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CASE REPORT

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Association of Autism Spectrum Disorder in an Iranian Pedigree with a Novel Hereditary Mutation in *SETD5*

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Abstract

Autism spectrum disorder has evolved from a rare childhood-onset disorder to a widely acknowledged, extensively researched, and heterogeneous lifelong condition. This study focuses on an Iranian pedigree affected by autism spectrum disorder. By employing whole-exome sequencing, we detected a novel heterozygous (c.3694T>A: p.Tyr1232Asn) in exon 22 (NM_001080517.3) of the *SETD5* gene. The presence of this mutation was consistent with observed clinical features, confirming the genetic basis of autism spectrum disorder in the patient and his father. In contrast, the mother, with a normal genotype, did not exhibit the identified mutation. Genetic counseling implications are underscored by the shared heterozygous mutation in both, emphasizing the importance of incorporating genetic insights into psychological counseling. This integration can empower families with informed strategies to navigate the challenges associated with autism spectrum disorder, fostering resilience and tailored support. **(International Journal of Biomedicine. 2024;14(1):170-174.)**

Keywords: Autism Spectrum Disorder • *SETD5* gene • mutation

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Introduction

Over the past fifty years, autism spectrum disorder (ASD) has experienced a profound transformation. Initially perceived as a rare childhood-onset disorder, it has evolved into a widely acknowledged and extensively studied lifelong condition. This shift reflects not only increased awareness but also a growing understanding of the remarkable diversity within the spectrum. ASD, now recognized as fairly common, is marked by enduring core features, including social communication deficits and repetitive, unconventional sensory-motor behaviors.[1] Autism is presently conceptualized as a spectrum, encompassing a spectrum of severity from mild to severe. Despite this diversity, it is important to recognize that a considerable number (though not all) of individuals with ASD necessitate some form of lifelong support.

Individuals with ASD exhibit a diverse range of characteristics, yet the disorder is primarily defined by core features in two key domains: social communication and restricted, repetitive sensory-motor behaviors. These fundamental traits persist consistently across various backgrounds, regardless of cultural, racial, ethnic, or socioeconomic factors.[2] The origins of ASD are rooted in early disruptions in brain development, leading to subsequent neural reorganization.[3,4] However, the absence of reliable biomarkers necessitates reliance on observable behavior for diagnosis. In an effort to improve diagnostic precision, the American Psychiatric Association introduced the Diagnostic and Statistical Manual of Mental Disorders (DSM)-5 criteria in 2013.[5] This revision aimed to streamline ASD diagnosis by creating a unified spectrum based on the two core

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domains: social communication and restricted, repetitive, or unusual sensory-motor behaviors. Notably, subtypes like Asperger's disorder and pervasive developmental disorder not otherwise specified, previously inconsistently utilized by clinicians, were amalgamated into the consolidated diagnosis of ASD. Additionally, the DSM-5 explicitly recognizes that ASD can co-occur with other conditions, including genetic disorders like fragile X syndrome and psychiatric conditions such as attention-deficit hyperactivity disorder (ADHD). This acknowledgment demonstrates a more comprehensive understanding of the intricacies and comorbidities associated with ASD.

SETD5, a widely expressed protein, belongs to the SET domain-containing protein family. While SET domains in most proteins catalyze protein lysine methylation, SETD5 and its paralog MLL5 diverge due to amino acid substitutions at critical positions, rendering them devoid of methyltransferase activity. Recent studies have unveiled that SETD5 interacts with two chromatin-regulating complexes—the polymerase-associated factor 1 (PAF1) and histone deacetylase 3 (HDAC3) complexes. This association underscores SETD5's role in epigenetic regulation and control of gene expression. Crucially, heterozygous loss-of-function mutations in genes encoding components of the HDAC3 complex have been identified in individuals with ASD or intellectual disability (ID). This observation suggests a functional link between *SETD5* and the HDAC3 complex in the pathogenesis of ASD and ID. Despite this insight, the precise mechanisms by which SETD5 regulates gene expression related to ASD and ID have remained elusive. Further exploration of SETD5's interactions and its impact on gene expression holds promise for advancing our understanding of the molecular underpinnings of ASD and ID.[6]

Whole-exome sequencing (WES), emerges as a highly valuable methodology, providing a comprehensive and efficient approach to identify causative mutations in individuals grappling with genetic disorders. Distinguished by its advanced capabilities, this technique facilitates the focused sequencing of the coding regions of genes, thereby ensuring a swift, precise, and economically feasible identification of mutations that underlie single-gene disorders.[7-9] Based on this evidence, we employed WES technique to discern the causative genetic defect in a nonconsanguineous Iranian family affected by ASD. Our primary diagnosis of ASD was initially established through comprehensive psychological assessments. Through these efforts, we identified a novel heterozygous mutation within the *SETD5* gene, shedding light on a potential genetic basis for the ASD phenotype observed within this family.

