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Original Article

Identification of novel single nucleotide polymorphisms in androgen receptor gene in two families with complete androgen insensitivity syndrome

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Abstract: The present study aimed to identify novel mutations in androgen receptor (AR) gene associated with complete androgen insensitivity syndrome (AIS). Genome DNA was extracted from peripheral blood of 35 suspected patients and their genetic relatives. All participants' karyotypes were analyzed. Single nucleotide polymorphisms (SNPs) in the *AR* gene (Genebank accession no.: NM_000044.2) were analyzed by sequencing. Among the patients, 3 SNPs in the *AR* gene were identified. Two out of the 3 SNPs were detected in 2 families with complete AIS. The proband of one family was a 3-year-old girl (a 46,XY karyotype) and normal intelligence. She was hospitalized for irreversible lump in the right inguinal canal. One SNP in the *AR* gene (c.2521C>A, p.R840S) was identified in the proband. The proband's mother (healthy) and her aunt IV2 (showing complete AIS) carried the same SNP in the *AR* gene. However, her father did not carry this variant. Another *AR* SNP (c.946T>V, p.Y316H) was detected in a 20-month-old boy (46,XY) and 3 other members of a second family. His mother and grandmother carried this SNP but did not present AIS. His uncle was a homozygous carrier and declared no signs of AIS. The 2 affected probands underwent surgery and pathological tests confirmed the excised tissue as the testis tissue. In conclusion, we report 2 novel mutations in AR gene (i.e., c.2521C>A and c.946T>V), which are associated with the development of complete AIS.

Keywords: Androgen insensitivity, androgen receptor, family, single nucleotide polymorphisms

Introduction

Androgen insensitivity syndrome (AIS) is one of the most common disorders of sex development (DSD) and has an incidence of 1/99000 to 1/13000 male neonates [1]. Although AIS patients have male (XY) chromosomes and a normal level of androgen, mutations in the androgen receptor (AR) gene lead to partial or complete loss of response to androgen [2, 3]. Accordingly, AIS patients commonly display a feminine appearance including female breast development [4].

The gene *AR* is located on q11-12 of X chromosome and contains 8 exons. To date, nearly 800 different variants of *AR* have been identified [5]. Exon 1 encodes the N-terminal transcription promoting domain, exons 2 and 3 the DNA binding domain (DBD), and exons 4-8 the ligand binding domain (LBD) [6, 7]. Pathogenic variants result in compromised AR function, e.g. loss of binding activity with androgen or transcription factor activity [8]. Complete AIS is associated with typical female external genitalia [9]. The incidence of complete AIS is about 1/20400 to 1/99100 male neonates [10]. Although the risk of testicular cancer can be eliminated by gonadectomy, the necessity of this surgery is still on debate [2]. Previous studies have estimated that >80% of complete AIS patients carry *AR* gene mutations, while only 16% of partial AIS patients carry *AR* mutations [12]. To provide more insight into the pathogenesis of AIS, in the present study, we sought to identify novel *AR* mutations associated with complete AIS.

Table 1. PCR primers

Primer ID	Primer sequence (5'-3')	Product length (bp)
AR-1F	AATCAGAGGTTGGGGAAGAGG	530
AR-1R	GACACTGGGCCATATGAGGAT	
AR-2F	GGGTCAAGTCTGTGGTCAGAA	281
AR-2R	GGCTCTATCAGGCTGTTCTCC	
AR-3F	GGGATGGCAATCAGAGACAT	312
AR-3R	AGGAGCTGGCTTTTCCCTAA	
AR-4F	TAGCTCAACCCGTCAGTACCC	270
AR-4R	AAGCTTCACTGTCACCCCATC	
AR-5F	TTGCATTGTGTGTTTTTGACC	367
AR-5R	TGATCCCCCTTATCTCATGCT	
AR-6F	CCCGAAGAAAGAGACTCTGG	263
AR-6R	CCTTGGAAGCATCAAAGAAGA	
AR-7F	TGCAGGTTAATGCTGAAGACC	298
AR-7R	GGGCCCTGAAAGGTTAGTGTC	
AR-8F	CCAACCCACTGTGTATTGCAG	319
AR-8R	GGCTGCGTAATATTAGGTCACAG	
AR-9F	AAAGGGCTAGAAGGCGAGAG	542
AR-9R	CACACGGTCCATACAACTGG	
AR-10F	AGACCTACCGAGGAGCTTTCC	633
AR-10R	TGTCAGAAATGGTCGAAGTGC	
AR-11F	AGCAGGAAGCAGTATCCGAAG	426
AR-11R	GAGACAGGGTAGACGGCAGTT	
AR-12F	AAAGGGCTAGAAGGCGAGAG	400
AR-12R	CACACGGTCCATACAACTGG	
AR-13F	CTCGCATCAAGCTGGAGAAC	531
AR-13R	GATACCCCAGAACACAGAGTGA	

Materials and methods

Patients

A total of 35 suspected patients under the age of 14 years were enrolled in this study. All of them presented micropenis. Written informed consent was obtained from each patient. This study was approved by the ethics committee of our hospital. Patient characteristics were retrieved from medical records.

