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A CASE REPORT OF A RARE NONSENSE *ZP1* VARIANT IN A PATIENT WITH OOCYTE MATURATION DEFECT*

OOSİT MATÜRASYON DEFEKTİ OLAN BİR OLGUDA NADİR GÖRÜLEN ANLAMSIZ *ZP1* VARYANTI

Ezgi Gizem BERKAY[1,2], Birsen KARAMAN[1,3], Zehra Oya UYGUNER[1], Seher BAŞARAN[1]

[1]Istanbul University, Istanbul Faculty of Medicine, Department of Medical Genetics, Istanbul, Turkiye
[2]Istanbul Kent University, Faculty of Dentistry, Department of Basic Science, Istanbul, Turkiye
[3]Istanbul University, Child Health Institute, Department of Pediatric Basic Sciences, Istanbul, Turkiye

ORCID IDs of the authors: E.G.B. 0000-0002-1967-705X; B.K. 0000-0001-8640-0176; Z.O.U. 0000-0002-2035-4338; S.B. 0000-0001-8668-4746

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ABSTRACT

After four unsuccessful assisted reproductive techniques trials, a female was referred for genetic analysis. In this case study, we aimed to investigate the genetic etiology of a female with infertility and oocyte maturation defect. Chromosome analysis and fluorescence in-situ hybridization (FISH) using X-centromeric (DXZ1) and SHOX-probe (SHOX/SE X) (CytoCell, Cambridge, UK) on interphase nuclei of lymphocytes and mucosal cells were performed. Exome sequencing using the Illumina platform and confirmatory studies, including intra-familial segregation analysis, was done by Sanger sequencing. Karyotyping and molecular cytogenetics studies were normal, and potential chromosomal abnormalities and mosaicism were excluded. WES data analysis identified a known, rare, nonsense pathogenic homozygous variant in exon 3 (NM_207341.4, c.628C>T; p.Q210*) of the *ZP1* gene. Additionally, her parents, who were first-degree cousins, were heterozygotes for this variant. Zona pellucida is an essential glycoprotein that surrounds oocytes and contains four types of receptor proteins (ZP1-4). The detected mutation in the *ZP1* gene leads to the premature stop codon, causing truncation of the ZP1 receptor protein. This is the first case report with a homozygous variant associated with oocyte maturation defect. Also, exome sequencing is a valuable method to identify the genetic etiology in complex, multigenic conditions like infertility.

Keywords: OOMD, zona pellucida, *ZP1*, female infertility, exome sequencing

ÖZET

Bu çalışmada yardımcı üreme tedavisi sonrasında dört başarısız denemesi olan kadın bir olguda, infertilite ve oosit olgunlaşma bozukluğunun genetik etiyolojisinin araştırılması amaçlanmıştır. Lenfositlerin ve mukozal hücrelerin interfaz çekirdekleri üzerinde X-sentromerik (DXZ1) ve SHOX-probu (SHOX/SE X) (CytoCell, Cambridge, UK) kullanılarak kromozom analizi ve floresans hibridizasyonu (FISH) yapıldı. Ekzom dizilemede Illumina platformu; bulunan varyantın doğrulaması ve aile içi segregasyon analizi için Sanger dizileme tekniği kullanıldı. Karyotip ve moleküler sitogenetik analiz sonuçları normaldi, potansiyel kromozomal anomaliler ve mozaiklik dışlandı. Tüm ekzom veri analizinde, *ZP1* geni 3. ekzonunda (NM_207341.4, c.628C>T; p.Q210*) bilinen, nadir, anlamsız bir patojenik homozigot varyant tanımladı. Segregasyon çalışmasında birinci derece kuzen olan ebeveynlerinin bu varyant için heterozigot oldukları bulundu. Erken durdurma kodonu bileşimindeki bu mutasyon, ZP1 reseptör proteininin kısa sentezlenmesine neden olmaktadır. Zona pellusida, oositleri çevreleyen ve dört tip reseptör proteini (ZP1-4) içeren temel bir glikoproteindir. Bu çalışma, tespit edilen homozigot varyantın oosit matürasyon defekti ve kadın infertilitesi ile ilişkili olduğunu gösteren ilk olgu sunumudur. Ayrıca, ekzom dizileme yönteminin infertilite gibi karmaşık, multigenik durumlarda genetik etiyolojiyi belirlemek için kullanılabilecek değerli bir yöntem olduğu görülmüştür.

