Case Report

Novel Androgen Receptor Gene Variant Containing a Premature Termination Codon in a Patient with Androgen Insensitivity Syndrome

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ABSTRACT

Background: Androgen receptor (AR) mutations, which cause androgen insensitivity syndrome, impair the actions of 5α-dihydrotestosterone and testosterone, resulting in abnormal sexual development. In most cases, genetic aberrations of the AR are caused by substitutions, but also can result from mutations in splicing regions and deletions in the *AR* gene.
Case: Our present report describes a female patient with 46,XY karyotype and normal female external genitalia. A novel de novo c.1669_1670insC insertion in the *AR* gene caused androgen insensitivity syndrome.
Summary and Conclusion: This report provides a detailed clinical characterization of the patient and a possible pathogenic mechanism leading to androgen insensitivity syndrome and should be particularly useful in genetic counseling.
Key Words: Androgen insensitivity syndrome, *AR* gene, Disorder of sexual development, 46,XY female

Introduction

Differences of sex development (DSD) are defined as congenital conditions with atypical development of chromosomal, gonadal, or anatomic sex.[1] DSD are categorized into 3 categories, 46,XY DSD, 46,XX DSD, and sex chromosome DSD.[2] Sex chromosome DSD includes conditions such as Turner (45,X) or Klinefelter (47,XXY) syndrome. The genetic causes of DSD are heterogeneous, with more than 60 genes implicated.[3] These defined variants are responsible for determining gonadal development in utero or disruption of sex differentiation because of defects in hormone production or sensitivity.[4] Female individuals with a 46,XY karyotype can be subdivided into 2 major subdivisions. The first group consists of individuals with abnormal testicular development, such as complete or pure gonadal dysgenesis. This includes chronic granulomatous disease and Swyer syndrome. Chronic granulomatous disease is caused by the absence of the sex-determining region on the Y chromosome or mutations in genes of this region, such as *SRY-Box 9*, *Doublesex and mab-3 related transcription factor 1*, or *Wnt family member 4*, and *orphan nuclear receptor Dax-1*.[5] These mutations result in a complete failure of testis development and a lack of gonadal hormone secretion starting in utero and continuing after. Absence of anti-Müllerian hormone in utero results in Müllerian tract persistence and development.[2] The second group consists of individuals with defective androgen action, including androgen insensitivity syndrome (AIS).[5] AIS is determined by mutations in the androgen receptor (*AR*) gene, which responds to circulating testosterone during development and maintains the Wölffian duct structures. *AR* gene mutations cause the ARs to become resistant to the binding of testosterone, and thus, development and maintenance of some Müllerian duct structures are permitted. Also, fetal ovaries do not secrete a significant amount of androgens, causing the Wölffian ducts to become atrophic.[6] AIS can be subdivided into 3 phenotypes according to the degree of external genital defects. Patients with complete AIS (CAIS) have typical female external genitalia, those with partial AIS (PAIS) have predominantly male or ambiguous external genitalia, and those with mild AIS have typical male external genitalia.[7] Herein we report a novel de novo pathogenic variant, c.1669_1670insC, in the *AR* gene in a female patient with 46,XY karyotype and CAIS. The patient's parents provided written informed consent to publish all clinical information.

Case

The 17-year-old patient is the first born female child of healthy nonconsanguineous parents and her family history was unremarkable. At the ages of 3 and 12 years, correction surgery was performed for an inguinal hernia. Her psychomotor development was normal with no educational problems. At age 16 years, the patient consulted a gynecologist about concerns relating to primary amenorrhea. Physical examination revealed normal breast development,

