Letter to the Editor

Preimplantation and prenatal genetic diagnosis for androgen insensitivity syndrome resulting from a novel deletion/insertion mutation

To the Editor:

Androgen insensitivity syndrome (AIS, testicular feminization; OMIM #300068) is an X-linked recessive genetic disorder caused by mutations in the androgen receptor gene (AR; OMIM# 313700) (1). The AR gene is a single-copy gene that is located on Xq11–12 and consists of eight exons. To date, more than 800 mutations in the AR gene have been found to cause AIS (http://androgendb.mcgill.ca/AR23C.pdf). Here, we report a case of AIS with a novel AR mutation and preimplantation and prenatal genetic diagnosis using AR mutational analysis.

A 39-year-old woman who was suspected to be a carrier of an AR gene mutation was referred to our hospital. The proband is her 11-year-old child who is phenotypically female with female external genitalia and presented to the local hospital with bilateral inguinal hernia. Chromosome analysis revealed a karyotype of 46, XY. Plasma testosterone and E₂ were 203.0 ng/dl and 24.1 pg/ml, respectively. A sonographic examination reported absence of uterus and presence of testes in the inguinal canals. On the basis of those findings, the child was diagnosed of AIS.

By analyzing all the eight exons and flanking intron regions of the AR gene using the primers listed in Table 1 (primer set 1–12), a novel deletion/insertion mutation c.933_1219del287ins77 (AGATTTATTTCTATATCTATAAAATTAGAATACATGTGGTTGTGA TAAGTATTTTTAAAGAATAGAAATAAAGG) was identified in exon 1 of the proband’s AR gene, which is a frameshift mutation resulting in an early stop codon (p.Phe312Aspfs*7). The primer set 13–14 was used to amplify the deletion/insertion sequence of exon 1. The product was 349 bp for the normal allele and the mutant polymerase chain reaction (PCR) product was a shorter band of 139 bp. The proband’s mother and maternal grandmother were heterozygous for the mutant allele, and his father, maternal grandfather and maternal uncle had the normal allele (Fig. 1a).

The couple decided to undergo preimplantation genetic diagnosis (PGD). In vitro fertilization (IVF) PGD cycle was initiated after the couples signed written informed consent. However, only four mature size follicles had developed after controlled ovarian stimulation due to advanced maternal age. The peak serum E₂ level was 203.0 ng/dl and 24.1 pg/ml, respectively. A sonographic examination reported absence of uterus and presence of testes in the inguinal canals. On the basis of those findings, the child was diagnosed of AIS.

By analyzing all the eight exons and flanking intron regions of the AR gene using the primers listed in Table 1 (primer set 1–12), a novel deletion/insertion mutation c.933_1219del287ins77 (AGATTTATTTCTATATCTATAAAATTAGAATACATGTGGTTGTGA TAAGTATTTTTAAAGAATAGAAATAAAGG) was identified in exon 1 of the proband’s AR gene, which is a frameshift mutation resulting in an early stop codon (p.Phe312Aspfs*7). The primer set 13–14 was used to amplify the deletion/insertion sequence of exon 1. The product was 349 bp for the normal allele and the mutant polymerase chain reaction (PCR) product was a shorter band of 139 bp. The proband’s mother and maternal grandmother were heterozygous for the mutant allele, and his father, maternal grandfather and maternal uncle had the normal allele (Fig. 1a).