Case Presentation

A 9.5-year-old boy patient was referred to Noor-Gene Genetic Laboratory in Ahvaz, Iran, based on developmental concerns noted by the parents (Figure 1). Born full-term without complications, the individual experienced delayed developmental milestones, particularly in language and social interaction. Parents reported challenges in communication, repetitive behaviors, and forming peer relationships. A comprehensive psychological evaluation, inclusive of standardized assessments, resulted in a provisional diagnosis of ASD.

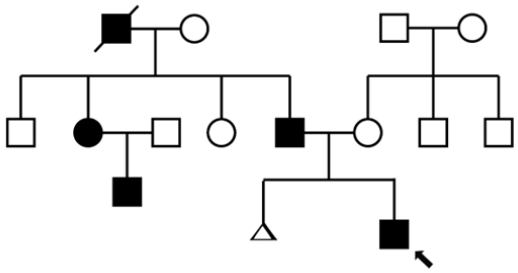

Fig. 1. *The pedigree of the studied family. Squares denote males, circles represent females, triangles signify spontaneous abortions, and slashes indicate deceased individuals, while enclosed symbols mark affected members, with the proband identified by an arrow.*

In a parallel context, the patient's father underwent psychological assessment due to a history of developmental challenges during childhood, marked by language delays and difficulties in forming social connections. Despite achieving academic milestones, persistent challenges in social interactions and communication were observed throughout adulthood. Conversely, the patient's mother has no reported history of neurodevelopmental challenges, achieving typical developmental milestones during childhood and exhibiting normal social communication and cognitive abilities. Given the notable resemblance in clinical characteristics between the patient and his father, we suspected a potential genetic basis for the observed neurodevelopmental phenotype. To validate this hypothesis and complement the psychological diagnosis, we employed WES, with confirmation from his father's statement that the individuals represented by the blackened symbol in the pedigree shown in Figure 1 have been diagnosed with ASD. This also indicates that they are affected by a hereditary condition such as ASD, emphasizing the genetic basis of the disease.

Blood samples were obtained with informed consent from his parents. Following established protocols, DNA extraction from the buffy coat was conducted using the FAVORGEN kit (Biotech Corp, Cat. No.: FABGK 001, Taiwan).

A comprehensive WES analysis, with a specific focus on genes associated with ASD, was performed by Macrogen in Seoul, South Korea. The intentional emphasis on ASD-related genes aimed to identify potential genetic variations and mutations contributing to the manifestation of this neurodevelopmental disorder. The analysis revealed a novel single heterozygous mutation in the affected son, specifically identified as a novel missense mutation in the *SETD5* gene (c.3694T>A: p.Tyr1232Asn) located in exon 22 (NM_001080517.3) of chromosome 3p. Sanger sequencing of coding exons confirmed the presence of this new Y1232N mutation, predicting a consequential alteration in codon

translation, leading to the conversion of tyrosine to asparagine. This transformation was identified as heterozygous in both the patient and his father, confirming the disease as they exhibited similar clinical manifestations along with the heterozygous mutation. The mother, on the other hand, exhibited a normal genotype (Figure 2). This missense mutation substitutes Tyrosine with Asparagine (TAC>AAC) at the 1232-position of the SETD5 protein (Figure 2D). Bioinformatic tools, such as PolyPhen-2, SIFT, FATHMM-MKL, LIST-S2, and MutationTaster (Table 1), collectively indicate the identified mutation as a probable pathogenic variant. Furthermore, mutations reported in the *SETD5* gene are compiled and summarized in Table 2 using data from the Human Gene Mutation Database (HGMD, http://www.hgmd.cf.ac.uk/ac/index.php).

