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A Novel Androgen Receptor Mutation in a Patient with Complete Androgen Insensitivity Syndrome[1]

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Abstract—Androgen insensitivity syndrome (AIS) occurs when target tissues are resistant to the effect of androgens resulting in phenotype with varying degrees of feminization ranging from male infertility to completely normal female external genitalia in patients with male karyotype. Androgen receptor (AR) following activation by androgenic hormones binds to DNA in cells of target tissues and induces biological changes leading to differentiation and development of male urogenital structures. To date, more than 800 mutations in AR gene have been described in patients with AIS with the majority being located in the ligand-binding domain. Here a detailed description of a family with two affected 46,XY females with complete androgen insensitivity is provided. Whole exome sequencing revealed a novel mutation in exon 1 (c.238C>T) of AR gene. The mutation was detected in a proband and her sister, both with normal male karyotype and phenotypic expression of complete androgen insensitivity syndrome.

Keywords: androgen insensitivity syndrome, androgen receptor mutation

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INTRODUCTION

Androgens play an important role in male phenotype determination by binding to androgen receptor and mediating the expression of androgen-dependent genes, which regulate differentiation and development of male urogenital structures [1]. The AR gene is mapped to Xq11-q12 region. It consists of 8 exons separated by introns up to 26 Kb in size and encodes a protein with 919 amino acids [2, 3]. Mutations in AR gene may lead to androgen insensitivity syndrome (AIS) (OMIM no. 300068) of different levels – from mild AIS (MAIS), partial AIS (PAIS) to complete AIS (CAIS) [4].

Carriers of AR mutations are 46,XY individuals with abnormal development of both internal and external genitalia. Clinical, hormonal and molecular investigations are required for the diagnosis of AIS, which is important for management of carriers, including appropriate gender assignment. Molecular diagnosis and genetic counseling of AIS carriers is complicated by the complexity of phenotypic expression of AIS as well as by genotype-phenotype variability of identical mutations. More than 800 mutations in AR genes have been reported to date [5]. Approximately 90% of alterations detected in AR gene are single-base mutations, mainly nonsense mutations [6]. We describe a novel nonsense mutation in exon 1 of AR gene (c. 238C>T) in a patient with CAIS.

MATERIALS AND METHODS

The proband (III-1) was enrolled in the study from Clinic of Reproductive Medicine "Nadiya" (Kyiv, Ukraine). An X-linked recessive inheritance was observed in the proband's family with the evidence of two affected individuals. Blood samples were collected from the proband (III-1), her sister (III-3) and her mother (II-2) for conventional cytogenetic analysis. Hormonal levels were studied in proband (III-1). Genomic DNA was extracted from peripheral blood of proband (III-1), her mother (II-2) and sister (III-3) by Macherey-Nagel NucleoSpin® Blood kit (GE). Whole exome sequencing (CentoXome, Centogen, GE) was performed.

Informed written consent was obtained from each member of the family participating in the study and the study was approved by the institutional ethics committee.

RESULTS AND DISCUSSION

A 31-year-old woman was referred to the Clinic of Reproductive Medicine "Nadiya" with the medical history of primary amenorrhea and infertility. She was 178 cm high with weight of 60 kg. The patient pre-

[1] The article was translated by the authors.

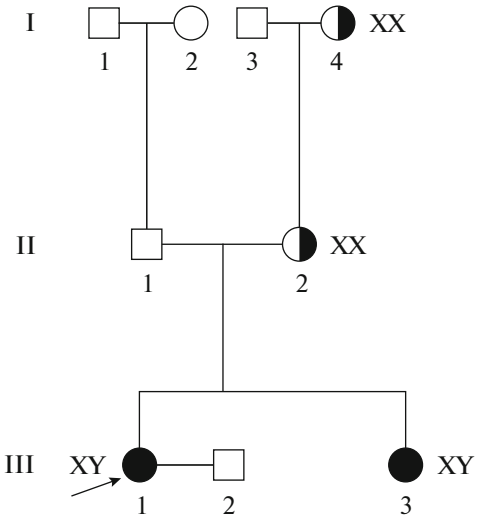

Fig. 1. Pedigree analysis of the family affected with CAIS.

sented normal female external genitalia and normal breast development. Ultrasound studies revealed a short (6 cm) blind-ending vagina. No uterus was observed. A cystic formation instead of uterus was visualized. Gonads were presented by two structures. The left one was 32 × 31 × 22 mm with heterogeneous echogenicity, resembling the ovary structure with focal alterations looking like follicles (8–10 mm). The right one was 29 × 22 × 25 mm, located higher with more homogenous structure.