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normal female external genitalia, an unformed hymen, and a short (4 cm in length) vagina. Sexual development status was B4 P1, determined using the Tanner scale of maturation. Diagnostic ultrasound imaging showed no uterus and an ovoid mass 2.55 × 1.68 cm in size at projection of the right ovary. Hormone analysis revealed low estradiol level (39 pg/mL), normal follicle-stimulating hormone (6.71 mIU/mL), elevated concentrations of luteinizing hormone (24.3 mIU/mL), and testosterone (12.76 ng/mL), and a decreased level of progesterone (0.8 mIU/mL). Magnetic resonance imaging of the pelvic organs showed complete uterine aplasia, nonfunctional ovarian or testicle structures at the projection of the right ovary, and in the left part of the pelvis close to the abdominal wall (Fig. 1A and B). During her last examination at age 17 years 3 months, her weight was 64 kg (50-75th percentile), height was 164 cm (50-75th percentile), and head circumference was 57 cm (75th percentile). She had hypopigmented spots on her forehead and waist. Cytogenetic analysis identified the 46,XY karyotype and Sanger sequencing of the *AR* gene revealed a de novo variant, c.1669_1670insC (Fig. 2), confirming the diagnosis of AIS. A gonadectomy was performed at age 17 years 9 months. Histopathological examination using light microscopy revealed testicular tissues containing only Sertoli cells and fallopian tube type tissue (Fig. 1C and D).

DNA extracted using the phenol-chloroform method from peripheral blood lymphocytes was used for polymerase chain reaction; conditions were as follows: 5 minutes at 95°C followed by 30 cycles of 1 minute at 95°C, 1 minute at 59°C, and 1 minute at 72°C with a final extension for 7 minutes at 72°C. A 2-μL aliquot of each product was loaded onto a 1.5% agarose gel and visualized using ethidium bromide (Sigma-Aldrich, St Louis, MO) to confirm the presence of an appropriately sized product. Direct sequencing of the coding region of the *AR* gene (8 exons) along with the surrounding area gene amplicons from the amplification of the *AR* gene from the patient was performed using an ABI PRISM 3130xl Genetic Analyzer (Thermo Fisher Scientific, Inc, Pittsburgh, PA) using the BigDyeTerminator v3.1 Cycle Sequencing kit (Thermo Fisher Scientific, Inc). Pathogenicity of the identified de novo variant was assessed using the prediction models SIFT and Mutation Taster.[8,9] Models of the wild type and mutated AR protein structures were formed using SWISS-MODEL software.[10]

Summary and Conclusions

AIS (OMIM300068), is the most common cause of the 46,XY DSD. Diagnosis of AIS is established in an individual with a 46,XY karyotype who has undermasculinization of the external genitalia, impaired spermatogenesis with otherwise normal testes, and absent or rudimentary Müllerian structures, such as a uterus, fallopian tubes, and upper vagina. Variable phenotypic expression divides the classification of AIS into CAIS and PAIS forms, as well as a rare group of phenotypically normal men with azoospermia.[11,12] The phenotype of PAIS varies depending on degree of end organ unresponsiveness to androgens, resulting in variable degrees of Wölffian duct development to external genital ambiguity. The diagnosis of CAIS was suspected at age 16 years in our patient, despite 2 hernia repairs in childhood. Lack of comprehensive investigation of a girl with an inguinal hernia could be the cause of failure to

