Research in Molecular Medicine

A Novel *ZC4H2* Gene Variant (c.197 T>C) Associated With Wieacker-Wolff Syndrome: A Case Report From North of Iran

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ABSTRACT

Background: The *ZC4H2* gene is highly expressed during brain development and its expression involves the nervous system, especially during the fetal period. The pathogenic variants in the *ZC4H2* gene are associated with X-linked intellectual disability (ID).

Materials and Methods: DNA analyses on a 3-year-old boy with mental retardation, muscular atrophy, developmental delay, movement and respiratory problems and joint contracture using whole exome sequencing and confirmatory Sanger sequencing method revealed a novel pathogenic variant (c. 197 T>C) in the *ZC4H2* gene.

Results: This variant affects the nervous system's development, leading to a better understanding of *ZC4H2* function.

Conclusion: Genotype/phenotype correlation analysis of new cases with pathogenic variants on the *ZC4H2* gene could be informative for a better understanding of the *ZC4H2* function in humans.

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Introduction

Located on the pericentric region of the X chromosome (Xq11), the *ZC4H2* gene is highly expressed during brain development and could affect the nervous system, especially during the fetal period. It is among the many candidate genes responsible for X-linked intellectual disability (ID) [1, 2]. The gene encodes a zinc-finger protein, which is a member of the family of proteins with a C-terminal zinc-finger domain containing four cysteine residues, two histidine residues, and one coiled-coil region [1, 3]. It has been reported that pathogenic variants in the *ZC4H2* gene will lead to Wieacker-Wolff syndrome, characterized by ID, motor developmental delay, poor growth, muscle weakness, and skeletal abnormalities. Affected patients usually experience multiple congenital joint contractures at birth due to muscle weakness beginning in utero [1-5].

Knockdown or knockout of the *ZC4H2* orthologue in zebrafish causes complications such as abnormal swimming, impaired α-motor neuron development, and a striking reduction of GABAergic interneurons [1]. This finding indicates that *ZC4H2* may be important in nervous system development. Introducing novel pathogenic variants on the *ZC4H2* gene can help better understand its function and related diseases and complications. In the present study, we introduce a novel mutation of the *ZC4H2* gene in a case with ID from Iran.

Case Presentation

The case was a 3-year-old boy with complaints of mental retardation, muscular atrophy, developmental delay, movement disabilities, respiratory problems, and joint contracture. The subject had a brother who died at age 3 (Figure 1). He was referred to Fjar Medical Genetics Lab (Sari City, Iran) for genetic counseling and DNA analysis.

A written informed consent was obtained to explore the possible disease-causing variants. The blood karyotype of the case showed a normal pattern. The results of the acylcarnitine profile showed no abnormality, and the subject had normal cranial magnetic resonance imaging.

The genomic DNA was extracted from peripheral blood for molecular analysis, and whole exome sequencing was conducted using the Illumina platform. A novel hemizygous missense variant in the *ZC4H2* gene [NM_018684.4, ENST00000374839.8: c.197T>C; p.(Leu66Pro)] was identified. This variant has not been reported in the gnomAD genomes, gnomAD exoms, ExAC, and Iranome databases. It was classified as a likely pathogenic variant (class 2) according to the American College of Medical Genetics (ACMG) recommendations based on the following rules: PM1, PM2, PP3 and PM5. However, the PP4 rule can also be applied because our patients' clinical symptoms were compatible with the previously reported patients caused by disease-causing variants in the *ZC4H2* gene. Since the disease is an X-linked inherited disorder and the mother has one normal allele, as expected, she has no clinical symptoms.

To predict the impact of the identified variant in the ZC4H2 protein, several in silico analyses were performed, including Mutation Taster [6] and PolyPhen-2 [7], and SIFT [8] were applied. The Mutation Taster predicted that the c.197 T>C variant would be "disease-causing," with a probability of 0.999999999999894. SIFT, which predicts whether an amino acid substitution affects the protein, indicated that p.L66P is "damaging" (score=0.00)—polyPhen-2 classified p.L66P as "probably damaging," with a score of 0.999. Moreover, the Franklin Genoox online tool has also categorized this variant as likely pathogenic.