Hormone analysis

Peripheral blood and urine samples were collected from the patients. The concentrations of sex hormones were measured by chemiluminostence assays.

Karyotype analysis

The lymphocytes in peripheral blood were isolated and subjected to karyotype analysis. After culturing with 0.2 g/mL of colchicin for 1 h, lymphocytes were observed under a microscope and analyzed by cytovision software.

Genetic analysis

For gene analysis, genomic DNA was extracted from peripheral blood using Qiagen DNeasy Blood & Tissue kit (Qiagen, Hamburg, Germany). The *AR* gene (*NM_000044.2*) fragments of interest were amplified by PCR using the specific primers (**Table 1**). PCR products were purified with the QIAquick PCR Purification Kit (Qiagen) and then subjected to DNA sequencing.

Results

Screening for novel mutations in AR gene

In the present study, 35 patients with micropenis were enrolled for genetic tests. All patients had a 46,XY karyotype. Among the 20 patients with coexisted hypospadia, 3 SNPs in the *AR* gene were identified. Most interestingly, 2 out of the 3 SNPs, which have not been reported in the literature, were detected in 2 families with complete AIS. In contrast, the 15 patients without hypospadia displayed no mutations in the *AR* gene.

Clinical and laboratory findings in family 1 with complete AIS

The proband was a 3-year-old girl with 46,XY, XY karyotype and normal intelligence. She was hospitalized for irreversible lump in the right inguinal canal. On physical examination, the vulva appeared normal (**Figure 1A**). Each side of the labium majus contained one touchable testis (1 × 0.9 cm). She had labia minora, but no vagina or scrotum. Type-B ultrasonography revealed absence of the uterus and ovary. The results of sex hormone examination were: follicle stimulating hormone (FSH): 1.61 mIU/mL, luteinizing hormone (LH): 0.13 mIU/mL, estradiol (E26III): 63.62 pmol/L, progesterone: 0.87 nmol/L, prolactin: 193.33 mIU/mL, and testosterone: 0.63 ng/mL. The urine level of 17-ketosteroide and 17-hydroxysteroid were normal. The proband's father and mother showed a 46,XY,XY and 46,XX karyotype, respectively, and were unrelated. The proband's mother experienced spontaneous abortion twice. Type-B ultrasonography indicated that both aborted fetus (V3 and V4) were physiologically