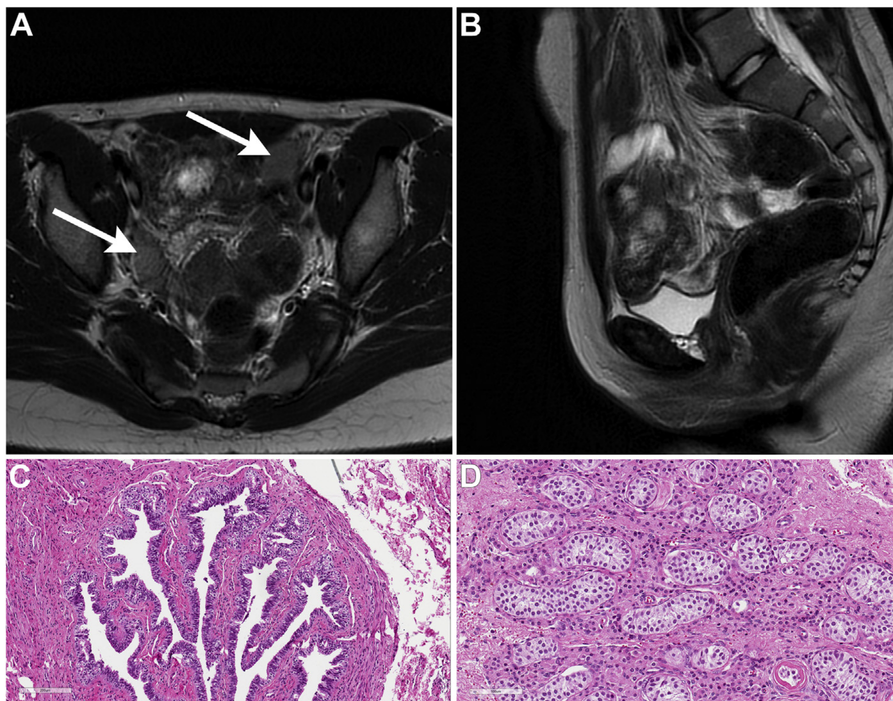

Fig. 1. Magnetic resonance image of the pelvic organs showed nonfunctional ovarian or testicle structures at the projection of the right ovary and in the left part of the pelvis close to the abdominal wall (A) and complete uterine aplasia (B). Histopathological examination using light microscopy revealed testicular tissues with slight and focal decrease of seminiferous tubules without lumens, containing only Sertoli cells, and abundant Leydig cells in interstitium (C), and fallopian tube type tissue (D).

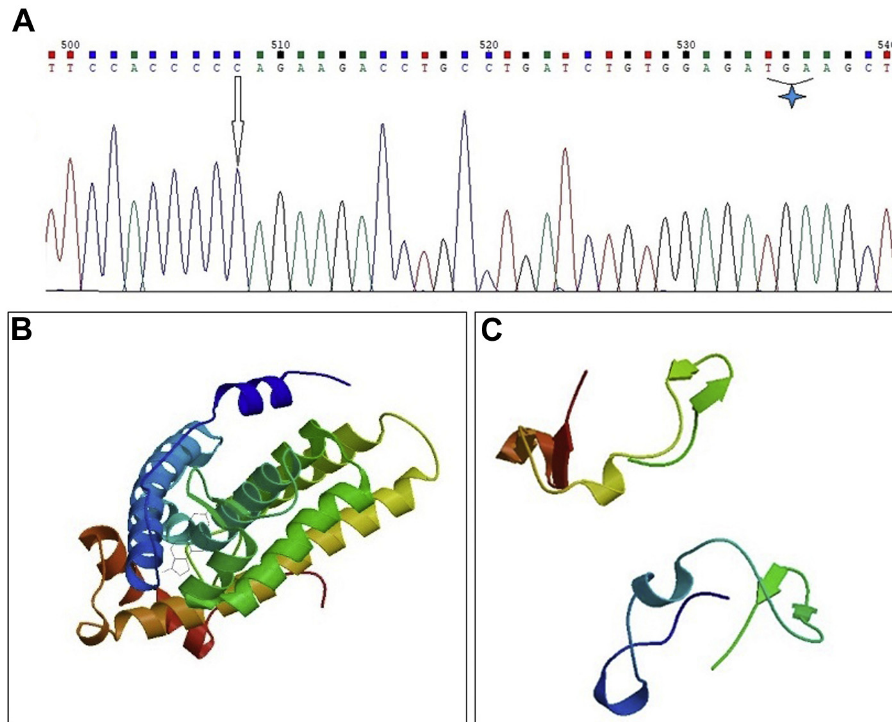

Fig. 2. An electropherogram shows a de novo insertion in the androgen receptor (*AR*) gene (C)1669_1670insC (marked with an arrow); stop codon is indicated with a blue star (A). Protein model of wild type AR structure (B). The structure of AR protein containing the p.Q557Pfs*10 frameshift insertion found in the patient analyzed in this study (C).