The couple decided to undergo preimplantation genetic diagnosis (PGD). In vitro fertilization (IVF) PGD cycle was initiated after the couples signed written informed consent. However, only four mature size follicles had developed after controlled ovarian stimulation due to advanced maternal age. The peak serum E₂ level was 203.0 ng/dl (basal Follicle-stimulating hormone (FSH) = 8.97 mIU/ml, luteinizing hormone (LH) = 5.4 mIU/ml and E₂ = 126.7 pmol/l). Totally three oocytes were retrieved and two were fertilized. On day 3, one good quality embryo with eight blastomeres was biopsied. Multiplex-nested PCR was used to identify the mutation of AR gene and determine the SRY gene. Unfortunately, that embryo was identified as AR mutant male embryo (Fig. 1b). Six months later, the woman became pregnant by natural conception. Ultrasound-guided abdominal amniocentesis was performed at 20 weeks of gestation. Mutational analysis using DNA extracted from amniocytes showed that the fetus was a normal female (Fig. 1c). The diagnosis was further confirmed with postnatal umbilical cord blood examination (Fig. 1d).
AIS is associated with a wide variety of molecular defects in \( AR \) gene (1, 2). \( AR \) mutations are mostly caused by single-base substitutions, less frequently by deletions or insertions. Deletion/insertion mutation of \( AR \) gene is exceptionally rare (1). To the best of our knowledge, there has been only one report of familial AIS attributed to a 7-base deletion with 11-base insertion in exon 5 of \( AR \) gene (3).

According to European Society of Human Reproduction and Embryology (ESHRE) data, currently more than 120 monogenic diseases can be diagnosed via PCR-PGD since its first application in early 1990s (4). It has been shown that maternal age above 38 adversely affects PGD outcome (5). The reason is that women of advanced maternal age may not have enough embryos for genetic testing, and their embryos showed a higher incidence of meiotic errors that may lead to a lower pregnancy rate (6). For these patients, ovarian function evaluation and genetic counseling are crucial.

In summary, we report on a novel large deletion/insertion mutation in the \( AR \) gene that results in AIS. This is the first report of application of PGD for AIS, although AIS is among the ESHRE list of indication for PGD of monogenic disease (4).

Acknowledgements

This study was funded by Natural Science Foundation of Zhejiang Province (Grant No. Y2090412), Special Program of Science and Technology Department of Zhejiang Province (Grant No. 2011C11033), National Basic Research Program of China (Grant No. 2012CB944901) and Natural Science Foundation (Grant No. 8170532 and 81170567).

References


Correspondence:
Fan Jin, MD
Department of Reproductive Endocrinology
Women’s Hospital
Zhejiang University School of Medicine,
Hangzhou, Zhejiang, China

Y Ye
P Cong

P Yu
M Q
F Jin

aDepartment of Reproductive Endocrinology, Reproductive Genetics Laboratory, Women’s Hospital, and
bDepartment of Biochemistry and Genetics, Zhejiang University School of Medicine, Hangzhou, Zhejiang, China

Fig. 1. (a) Agarose gel analysis of the polymerase chain reaction products obtained from genomics DNA extracted from the family members. The 349-bp band is a normal sequence in the control and carriers. The shorter 139-bp band is the deletion/insertion sequence in the patient and carriers. 1 = proband; 2 = mother; 3 = father; 4 = maternal uncle; 5 = maternal grandfather; 6 = maternal grandmother; 7 = normal. (b) PGD results. \( AR \) mutation (c.933_1219del287ins77) was tested in single blastomere and \( SRY \) gene amplification results were shown. 1, 2 = normal father; 3, 4 = mutant proband; 5 = mutant embryo; 6, 7 = heterozygous mother; 8 = normal; 9 = negative control. (c) Prenatal diagnosis results. \( AR \) mutation (c.933_1219del287ins77) was tested in amniocytes and \( SRY \) gene amplification results were shown. 1 = heterozygous mother; 2 = normal fetus; 3 = mutant proband; 4 = normal father; 5 = negative control. (d) Postnatal umbilical cord blood examination results. \( AR \) mutation (c.933_1219del287ins77) was tested in umbilical cord blood cells of the newborn and \( SRY \) gene amplification results were shown. 1 = heterozygous mother; 2 = amniocytes of the newborn; 3 = umbilical cord blood cells of the newborn; 4 = mutant proband; 5 = normal father; 6 = negative control.