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CLINICAL REPORT

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Somatic mosaicism of androgen receptor gene in an androgen insensitivity syndrome patient conceived through assisted reproduction technique

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Abstract

Background: Mutations of human androgen receptor (*AR*) gene are responsible for androgen insensitivity syndrome (AIS). Variable phenotypes and androgen receptor binding activity have permitted the classification of AIS into complete (CAIS), partial (PAIS), and minimal or mild (MAIS) forms. Somatic mosaicism in AIS is a rare condition which happened when de novo mutations occur after the zygotic stage.

Methods: Clinical evaluation, hormone measurements, and molecular analysis were performed to diagnose the patient in the study.

Results: A 46, XY girl who conceived through in vitro fertilization (IVF), presented with partial virilization of external genitalia, was found to have the p.C620R in heterozygosity. The variant p.C620R of *AR* has been previously reported in a patient with completely feminized external genitalia, which was inherited from the heterozygote carrier mother. Mutation analysis of the mother of our patient revealed that the variant was de novo and presented as a somatic mosaicism which indicated an insufficient amount of wild-type AR in our patient.

Conclusion: This is the first case that AIS was caused by de novo mutation of *AR* in a 46, XY Disorder of Sexual Development (DSD) patient by the assisted reproduction technique (ART). The phenotype of partial virilization could be explained by *AR* mutation in somatic mosaicism.

KEYWORDS

AR: androgen insensitivity syndrome, assisted reproduction technique, somatic mosaicism

1 | INTRODUCTION

Androgen insensitivity syndrome (AIS) is a disorder of male sexual development caused by defect in the androgen receptor (*AR*) gene (OMIM accession number: 313,700, NC_000023.11) (Brown et al., 1988). According to phenotypic heterogeneity, AIS can be divided into complete (CAIS), partial (PAIS), and minimal or mild (MAIS) forms (Mongan, Tadokoro-Cuccaro, Bunch, & Hughes, 2015). CAIS presents as breast development but no menarche in

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an adolescent female commonly and a substantial portion of CAIS patients presented firstly by inguinal hernia, while patients with MAIS mainly present as gynecomastia and infertility in adulthood with normal male sexual development. The phenotypes of PAIS are more intricate which range from severe undervirilization presenting as nearly female external genitalia to mild undermasculinization such as micropenis, variable degrees of hypospadias, and cryptorchidism depending on the residual AR function.

Androgen receptor, located on X chromosome, encodes the protein functioning as a steroid-hormone activated transcription factor during androgenization of external genitalia (Quigley et al., 1995). Androgen binding to AR mediates the male sex differentiation, the subsequent development of secondary sex characteristics after puberty, and spermatogenesis. Mutations of *AR* hamper the combination of androgen and AR, and result in the abnormal male sexual development. To date, about 1,000 mutations of *AR* have been identified in patients with AIS and other AR-related diseases as prostate cancer and over 500 different mutations were in AIS, of which a rare proportion was detected with somatic mosaicism(Gottlieb, Beitel, Nadarajah, Paliouras, & Trifiro, 2012; Köhler et al., 2005).

Nowadays, the application of assisted reproduction technique (ART), such as intracytoplasmic sperm injection and in vitro fertilization (IVF), becomes more and more frequent and the proportion of ART children is substantial worldwide. In many developed countries, the infants born from ART account for more than 1% of the birth cohorts (Pinborg, Henningsen, Malchau, & Loft, 2013). Meanwhile, a higher incidence of congenital malformations was observed as the concerns for epigenetic changes (Manipalviratn, DeCherney, & Segars, 2009) or the effect of parental characteristics (Pinborg et al., 2013).

Here, we reported the first case that AIS was caused by de novo mutation of *AR* in a 46, XY Disorder of Sexual Development (46, XY DSD) patient by the ART.

2 | MATERIALS AND METHODS

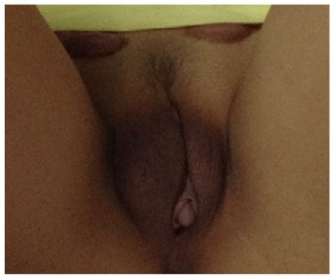
2.1 | Ethical compliance

This study was approved by the Ethics Committee of our institute. Informed consent was obtained from the participants prior to DNA extraction and molecular research.

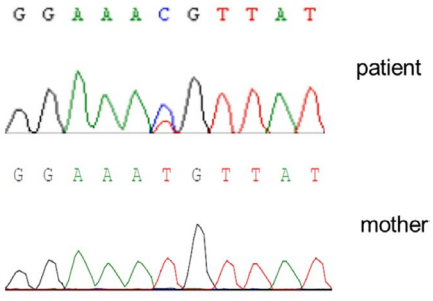
A 14-year-old girl was admitted to our hospital for abnormal external genitalia (clitoromegaly and hypertrophic labia majora) (Figure 1a). Physical examination showed an enlarged clitoris (2 cm in length) and pigmentation in the hypertrophic labia majora. She was born in nonconsanguineous family via Cesarian section. The patient was conceived through IVF and her mother had a history of progesterone usage during pregnancy. At birth, the patient manifested ambiguous external genitalia and was raised as a girl. At the age of 1, she was presented with bilateral inguinal masses, which were proved to be testis by ultrasound. And, the karyotype of 46, XY was confirmed. Computed tomography (CT) scan of pelvic showed normal adrenal, no uterus, and ovarian when she was 6 years old. She underwent bilateral orchiopexy at the age of 8.