Hormonal evaluation revealed FSH of 3.79 U/L (reference value: 3.5–12.5), LH of 19.34 U/L (reference value: 2.4–12.6), total testosterone of 2.7 nmol/L (reference value: 0.29–1.67), AMH of 18.5 ng/mL (reference value: 0.01–0.9 low; 1.0–2.5 medium; above 2.5 – high).

After ultrasound and hormonal studies Mayer-Rokitansky-Kuster-Hauser Syndrome (OMIM no. 277000) was suspected as a primary diagnosis.

Genetic counseling was performed and family history gathered (Fig. 1).

Proband`s sister (III-3), 28-years old, was also characterized by female external genitalia and normal breast development, amenorrhea and short-blinded vagina. Since the mother (II-2) and grandmother (I-4) had late menarche, the proband and her sister have not been referred for medical investigation and diagnosis by the age of 31 and 28.

Standard cytogenetic studies performed on GTG-banded metaphase chromosomes of peripheral lymphocytes with the resolution of 450–550 bands per haploid set revealed normal male karyotype in proband (III-1) and her sister (III-3) – 46,XY. The proband's mother (II-2) had normal female karyotype 46,XX.

Whole exome sequencing revealed a heterozygous mutation in exon 1 of AR gene (c.238C>T) of the proband's mother (II-2). Proband (III-1) and her sister (III-3) were hemizygous carriers of c.238C>T mutation (Fig. 2).

CAIS diagnosis was established based on the results of genetic analysis and clinical investigation.

DISCUSSION

AIS is the most common known specific cause of 46,XY disorder of sex development with the preva-

