Score	Interpretation
LRT	0.412	Deleterious
Mutation Taster	1	Disease causing
CADD	42	Deleterious
DANN	0.998	Deleterious
FATHMM	0.936	Deleterious
PredictSNP2	0.658	Deleterious
FunSeq2	4	Deleterious
BayesDel	0.625	Deleterious
GERP++	5.77	Highly conserved residue
phyloP	7.495	Highly conserved residue

4. Discussion

Pathogenic variants disrupting the *AUTS2* gene have been identified in more than 60 cases with AUTS2 syndrome, an autosomal dominant disorder characterized by developmental delay (DD), intellectual (ID) and mental dysfunction, and various neurodevelopmental manifestations (Sanchez-Jimeno *et al.*, 2021).

In the current study, we described a Syrian family with one affected 5-year-old female who harbors a heterozygous *de novo* nonsense variant (c.1606C>T; p.Gln536*), as it was absent in her parents and the two healthy siblings. According to ACMG guidelines, this variant is classified as a likely pathogenic (Richards *et al.*, 2015) and is predicted to cause a premature termination at (p.Gln536) of the AUTS2 protein. The resultant protein will likely lack the C-terminal domain, suggesting a loss-of-function effect of this variant. Moreover, the altered mRNA transcript could be subjected to nonsense-mediated mRNA decay (NMD) (Maquat, 2004).

The p.Gln536 is located within the histidine-rich region (HX) that contains alternating Histidine-Glutamine (HQ) and Histidine-Threonine (HT) residues (aa 525-542), a highly conserved region in the C-terminal domain, which has a crucial role in neuronal differentiation (Liu *et al.*, 2021).

Most AUTS2 patients carry *de novo* intragenic deletions, whereas missense, nonsense variants, and indels have been reported in a small number of cases (Sanchez-Jimeno *et al.*, 2021; Fair *et al.*, 2023). In the ClinVar database, around 129 AUTS2 variants have been classified as likely pathogenic or pathogenic (accessed on 10 March 2023), of which only 19 are single nucleotide nonsense variants lying upstream or downstream of the identified variant; however, no clinical descriptions were provided. Only two nonsense pathogenic variants have been reported in the literature so far, one of these variants (c.976C>T; p.Gln326*) was reported twice in patients with DD, ID and ASD (Fitzgerald *et al.*, 2015; Kosmicki *et al.*, 2017). The second variant (c.317C>T; p.Gln107*), however, shares some clinical features of our patient (Beunders *et al.*, 2016). The patient in the current study displays typical clinical manifestations of AUTS2 including intellectual developmental disability, microcephaly, substantial motor and language delay, hyperactive behavior, and mild dysmorphic facial features similar to the previously reported case. Additional features such as recurrent seizures, strabismus, scoliosis, frequent salivation, and tight heel cords were only observed in our patient.

In 2013, *Beunders* et al. suggested the AUTS2 syndrome severity score (ASSS) that measures the phenotype's severity and specificity and is categorized into four grades: 0-7, 8-12, 13-18, and 19-31 (Beunders *et al.*, 2013). The ASSS score focuses on around 32 clinical features reported in more than 10% of AUTS2 cases that affect growth parameters, feeding problems, dysmorphic features, skeletal disorders, neurodevelopmental features, as well as congenital anomalies (Hori *et al.*, 2022). The genotype-phenotype correlation (as measured by ASSS means) and the variant site in the *AUTS2* gene have been well established. The ASSS in our case was 14, which is considered high. This value is mostly associated with neurodevelopmental and growth defects. In comparison to previous cases, the median ASSS was 8.5 and 15 for mutations lie in the 5' end (exons 1-8) and 3' end (exons 9-19), respectively.

Our patient carries a truncated mutation in the C-terminal region of AUTS2 protein, which causes severe phenotypes, such as feeding difficulty. Other features were also observed such as squint, ataxia, and frequent salivation, in addition to other manifestations that are rarely seen in AUTS2 patients such as seizures, eczema, and sleeping difficulties. Previous studies have shown that the 3′ end of AUTS2 comprises significant functional domains and cases harboring pathogenic variants affecting the C-terminal region of the AUTS2, particularly the HX repeat are significantly associated with more severe manifestations such as microcephaly, feeding difficulty, intellectual disability, and mental retardation (Beunders *et al.*, 2013; Saeki *et al.*, 2019; Martinez-Delgado *et al.*, 2021; Brunet *et al.*, 2021; Fair *et al.*, 2023).

5. Conclusions

In conclusion, this study reports a novel *de novo* loss-of-function variant in a patient with typical features of AUTS2 syndrome. Since this is the third nonsense variant that will be reported in the literature, our findings will expand the mutation spectrum in *AUTS2* gene and its clinical manifestations. However, additional functional experiments are needed to confirm the impact of the identified variant. These results will be helpful in genetic counseling as well as future prenatal testing and preimplantation genetic diagnosis for families with clinical phenotypes related to AUTS2.

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Conflicts of Interest

None.

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