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NEÜ YAYINEVİ

Rare Mutation in Androjen Receptor Gene with a Case of Primary Amonerrhoea

Primer Amenoreli Vakada Nadir Bir Androjen Reseptör Gen Mutasyonu

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Öz

Androjen duyarsızlık sendromu (AIS), Xq11-q12'de yer alan androjen reseptör (*AR*) genindeki mutasyonların neden olduğu genotip/fenotip uyumsuzluğu ile seyreden X'e bağlı çekinik bir hastalıktır. Bu çalışmada *AR* geninde literatürde daha önce bildirilmemiş nadir bir mutasyon saptadık. AIS'nun temel klinik bulguları; 46,XY bireylerde, doğumda dişi dış genitalya, puberte döneminde anormal sekonder seks karakter gelişimi ve erişkinlik döneminde görülen infertilitedir. 17 yaşında, dişi dış genitalyaya sahip hasta primer amenore nedeniyle kromozom analizi yapılmak üzere kliniğimize yönlendirildi. Hastanın genotipi 46,XY olarak saptandı. Hastanın *AR* geni sekans analizinde ekzon 1'de; daha önce ExAc, 1000genome ve diğer popülasyon veri tabanlarında bulunmayan NM000044 c.5A>G varyantı tespit edildi. Bu varyant 2. pozisyonda glutamik asidin glisine dönüşümüne neden olmaktadır. Negatif yüklü, polar bir aminoasit olan glutamik asidin; hidrofobik özelliğe sahip glisine dönüşümü nedeniyle, bu mutasyonun *AR* geninin fiziksel yapısını ve proteinin 3 boyutlu yapısını bozabileceğini düşündük.

Anahtar Kelimeler: Androjen reseptör, primer amenore, sekans analizi

Abstract

Androgen insensitivity syndrome (AIS) is an X-linked recessive disorder associated with incompatible genotypes and phenotypes caused by mutations in the androgen receptor (*AR*) gene is located at Xq11-q12. We have detected a rare mutation in the *AR* gene that has not been reported in the literatüre. Clinical findings are female external genitalia at birth, abnormal secondary sexual development in puberty, infertility in individuals with a 46, XY karyotype as typically characterized AIS. In our case, a 17-year-old female phenotype presented with primary amenorrhoea and predominantly female external genitalia. The patient had 46,XY with female phenotype. We detected a missense rare mutation in the first exon as NM000044 c.5A>G variant was not found in ExAc or 1000genome population database. Substituting at position 2 to glutamic acid exchange to glycine. Glutamic acid is a polar amino acid with a negative charge while glycine has stayed in a nonpolar hydrophobic group. So, we thought that the mutation may cause a physical defect in protein and the native three-dimensional structure of the *AR* gene

Key words: Androgen receptor, primary amenorrhoea, sequence analysis

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INTRODUCTION

Androgen insensitivity syndrome (AIS) is an X-linked recessive disorder, associated with incompatible genotypes and phenotypes caused by mutations in the Androgen receptor (*AR*) gene. Mostly, this molecular pathology in the gene results from androgen receptor dysfunction in a failure of normal masculinization of the external genitalia. Depending on the pathology of the virilization, the disease is observed in three phenotypes. Partial androgen insensitivity syndrome (PAIS) exhibits a broad spectrum phenotype as predominantly male external genitalia from the female external genital phenotype to the masculine. Mild androgen insensitivity syndrome (MAIS) is characterized by decreased virilization, male external genitalia usually presenting with gynecomastia at puberty. 46,XY individuals with complete androgen insensitivity syndrome (CAIS) are phenotypic normal females with a short, blunt-ended vagina, bilateral testes (intra-abdominal or inguinal hernia), and absence of Wolffian structures. Many patients are diagnosed later in life due to primary amenorrhea or the finding of testes during hernia repair. The incidence is 1 in 20,000–64,000 male births. *AR* disorders are caused by testicular feminizing resulting in female phenotypic differentiation and disruption of spermatogenesis lead to subfertility or infertility. The target organs are unresponsive to androgens (1).