make an early diagnosis. As in other individuals with CAIS, our patient presented with a female phenotype and normal female gender identity. At puberty, these patients have normal breast development and pubertal growth occurs because these XY women have the necessary level of estradiol derived from aromatization of testosterone. Female internal genitalia are absent, and hence, CAIS commonly presents as primary amenorrhea in a female adolescent.[11] Pubic hair is androgen-dependent and therefore reduced.[12] Women with CAIS are taller than average, illustrating the effect of the Y chromosome, but not as tall as the average male person.[6]

AIS is an X-linked inherited disorder caused by pathogenic variants in the *AR* gene.[12] This gene is a member of the ligand-dependent transcription factor superfamily of nuclear receptors.[13] The protein functions as a steroid hormone-activated transcription factor and contains 3 major functional domains (Fig. 3).[14] There is the N-terminal domain, the DNA-binding domain, and the ligand-binding domain, which is involved in binding to androgens and relevant coactivator proteins. In more than 95% of patients with CAIS an *AR* mutation is identified.[15] Therefore to date, more than 900 different *AR* pathogenic variants have been reported.[13,16] The most common type of *AR* gene pathogenic variants result from base substitutions.[16] However, according to the *AR* mutation database (http://androgendb.mcgill.ca/), more than 118 *AR* gene pathogenic variants causing premature terminations have also been identified.

According to in silico analysis of pathogenicity, SIFT and Mutation Taster algorithms predicted our identified de novo c.1669_1670insC mutation as a determinant of CAIS due to a premature stop codon in exon 2 (p.Q557Pfs*10). Exon 2 and

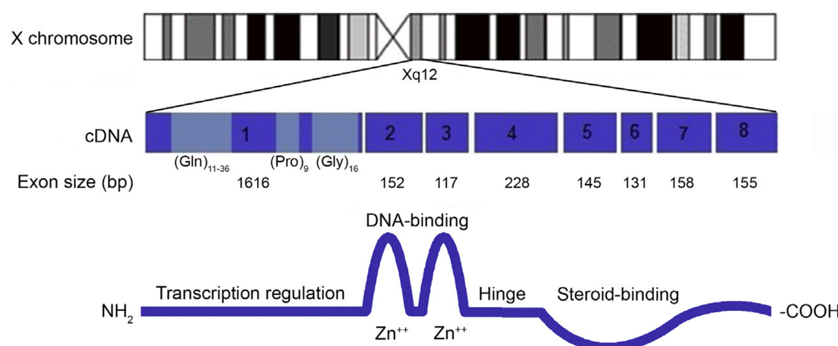

Fig. 3. Schematic representation of the androgen receptor (*AR*) gene and protein. *AR* located on the long arm of X chromosome (q11-12) and consists of 8 exons. The AR consists of 4 structurally and functionally distinct domains, an N-terminal domain, a highly conserved DNA binding domain, and a ligand binding domain. A short amino acid sequence called the "hinge region" separates the ligand binding domain from the DNA binding domain.

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exon 3 encode 2 zinc fingers in the AR DNA binding domain, which is responsible for binding to the androgen-responsive element sites (Fig. 3).[17] Structural analysis of the pathogenic variant detected in our patient showed that the mutation causes a truncated nonfunctional AR protein split into 2 monomers instead of 1 functional protein with an active center (Fig. 2B and C). Therefore, in summary, our patient with de novo pathogenic variant c.1669_1670insC in the *AR* gene provides additional molecular and clinical information about AIS. Further reports of novel and recurrent pathogenic variants in the *AR* gene might aid in the identification of mutational hot spots, which might be useful in genetic counseling.

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