Anahtar Kelimeler: OOMD, zona pellusida, ZP1, kadın infertilitesi, ekzom dizileme

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Corresponding author/İletişim kurulacak yazar: Ezgi Gizem BERKAY – ezgi.berkay@kent.edu.tr

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INTRODUCTION

Oocyte maturation defect (OOMD) is a rare condition that occurs due to disruptions in the maturation process of oocytes and leads to primary female infertility, which is diagnosed mainly during assisted reproduction techniques (ART) (1). Twelve genes related to OOMD have been identified, and both autosomal dominant and recessive inheritance have been observed (*ZP1, TUBB8, ZP3, PATL2, WEE2, ZP2, PANX1, BTG4, TRIP13, REC114, ASTL, FBXO43*).

ZP1 pathogenic gene variants lead to OOMD with AR inheritance in homozygous or compound heterozygous form. Deletions leading to a frameshift and protein truncation causing variants (c.170-174del, c.507del, c. 508del, c.1129-1130del, c.1169-1176del), splice-site variants (c.1014+1G>A, c.1430+1G>T, c.1775-3C>A), missense variants (c.181C>T, c.1228C>T, c.1708G>A) and nonsense mutations (c.1413G>A, c.1510C>T, c.1663C>T) have been reported (2-8). Here, we present a female primary infertility case with four unsuccessful ART trials due to empty follicle syndrome and OOMD, and who has a homozygous nonsense variant in the *ZP1* gene.

MATERIAL and METHODS

Karyotyping, FISH analysis, and whole exome sequencing (WES) were performed. The xGen Exome Research Panel IDT Kit was used for library preparation, and the proband's sample was sequenced on the Illumina NextSeq® platform. An average reading depth of 65x was obtained for the target exome regions. The data were aligned with the reference genome data using the Burrows-Wheeler Aligner, and the shooting of the variants was done with the GATK Unified Genotyper. The alignment corrections in the in/del regions with realignment and filtering processes were carried out according to the percentage of variant detection (≥30%) and reading depth (≥10x). Sanger sequencing was performed for confirmation and segregation studies. Sequence data were analyzed using Seq Scape v3 and Chromas v2.6.6 analysis programs.

The bioinformatics analysis started with creating a list of active pathway genes through data mining, and genes related to female infertility were examined as the first filtering step. Secondly, variants classified as pathogenic or likely pathogenic were evaluated (≥20x of reading depth, minor allele frequency (MAF) of <0.01). The variant analysis criteria from the American College of Medical Genetics and Genomics (ACMG) were considered (9). Varsome and GnomAD databases were used for *in silico* analysis. This study was approved by Istanbul Faculty of Medicine Clinical Research Ethics Committee (Date: 09.02. 2018, No:249). The case and family members both signed the informed consent form. This study was funded by the Scientific Research Projects Coordination Unit of Istanbul University (Grant number: TSA-2018-32135).

RESULTS

The 31-year-old patient was referred to Istanbul University, Istanbul Faculty of Medicine, Department of Medical Genetics, to clarify the genetic etiology. Her parents were first cousins. Her pubertal development was normal, and anatomical, endocrinological, and autoimmune defects were excluded. Her physical examination was normal, but short stature with -3 SD was observed with limited extension flexion on bilateral elbows. Bilateral proximal radioulnar synostosis and slightly short ulnas were detected on the radiograph. Four ART trials were done, but follicles never achieved maturation.