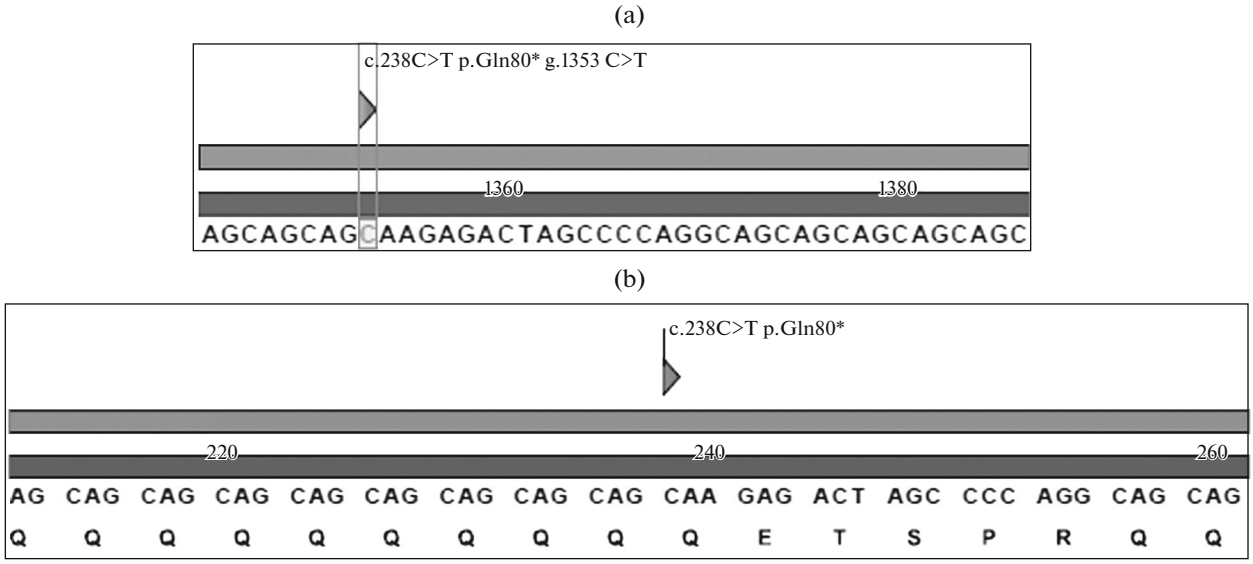

Fig. 2. Mutation in AR gene of proband. (a) DNA nomenclature (NG_009014.2(AR):g.1353C>T); (b) RNA nomenclature (NM_000044.2(AR):c.238C>T).

lence ranging from 1 in 20 400 to 1 in 99100 [4]. The AR gene has been proven to be involved in AIS and a number of mutations have been reported, resulting in different levels of androgen insensitivity in phenotypically female individuals with male karyotype [1, 4]. The AR consists of four domains: the N-terminal domain (NTD), the DNA-binding domain (DBD), the hinge region (HR), the ligand-binding domain (LBD) [7, 8]. NTD is involved in transcriptional activation of target genes by androgens. Exon 1 encodes NTD, which is the least conserved of the four AR domains, allowing AR for differential recruitment of co-regulators for androgen specific transactivation [9]. DBD is the region of the protein that interacts with DNA. The LBD domain represents the site of interaction with androgen hormone, binding of each leads to a migration of the AR to the cellular nucleus and activation of the target genes [10].

Mutations in AR exons encoding different domains of AR result either in CAIS of PAIS. Although mutations have been reported in every exon of AR gene, most of them are localized in LBD [5]. In our case, the mutation was located in NTD and constituted a single nucleotide substitution c.238C>T. This substitution resulted in a conversion of CAA codon encoding Gln80 into TAA stop codon and termination of further transcription and translation.

To the best of our knowledge, such a mutation has not been described previously. A search in a number of databases available to us (The Androgen Receptor Gene Mutations Database (ARDB), The Allele FREquency Database (ALFRED), The UniProt Knowledgebase (UniProtKB), Database of Genomic variants, Cento MD, 1000 genomes, The Singapore Human Mutation and Polymorphism Database, Human Gene Mutation Database (HGMD) gave no result. According to Mutation Tester modulator c.238C>T mutation is a disease-causing mutation [11].

The detected mutation is situated in a poly-Glutamine stretch (p.Gln80). Mutations described in the close proximity include GCA-codon duplication (c.237_239dupGCA) leading to p.Gln80dup [12] and c.240dupA mutation resulting in frameshift mutation p.Gln80fs*83 with formation of a new stop codon in 83 position [13]. A case with the description of a mutation in poly-Glutamine stretch causing an exchange of a glutamine to a termination codon (p.Gln60) has also been previously described as disease-causing for AIS [14].

The phenotypic expression of the mutation in hemizugous state in proband (III-1) and her sister (III-3) included female external genitalia and normal breast development, short blind-ended vagina and no uterus. The mother (II-2) was a heterozygous carrier of c.238C>T mutation and was characterized by late menarche.

It should also be noted that whole exome sequencing revealed an incidental finding in *FBN1* gene of the proband. The single nucleotide heterozygous substitution c.2956G>A representing a missense mutation (p.Ala986Thr) was observed. This mutation was considered as a variant of uncertain clinical significance of Marfan syndrome (OMIM no. 154700), since no eventual symptoms of Marfan syndrome were observed.

Since the proband was referred to the clinic for infertility treatment, oocyte donation with surrogate mother was considered as the optimal reproductive solution for the couple.

CONCLUSIONS

In summary, we describe a novel nonsense mutation c.238C>T in exon 1 of AR gene in a phenotypically female individual with 46,XY karyotype and phenotype corresponding to CAIS. This case report enriches the AR gene mutation database and provides further evidence for the correlation between AR mutations and AIS.

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