Androgens as testosterone and dihydrotestosterone are mediated with the *AR* is a ligand-dependent nuclear transcription factor and member of the steroid hormone nuclear receptor famiItsIt's activation is essential for normal primary male sexual development and differentiation in fetal life and virilization in puberty, whereas in females, androgens also participate in sexual development in puberty and adult female sexual function. *AR* gene is localized on chromosome Xq11-12 has four domains: The first one is the amino-terminal activation domain (NTD); the second is the DNA-binding domain (DBD); the third one is the hinge region (HR) and the last is carboxyl ligand-binding domain (LBD). NTD contains exon 1 encodes 1-556 amino acids. The first 30 aa plays a major role in the N/T interaction required for AR gene receptor activation (2).

Mutations in the *AR* gendefectscause defect or loss of function in the receptor. *AR* gene mutation database was also last updated in 2014. Only 15% of mutations have been reported in exon 1. Accordingly, *AR* gene mutations database around 1000 different *AR* mutations have been detected in cases with AIS. More than 70% of these mutations are observed in exon 1, causing the CAIS phenotype. Although, it has been reported that the first exon encodes the majority of the protein, 25% of all mutations in AIS patients are associated with the exon 1 region (3,4).

CAIS diagnosis is based on clinical and laboratory findings and can be confirmed by the detection of a defect in the *AR* gene. An accurate approach involving an elaborate family history that suggests a feature X-linked disease is an important marker for rapid diagnosis. In this study, we present a patient with CAIS in that a rare mutation of the *AR* gene was detected. We report a case of CAIS presenting with the same mutation described across three generations. To the best of our knowledge, this mutation is the first such report citing this mutation in the literature.

CASE

We report a case of an 18-year-old girl. She had a female phenotypic appearance with typical female genitalia, normal breast development, small labia, and absent or sparse pubic hair. Sexual hormones in blood were measured.Gonadotropins were found normal, FSH:5,89 mUI/ml, LH:39,95 mUI/mL, progesterone:1,14 ng/ml and estradiol: 37,858 pg/mL, nevertheless total testosterone was high for a woman:887,8 ng/ml. A pelvic ultrasound examination was done. Testicles in the inguinal channel and only 1/3 part of the vagina were seen but any müllerian structures, such as the uterus or fallopian tubes were not detected. The pelvic MRI revealed the absence of uterus and ovaries, hypoplastic vagina, and intraabdominal testes too. Surgery was planned for resection of testicles. While taking family history for pedigree analysis, it was learned that her cousin had the same diagnosis, too. Due to the family history suggesting a disorder of X-linked disease, AIS was considered so detection of the *AR* gene was planned. Firstly, chromosome analysis was performed on metaphases prepared from peripheral blood lymphocytes, applying standard GTG banding technique for the proband. The presence of a Y chromosome was confirmed by *AZF* and *SRY* gene region fragman analysis. Also, the MLPA technique was used to exclude *AR* gene large deletions and duplications. Then, all exons of the *AR* gene were amplified and sequenced, and checked for variations. DNA sequence result appeared a rare hemizygous *AR* gene mutation in exon 1. Then family investigations were done according to pedigree (Fig.1). The patient