According to the phenotypes of this patient, 46, XY DSD, such as AIS, 5α-reductase type 2 (SRD5A2) deficiency, and 17β-hydroxysteroid dehydrogenase 3 (HSD17B3) deficiency etc, should be suspected. Hormone measurements and molecular diagnosis were performed. The levels of FSH, LH, E2, P,

(a)



(c)



(b)

Laboratory index	Results in patient	Normal values
FSH (mIU/mL)	3.640	0.95-11.95
LH_2 (mIU/mL)	4.220	0.57-12.07
E2 (pg/mL)	17.000	11-44
P (ng/mL)	0.100	0-0.2
T (ng/mL) (B-A)	2.680-5.340	1.42-9.23(male)
FT (pg/mL)	11.840	8.69-54.69(male)
AD (ng/ml) (B-A)	1.3-2.01	0.30-2.63
DHT (pg/ml) (B-A)	91.19-207.38	17.9-579

FIGURE 1 (a) The phenotypes of external genitalia in the patient. (b) Laboratory hormone values for the proband. B, before the hCG stimulate; A, after the hCG stimulate; FSH, follicle-stimulating hormone; LH_2, luteinizing hormone; E2, estradiol; P, progesterone; T, testosterone; FT, free testosterone; AD, androstenedione; DHT, dihydrotestosterone. (c) Direct sequencing of *AR* in patient and her mother

and T were measured by ARCHITECT i2000SR Immunoassay Analyzer (Abbott Laboratories). Serum dihydrotestosterone (DHT) concentrations were assayed using a radioimmunoassay kit (Beckman Coulter). Androstenedione (AD) was assayed by ADVIA Centaur CP (Siemens Healthineers). The patient was subjected to molecular diagnosis by Sanger sequencing using the genomic DNA extracted from peripheral blood lymphocytes.

3 | RESULTS

Serum hormone test in this patient showed that the level of FSH and LH was normal, while her basal serum testosterone, AD and DHT, levels were detected in the normal range of male and significantly elevated after human chorionic gonadotropin (hCG) stimulation (Figure 1b).

The patient was subjected to molecular diagnosis by Sanger sequencing using the genomic DNA extracted from peripheral blood lymphocytes. As shown in Figure 1c, a variant p.C620R in *AR* was identified, which was previously reported in a patient with CAIS and inherited from the heterozygote carrier mother (Audi et al., 2010). Mutation analysis of her parent revealed that the variant was de novo.

4 | DISCUSSION

Among 70% of patients with AIS, the *AR* variants were germline mutations and transmitted in an X-linked recessive pattern from carrier mothers. In the remaining 30% cases, the mutations appeared de novo and a rare proportion was presented as somatic mosaicism (Köhler et al., 2005). As previously reported, somatic mosaicism of *AR* was suspected of the mutations occurring after the zygotic stage (Holterhus, Bruggenwirth, Brinkmann, & Hiort, 2001), resulting in a great significant impact on patients with AIS, especially in gender assignment, genetic counseling, and selection of treatment strategy, because of the possibility of further virilization after birth (Köhler et al., 2005). The partial virilization presentation of our patient was different from previously reported phenotype of the patient with the same p.C620R variant (Audi et al., 2010), which might be caused by expression of the residual wild-type AR due to the somatic mosaic. In addition, other previously reported cases with PAIS with somatic mosaicism presented different clinical manifestations (Batista et al., 2018). In other words, the presence of AR mutation in somatic mosaicism apparently changes the phenotype. Therefore, clinical trials with testosterone supplement to assess the possibility of virilization during puberty might facilitate the crucial decision of treatment, including the sex assignment and the optimal time for operation (Holterhus, 1997). For patients with CAIS, it was suggested that gonadectomy may be deferred until postpubertal stage for better breast development owing to the higher serum estrogen converted from increased testosterone through aromatization (Dohnert, Wunsch, & Hiort, 2017). While for female patients with PAIS who refused gender reassignment, early gonadectomy was recommended to prevent the virilization in puberty. But there is no reported AIS case caused by somatic mosaicism of *AR* mutation and traced the history of parents' ART procedure.

Assisted reproduction technique, such as IVF, might influence DNA stability, leading to the disruption of DNA or chromatin modification. Previous studies revealed an increased incidence of certain imprinting disorders after ART, such as retinoblastoma, Angelman syndrome, and Beckwith–Wiedemann syndrome et al (Manipalviratn et al., 2009). In addition, several research groups had reported an increased risk of karyotypic abnormalities or diploid-aneuploid mosaicism (Aboulghar et al., 2001; Bielanska, Jin, Bernier, Tan, & Ao, 2005; Bonduelle, 2002) and dynamic mutation of trinucleotide repeats in ART-derived offspring (Zheng et al., 2013). To date, monogenic missense mutation in somatic mosaicism has almost not been reported in IVF babies, especially in the condition of AIS. To our best knowledge, it is for the first time that de novo mutation in *AR* was detected in a patient with AIS conceived by IVF.

In conclusion, we reported the first AIS case whose *AR* mutation might be induced by IVF. Early gonadectomy might be helpful for the PAIS patient rearing as females to prevent the virilization during puberty. It should be noted that regular preimplantation genetic screening technique, which performed in couples at increased risk for chromosome abnormalities or specific genetic diseases, could not avoid other rare genetic disorders.

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CONFLICT OF INTEREST

The authors have no conflict of interest.

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