Targeted sequencing for the detected variant was used to confirm the identified mutation on the case and screen other family members. Locus-specific primers (5-GCAAATTGCAGGAACAAG-3 and 5-AAAGGCCCTATCATTCACG-3) that amplify 252 bp of the *ZC4H2* gene containing the variant were designed using the Oligo software, version 7. Then, the samples were sequenced using the Sanger sequencing method via Applied Biosystems 3130xl Genetic Analyzers. The presence of the c.197 T>C variant was confirmed in the case and was also detected in the heterozygote state in his mother (Figure 2).

Discussion

For the first time in 1985 and in six male members of one family from three generations, an X-linked form of arthrogryposis was reported as Wieacker-Wolff syndrome. All affected cases had the same clinical manifestations, including congenital contractures of the feet, slowly progressive predominantly distal muscle atrophy, visual dyspraxia, facial weakness and ID [9]. In 2013, a pathogenic missense variant on the *ZC4H2* gene was detected in all suspected cases [1]. Later, several families were introduced with a mutation on the *ZC4H2* gene [10, 11].

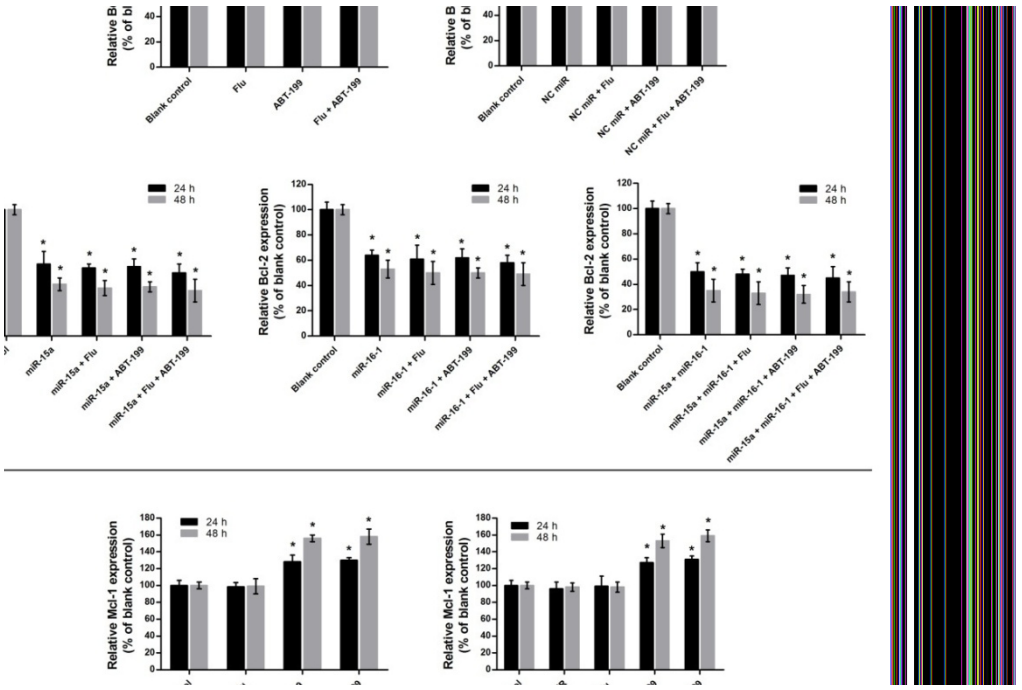

Figure 1. Pedigree chart of the family with c.197T>C variant in the *ZC4H2* gene

III-1: Confirmed hemizygote, III-2: Suspected hemizygote, II-1: Confirmed heterozygote.

The typical clinical manifestations of the patients carrying *ZC4H2* gene mutations which are presented in 30% of the cases are postnatal growth retardation, generalized hypotonia, motor delay, inability to walk, spasticity, hyperreflexia, urinary incontinence, dysarthria-deficit in expressive language, poor or absent speech, ID, drooling, dysphagia, chewing difficulties including oral motor dysfunction, feeding difficulties, facial weakness-palsy, high forehead, high anterior hairline, ocular motor apraxia, strabismus, anteverted nares, microretrogna-