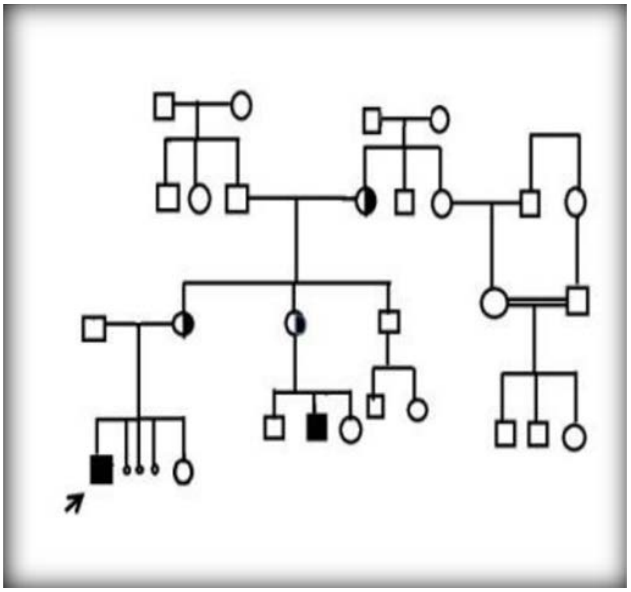

Figure 1. Family pedigree, illustrated X linked autosomal recessive inheritance pattern in our family study of the *AR* gene

was 46,XY karyotype. MLPA and *AZF-SRY* gene region analyses were normal. Screening all exons of the AR gene revealed a missense mutation at position g.529A>G(c.5A>G) in the *AR* gene (Fig.2), which substitutes glutamine to glycine. NM000044 c.5A>G variant was not found in population database as ExAc or 1000 genome however it was observed in-slice database as Varsome and Clinvar. The patient's mother and grandmother were carrying the same mutation in a heterozygous manner but her sister had a wild type (Fig.3). All three were normal female genotypes and phenotypes. Also, while the family history was taken it was learned that the cousin had a female phenotype with 46,XY genotype was

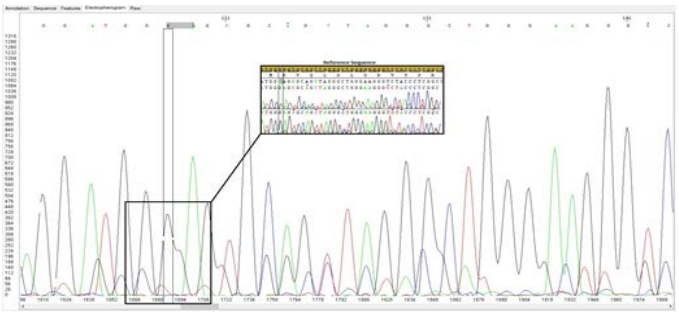

Figure 2. Patient: Hemizygous mutant with 46, XY;

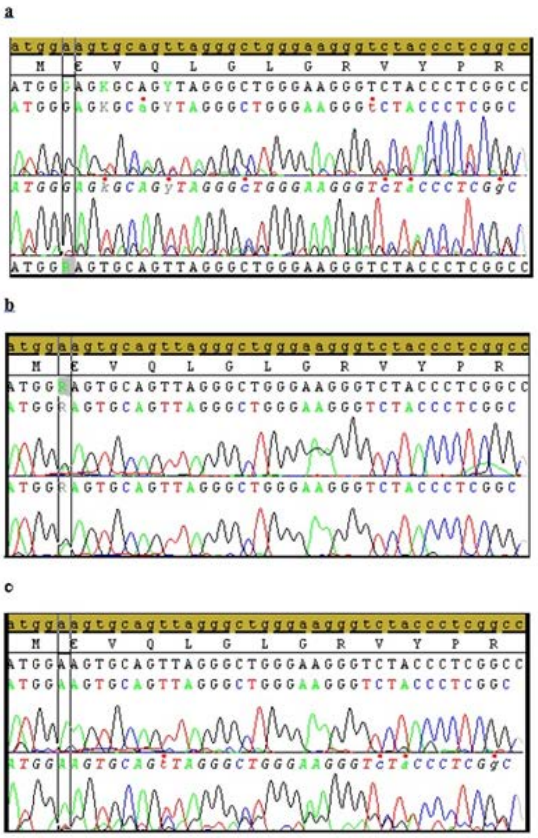

Figure 3. a- Mother: Heterozygous carrier with 46,XX; **b-** Grandmother: Heterozygous carrier with 46,XX; **c-** Sister: Wild type, with 46,XX; this figure shows that the sanger sequence analysis of *AR* gene in the family

laparoscopic gonadectomy. However, we thought that the cousin and the aunt could have the same mutation but they didn't come for sequence analysis.