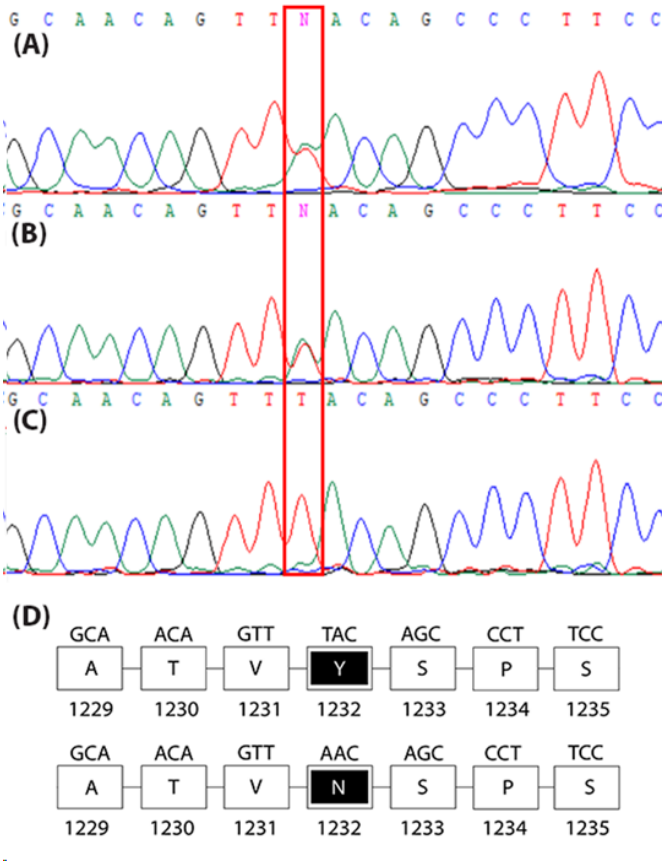

Fig. 2. *Sequence chromatograms portray a novel heterozygous SETD5 mutation (c.3694T>A) in the affected son (A) and his father (B), while the mother (C) exhibits a normal genotype in the sequence analysis. (D) the consequential amino acid modification arising from the substitution of T with A at position 3694 within the SETD5 gene. This genetic alteration results in the replacement of Y by N at position 1232 in the corresponding protein.*

Table 1.

Pathogenicity assessment of the new variant identified by WES.

Gene	Variant	Polyphen-2 HDIV score	SIFT score	FATHMM-MKL score	LIST-S2 score	Mutation Taster
SETD5	Y1232N	0.998 (Probably damaging)	0 (Pathogenic supporting)	0.9902 (Pathogenic supporting)	0.9902 (Pathogenic supporting)	Disease -causing

Table 2.

Reported mutations in SETD5 gene.

Pathogenic variant	Protein effect	Type of mutation	Phenotype
c.179C>T	p.Thr60Met	Missense	Autism spectrum disorder
c.509A>G	p.Lys170Arg	Missense	Autism spectrum disorder
c.922C>T	p.Arg308Ter	Nonsense	Autism spectrum disorder
c.1405G>A	p.Val469Ile	Missense	Autism spectrum disorder
c.2005G>A	p.Gly669Arg	Missense	Autism spectrum disorder
c.3773G>C	p.Ser1258Thr	Missense	Autism spectrum disorder
c.4029T>G	p.Ser1343Arg	Missense	Autism spectrum disorder
IVS14 as -2 A>T	-	Splicing	Autism spectrum disorder

Discussion

ASD is divided into syndromic and nonsyndromic types. Syndromic cases often present additional phenotypes, including ID, ADHD, epilepsy, and craniofacial dysmorphology. Nonsyndromic ASD is believed to have a predominantly polygenic etiology, while syndromic cases are often associated with chromosomal abnormalities, copy number variations, or single-gene mutations. Investigating molecular mechanisms in syndromic ASD, using targeted gene modification in cellular and animal models, sheds light on the disorder's pathophysiology. ASD-related mutations commonly disrupt cellular processes like neurogenesis, neurite growth, and synaptic plasticity. Evidence suggests that ASD-related gene mutations recurrently affect three major cellular activities: protein translation, WNT signaling, and synaptic signaling.[10-12]

At the forefront of this study, the identified *SETD5* gene mutation adds to the growing body of evidence linking this gene to ID and developmental disorders, particularly ASD. The familial context emphasizes the role of genetics in the manifestation of neurodevelopmental disorders. Beyond its genetic implications, this study underscores the potential benefits of genetic findings in psychosocial counseling for affected families.

Numerous studies have investigated the varied manifestations of ASD, emphasizing the heterogeneity of symptoms and the importance of recognizing early indicators. According to Jones et al.,[13] delayed language development is a prevalent characteristic in children later diagnosed with ASD. Social communication difficulties, such as challenges in understanding and responding to social cues, have also been extensively documented.[14] Repetitive behaviors are considered a core feature of ASD, as highlighted in studies by Leekam et al.[15] Challenges in communication, particularly nonverbal communication, have been linked to social interaction deficits in ASD.[16] The impact on forming peer relationships is a consistent theme in the literature, with studies suggesting that these difficulties often persist into adolescence

and adulthood.[17] In our case, the individual exhibited delayed language and social milestones, consistent with findings by Jones et al.[13] The challenges in communication, nonverbal cues, and forming peer relationships align with broader patterns observed in the literature.[14,16,17] Additionally, the presence of repetitive behaviors in our case mirrors the core characteristics outlined by Leekam et al.[15]