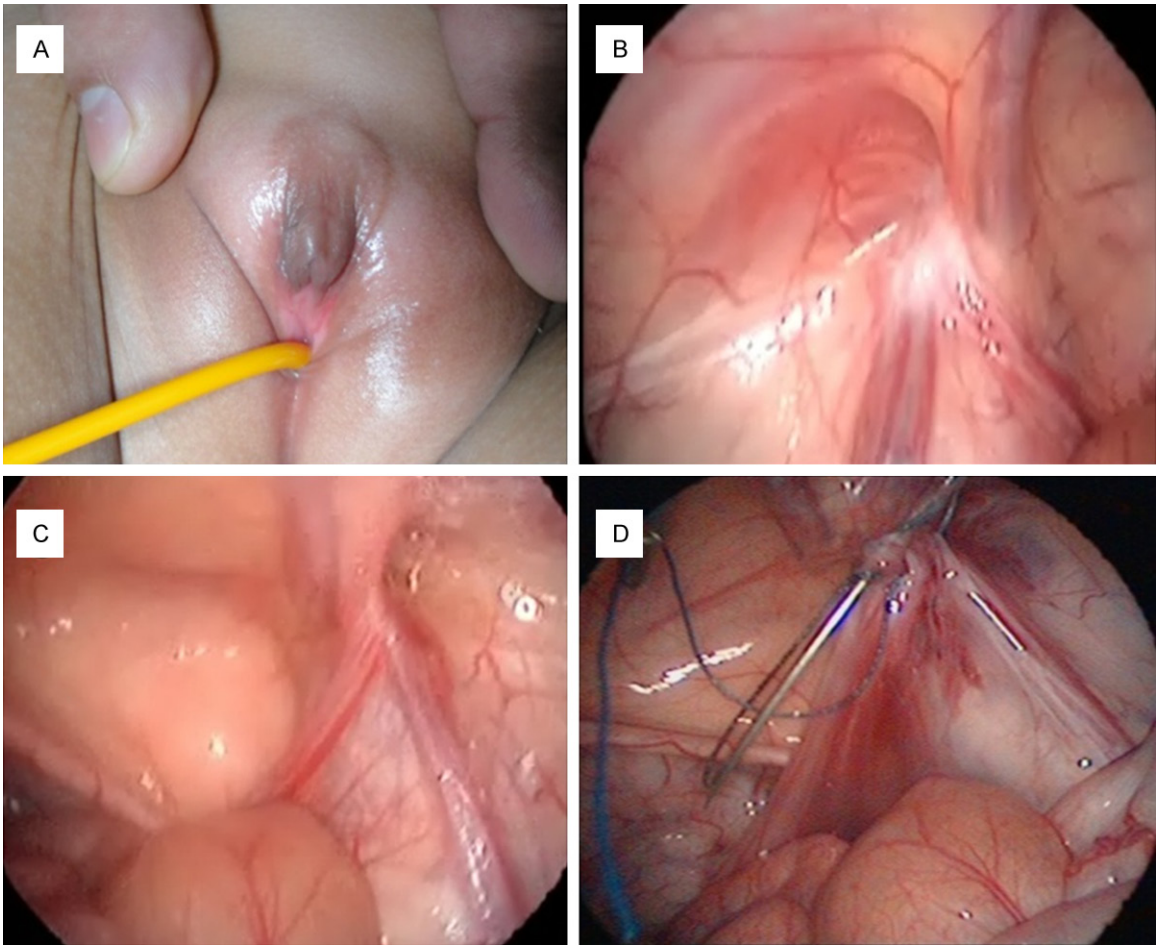

Figure 1. Extrinsic feature of the perineum of the proband in the family 1 and plastic surgery of vulva. A. The extrinsic feature of perineum. B. Left-opened processus vaginalis. C. The sac neck of right inguinal hernia. D. Laparoscopic ligation for the sac neck of right inguinal hernia.

female. The proband had a normal sister (V2), who presented a 46,XX karyotype. The proband's cousin (the son of the proband's aunt, V1), a karyotype of 46,XY, was admitted to hospital due to micropenis, enorchia and hypospadia at the age of 19 years. Neither the uterus nor the ovary was noted on Type-B ultrasound examination. The proband had 3 aunts (II3, IV1, and IV2) and 1 grandaunt, all presenting a karyotype of 46,XY. They had a vulva and short vagina, as well as breast development. The subject IV2 underwent testisectomy at the age of 25 years and the excised tissue was diagnosed as testis tissue. After surgery, estrogen-replacement treatment continues till now. No testicular cancer occurred in the subjects II3, IV1, IV2 and IV7. The proband's uncle (III6) got married and had a son without signs of AIS.

A novel mutation in the *AR* gene (c.2521C>A, p.R840S) was identified in the proband. Moreover, the proband's mother (healthy) and her aunt IV2 (showing complete AIS) carried the same SNP in the *AR* gene (**Table 2**). However, her father did not carry this variant.

The proband underwent laparoscopic ligation of the left hernia sac orifice and right hydrocele patent processus vaginalis with a 7-0 silk suture, which was threaded through an 18-gauge Vasocan hollow needle without an outer plastic sheath (**Figure 1B-D**). No recrudescence occurred until now. The proband is now living as female.

Clinical and laboratory findings in family 2 with complete AIS

The proband for a second family with complete AIS was a 20-month-old boy (46,XY) who was admitted to hospital for micropenis and bilateral abdominal cryptorchidism (**Figure 2**). Six

Table 2. The presentation of *AR* variant c.2521C>A in family 1

ID	Gender	Family relation to the proband	Karyotype	Phenotype	Genotype
F	Male	The proband's father	46,XY	No AIS	Wild type
M	Female	The proband's mother	46,XX	No AIS	Heterozygous
IV2	Female	The proband's mother's sister	46,XY	CAIS	Hemizygous