DISCUSSION

Androgen insensitivity syndrome (AIS) is a genetic disorder that is X-linked recessive inheritance characterized by androgen unresponsiveness which can incompatible correlation of the genotype and phenotype. Clinical phenotypic manifestations of AIS vary widely and form a broad scale. However, in general, there are three groups as complete androgen insensitivity syndrome,e (CAIS), partial androgen insensitivity syndrome (PAIS), and mild androgen insensitivity syndrome (MAIS) based on phenotype. CAIS is characterized by the presence of female external genitalia in a 46, XY genotype, which is a hormone-resistance syndrome due to mutations

in *AR* gene that affects sexual development before birth and during puberty.

Androgen receptor (*AR*) belongs to the steroid receptor subfamily of nuclear receptors (NRs) its it's function is important for the normal development of male and female reproductive organs and their physiology (1). *AR* gene at Xq11-12 locus has to encode 8 exons and contains 920 amino acid residues. The *AR* gene has 4 functional domains: an N-terminal domain (NTD) which mediates transcriptional function in exon 1; a central deoxyribonucleic acid (DNA) binding domain (DBD) has cysteine residues in exons 2-3, a hinge region containing the nuclear signal and a C-terminal ligand-binding domain (LBD) in exons 4–8 (1,2).

The NTD is the least conserved region but is an important regulatory part for androgen receptor activity. Most studies have been showing that the NTD domain is a critical region for androgen transactivation and function (5). Activation function-1 (AF1) region, which is for *AR* transactivation essentially. Deletions in this region have been caused by the inactivity of androgen receptors (6). Amino acid sequence alignment analysis between species has revealed 3 highly conserved regions in the NTD, residues 1–30, 224–258, and 500–541. The first 30 amino acids include the FQNLF motif, important for N-C interaction. The NTD domain also contains activation function (AF)-2 for interaction between N- and C-terminal of the receptor which facilitates cross-talk between receptor domains (7). For all these reasons, the NTD region has recently been shown as a therapeutic target in *AR*-related diseases. Therefore, defining mutations in this region is of great importance both in identifying the diagnosis of *AR*-related diseases and in explaining the possible resistance mechanisms of drugs developed for this region (8).

CONCLUSIONS

Mutations of *AR* are distributed throughout the eight exons of *AR* gene, but 85% of more stations are detected mainly in exons 2–8. Only 15% of mutations have been found in exon 1 and more than 50 different androgen receptor mutations in exon 1 were defined as the CAIS cause. Most of these mutations were stop codon mutations (2,3). Here, we describe a case of CAIS in which a rare mutation in the first exon of the *AR* gene was identified. NM000044 c.5A>G variant was not found in ExAc or 1000 genome population database however ıt was observed in-slico as Clinvar and Varsome. Two known GC-rich variable trinucleotide repeats are a challenge but we showed that these repeats are normal in size with MLPA analysis. Even though this mutation had not been previously reported, the clinical and laboratory findings overlapping with CAIS showed us that the receptor lost its function. Variants in this residue exchange the p.Glu2 position in *AR* gene. It is reported that the variants in this residue disruption have been detected to be pathogenic (20). However, this variant has been evaluated that interpretation uncertain significance in ClinVar database. So, we think that glutamine (polar amino acid) conversion to glycine (nonpolar amino acid) may prevent transcription or interrupts cross-talk between receptor domains. This rare mutation extends the spectrum of exon1 mutations in the androgen receptor gene.CAIS is an X-linked inheritance pattern so genetic counseling is important for both patients and next generations. Due to the risk of testicular malignancies, removal of the gonads by surgery was planned and the psychological counselor was arranged.

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