Initially, chromosome analysis was investigated and revealed 46,XX karyotype. To exclude monosomy X mosaicism and *SHOX* gene deletion, fluorescence in-situ hybridization (FISH) using X chromosome centromeric (DXZ1) and SHOX-probe (SHOX/SE X) (CytoCell, Cambridge, UK) on interphase nuclei of lymphocytes and mucosal cells was performed and the result was normal. WES data analysis identified a known, rare, nonsense pathogenic homozygous variant in exon 3 (NM_207341.4, c.628C>T; p.Q210*) in the the OOMD-associated ZP1 gene. This variant was classified as pathogenic according to the ACMG criteria. This variant's frequency is 0.000008508 in heterozygous form, according to the gnomAD database. Sanger sequencing studies were performed with DNA obtained from the peripheral blood of first-degree cousin parents of the patient. They were both carrier for this variant (Figure 1).

DISCUSSION

ZP1 protein and three other zona pellucida proteins form the zona pellucida. This thick glycoprotein layer is effective in oocyte maturation, sperm binding at fertilization, acrosome reaction initiation, polyspermy inhibition after fertilization, and preimplantation embryo protection (10). The proteins in the zona pellucida complex are synthesized independently of each other and transported to the cell surface by the endomembrane system. Cross-links formed by ZP1 proteins also form connections in the complex (2). Supporting this, in the studies conducted with mice without ZP1 protein, zona pellucida formed lankly around oocytes, and reproductivity was reduced (11).

The *ZP1* gene, located on the long arm of chromosome 11 (11q12.2), contains 12 exons. The encoded protein has seven domains: signal peptide (SP; the residues of 1-26), ZP-N1 domain (36-138), P-type Trefoil domain (232-277), ZP-N domain (277-380), ZP-C domain (402-522), Consensus furin cleavage site (CFCS; 522-525) and transmembrane domain (TM; 602-624). The absence of zona pellucida can be observed under the microscope in unsuccessful ART trials. Homozygous variants of the *ZP1* gene with autosomal recessive inheritance (MIM_615774)