In an animal model study, Setd5$^{+/-}$ mice underwent a comprehensive behavioral analysis, demonstrating phenotypes analogous to those observed in individuals with ASD and ID. The transcriptomics analysis of the Setd5$^{+/-}$ mouse brain unveiled disruptions in the expression of both rDNA and ribosomal protein genes. Examining the regulation of rDNA expression by *SETD5* in neuroblastoma cell lines, the study revealed that *SETD5* binds to the rDNA promoter, recruiting HDAC3. This recruitment leads to a reduction in the acetylated form of histone 4 at Lys16 (H4K16ac), subsequently inducing the dissociation of TIP5 and facilitating rDNA expression. Furthermore, the study observed a depletion of rRNA due to *SETD5* deficiency, resulting in a diminished cell proliferation in cultured neuroblastoma cells. This reduction in cell proliferation extended to Setd5$^{+/-}$ mouse embryos in vivo and adult neural stem cells in vitro. Additionally, the translation of cyclin D1 mRNA was specifically down-regulated in *SETD5*-deficient cells. Altogether, these findings emphasize the crucial role of *SETD5* in regulating neural cell proliferation through epigenetic control of rDNA expression.[6]

In a comprehensive study, the researchers conducted a systematic literature review and analyzed public databanks to identify all mutations within the *SETD5* gene. The outcomes revealed a noteworthy association between these mutations and a diverse spectrum of ID and ASD-like symptoms. Notably, the observed high penetrance in males indicated the pathogenic nature of these mutations. Interestingly, within the female population, the majority of reported cases exhibited a similarly elevated penetrance. However, the study brought to light two instances of female carriers with normal phenotypes, yet their offspring, specifically male children, manifested symptoms of ID and ASD after inheriting the mutation.[13] This intriguing finding adds a layer of complexity to our understanding of *SETD5* mutations, highlighting their potential as causative factors in the development of ID and ASD, particularly in males. Also, the results of our study have contributed significantly to the existing body of knowledge by identifying a novel missense heterozygous *SETD5* mutation. This newfound genetic variation is a noteworthy addition to the current understanding of the genetic landscape associated with ASD. The clinical manifestations of ASD were observed in both the patient and the father, both of whom carry the identified heterozygous mutation. Intriguingly, however, the mother exhibited a normal genotype, suggesting a potential link between the identified genetic mutation and the manifestation of ASD symptoms.

This particular heterozygous *SETD5*: c.3694T>A: p.Tyr1232Asn mutation, previously unreported in mutation databases, represents the first documented instance of a mutation in the SETD gene in a patient affected by ASD. Several lines of evidence substantiate the assertion that this mutation is causally linked to ASD: 1- WES exclusively identified this mutation as the primary factor underlying ASD in the patient. 2- Figure 2 illustrates that direct Sanger sequencing verified the presence of the mutation in both the proband and the affected father within the family, but his healthy mother has a normal genotype. The recognized heterozygote mutation in the father suggests an autosomal dominant pattern of inheritance for the *SETD5* gene. 3- Bioinformatics tools, including PolyPhen-2, SIFT, FATHMM-MKL, LIST-S2, and MutationTaster, collectively suggest that these genetic variants are likely to be deleterious and associated with disease development. 4. The new c.3694T>A: p.Tyr1232Asn mutation in exon 22 of the *SET5* gene results in a missense alteration, replacing tyrosine with asparagine at position 1232 in the protein. This amino acid substitution has the potential to impact the three-dimensional structure of the SET5 protein, affecting its folding, stability, and interactions. 5- Significantly, this alteration was absent in the healthy mother, reinforcing the hypothesis that it may contribute to the phenotype observed in these patients. Consequently, the identified mutation in the *SETD5* gene is deemed pathogenic in our patient with ASD.

Conclusion

The discovery of the novel heterozygous *SETD5* mutation (c.3694T>A: p.Tyr1232Asn) in an Iranian pedigree affected by ASD using WES sheds light on the genetic basis of ASD in this population. The identification of a heterozygous mutation shared by the patient and his father, with a normal genotype in the mother, underscores the importance of genetic counseling. Beyond its genetic implications, this information holds significant value for psychological counseling, offering a deeper understanding of the condition. Incorporating genetic insights into psychological counseling can empower families, providing them with informed strategies to navigate the emotional and practical challenges associated with ASD, fostering resilience and tailored support.

Competing Interests

The authors declare that they have no competing interests.

Ethical Considerations

All procedures performed in this study were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments. Written informed consent was obtained from the family members for this publication.

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