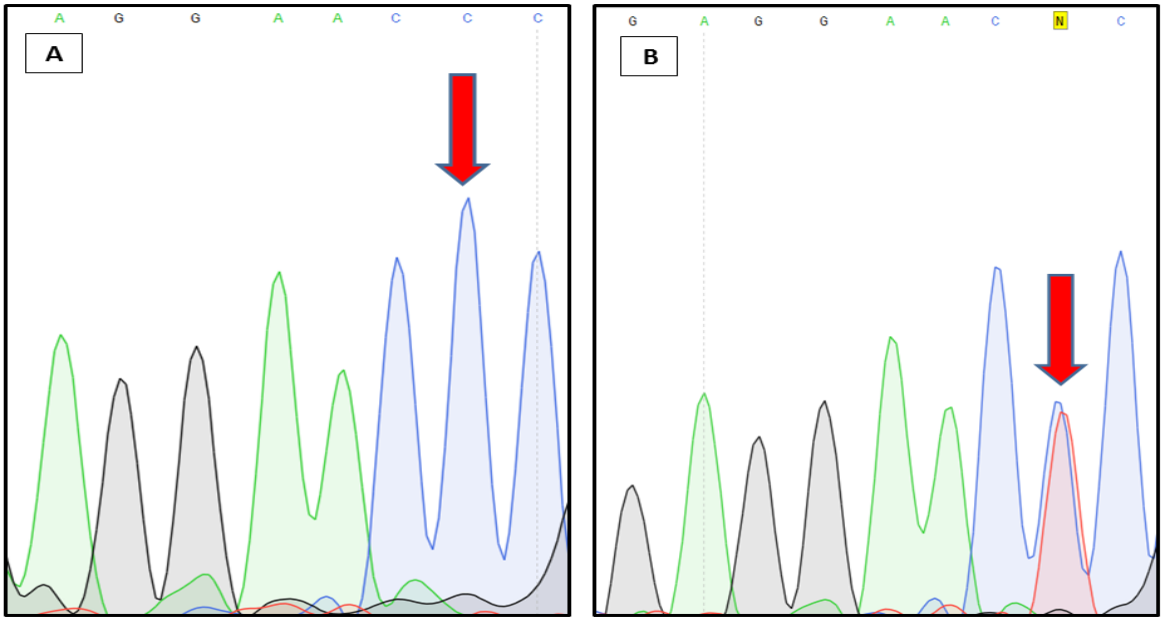

Figure 2. The sequencing results showing c. 197 T>C

A) Variant in hemizygote, B) Heterozygote states in the subject and her mother, respectively

thia, arthrogryposis multiplex congenita (AMC), limited shoulder movement, elbow, wrist contractures, metacarpophalangeal joint contractures, camptodactyly, radial deviation of any finger, knee flexion contractures, equinovarus deformity-club feet, Achilles tendon contracture, distal limb muscle atrophy or weakness and micropenis or cryptorchidism in males [12, 13].

Thus far, few missense variants in the *ZC4H2* gene have been reported to cause X-linked ID in hemizygous male and heterozygous female patients, including R18K, V63L, L66H, R198Q, P201S, R213W and K209N [1, 2]. Moreover, a de novo X-chromosome inversion and a splicing defect caused by the c225+5G>A mutation in the *ZC4H2* gene leads to an in-frame insertion of 15 novel amino acids, p.75_76insVHGCLEPISKAFEKE, and deletions have also been described as another type of disease-causing mutation [1, 2, 10]. In the present study, we reported the p.L66P as a new variant on the *ZC4H2* gene leading ID.

With the advent of the next-generation sequencing technique, whole genome sequencing is possible and helps diagnose rare and complex genetic disorders. This test is recommended for patients, especially those with an unknown cause of ID. Several hereditary diseases cannot be properly diagnosed based on clinical manifestations alone; new technologies such as next-generation sequencing for genetic analysis of these patients would help identify the cause of the disorders. Here, we described a case with Wieacker-Wolff syndrome due to a novel mutation on the *ZC4H2* gene, highlighting the role of novel technologies in the genetic diagnosis of rare diseases. Genotype/phenotype correlation analysis of new cases with a mutation on the *ZC4H2* gene could be informative for a better understanding the *ZC4H2* function in humans.

Ethical Considerations

Compliance with ethical guidelines

The family filled an informed questionnaire and agreed to attend the study.

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Authors contribution's

Performing the test: Hossein Jalali and Mahan Mahdavi; Data analysis and writing the original draft: Hossein Jalali; Review and editing: Mohammad Reza Mahdavi; Visiting the case as a physician: Fatemeh Pourfatemi.

Conflict of interest

The authors declared no conflict of interest.

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