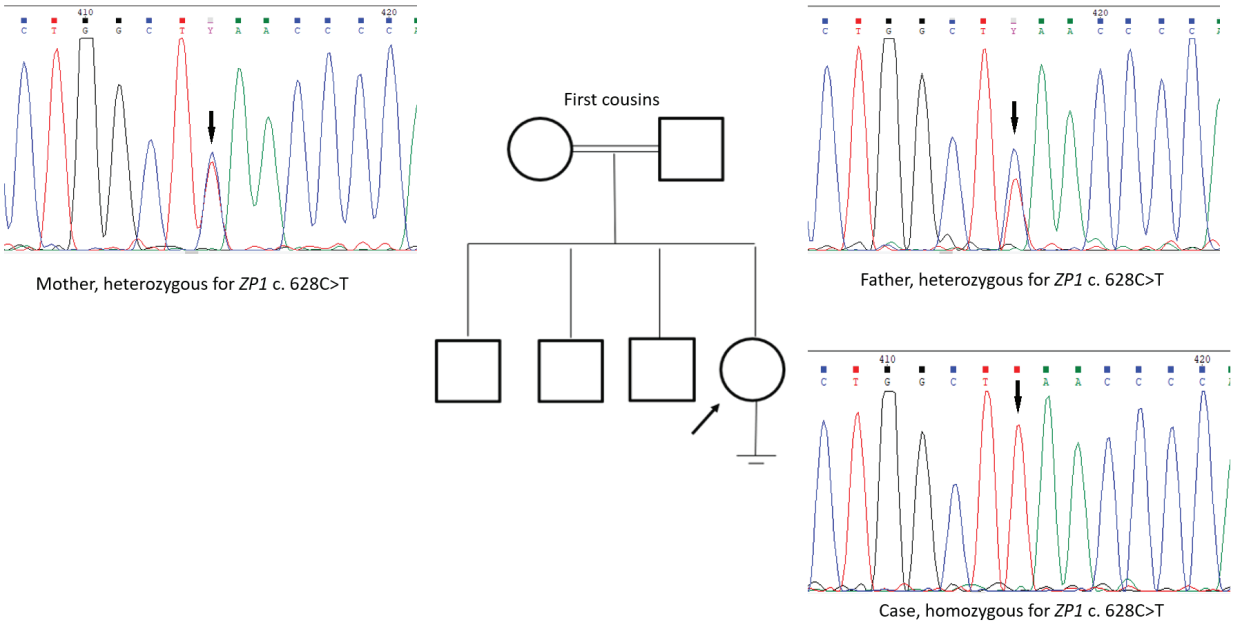

Figure 1: Confirmation and segregation of the ZP1 variant. Proband was homozygous for the *ZP1* c.628C>T variant, and her parents, who were first cousins, were detected as carriers.

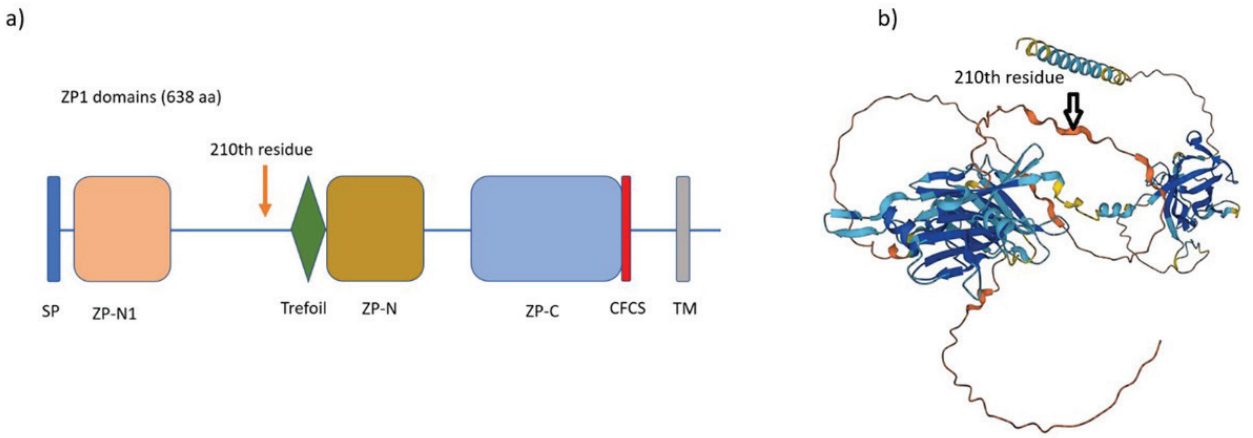

Figure 2: ZP1 protein domains and the location of the detected variant a) Nonsense mutation in ZP1 leading to truncated protein at the 210th residue. This results in the loss of trefoil (1-26), ZP-N (277-380), ZP-C (402-522), CFCS (522-525), and TM (602-624) domains. SP: signal peptide, CFCS: consensus furin cleavage site, TM: transmembrane **b)** The position of the 210th residue in ZP1 protein's 3D structure (AF-P60852-F1)

are related to OOMD-1. Our variant was in the 3rd exon and affected the protein between the ZP-N1 and Trefoil domains. All other domains were lost except for the signal peptide ZP-N1 domain due to premature protein termination (Figure 2).

In our case, empty follicle syndrome and oocyte maturation defect were diagnosed during ART trials. The detected variant was classified as pathogenic according to the ACMG and segregated in the family. The case's brothers weren't investigated for this variant due to not accepting to join the study. The mother and father of the proband were heterozygous carriers for the same variant. This article is the first report that shows homozygous c.628C>T (p.Q210*; rs776515172) mutation associated with OOMD. We conclude that exome data analysis is an efficient tool for identifying the mutations and genes related to infertility.

Ethics Committee Approval: This study was approved by Istanbul Faculty of Medicine Clinical Research Ethics Committee (Date: 09.02. 2018, No:249).

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Conflict of Interest: The authors have no conflict of interest to declare.

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