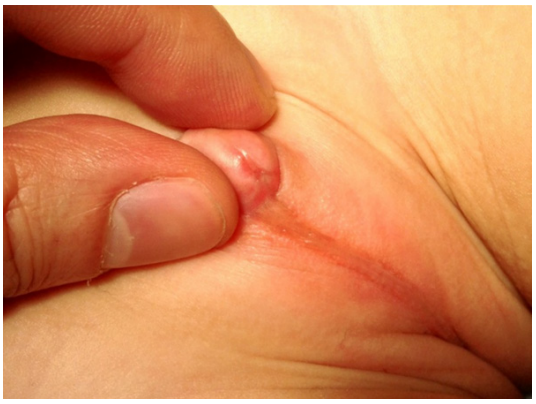

Figure 2. Extrinsic feature of the vulva of the proband in the family 2.

family members of the boy participated in the genetic analysis for *AR* gene variants. A SNP (c.946T>V, p.Y316H) was identified in the boy and 3 other members of his family (**Table 3**). His mother and grandmother carried this SNP but did not present AIS. His uncle was a homozygous carrier, but declared no signs of AIS.

After the approval by the ethics committee of our hospital, gonadectomy and plastic surgery of vulva were carried out with clitoridectomy and labia plasty separation. Pathological tests confirmed the excised tissue as the testis tissue (**Figure 3**).

Discussion

Mutations in *AR* gene play a pivotal role in the pathogenesis of AIS. A previous study demonstrated that 3 missense variants of *AR*, including c.1713C>G (p.H571E), c.1715A>G (p.Y572C), and c.2599G>A (p.V867M) were detected in 9 complete and partial AIS patients [7]. Two of them (c.1713C>G and c.1715A>G) was carried by probands' mother, whereas the c.2599G>A variant appeared to be sporadic. Another research identified a rare *AR* variant in one of the cryptic splice donor sites of exon 4 (c.2173+2T>C), which causes an in-frame deletion of 41 amino acids [8]. Another mutation in exon 4, c.2107T>C (p.S703P), is predicted to be deleterious for protein structure [13]. These studies suggest that genetic analysis of *AR* mutations may represent a non-invasive diagnostic tool for AIS [14]. Another significance for genetic analysis of *AR* mutations is decision-making for testisectomy. AIS is associated with an increased risk of developing testicular cancer in males due to gene mutations [3]. There is evidence that mutations in *AR* gene likely contribute to the development of ovarian cancer [15], prostate cancer [16], and breast cancer [17]. Therefore, some researchers suggest that an early gonadectomy may prevent from testicular carcinogenesis.

In the present study, the probands of 2 CAIS families both showed the karyotype as 46,XY and female-like extrinsic features of vulva. Suspected diagnosis for CAIS was made on physical examination. Further analysis of *AR* gene revealed 2 novel variants carried in these 2 families. The proband of family 1 carries the homozygous *AR* variant c.2521C>A, which was inherited from her mother. Another patient in this family underwent surgery to remove testicular tissues, followed by estrogen-replacement treatment. In family 2, a homozygous *AR* variant c.946T>C was identified in the proband. Both her mother and grandmother were carriers. Before diagnosis, the proband's parents hoped to correct her micropenis through an orthopedic surgery. The presence of *AR* mutations suggest that she had an AIS. Therefore, she underwent a radical therapeutic regimen with bilateral gonadectomy and a plastic surgery of vulva. The proband recovered well postoperatively. It was predicted that such mutations could cause amino acid substitutions in the AR protein. Overall, in this work, we showed 2 novel pathogenic *AR* mutations. However, it is still unclear to what extent the 2 SNPs affect the activity of AR.

It should be noted that an early gonadectomy may have adverse effects. Apart from androgen, the testes can also secrete estrogen,

Table 3. The presentation of *AR* variant c.946T>V in family 2

ID	Gender	Family relation to the proband	Karyotype	Phenotype	Genotype
MF	Male	The proband's mother's father	46,XY	No AIS	Wild type
MM	Female	The proband's mother's mother	46,XX	No AIS	Heterozygous
MB	Male	The proband's mother's brother	46,XY	No AIS[a]	Hemizygous
MS	Female	The proband's mother's sister	46,XX	No AIS	Wild type
M	Female	The proband's mother	46,XX	No AIS	Heterozygous
F	Male	The proband's father	46,XY	No AIS	Wild type

[a]MB dictated that he had no AIS phenotypes with any medical examination. Since he had no experience of marriage and procreation, no enough evidence can exclude him as an AIS patient. So the pathogenicity of the *AR* variant c.946T>V can still not be negated by this case.

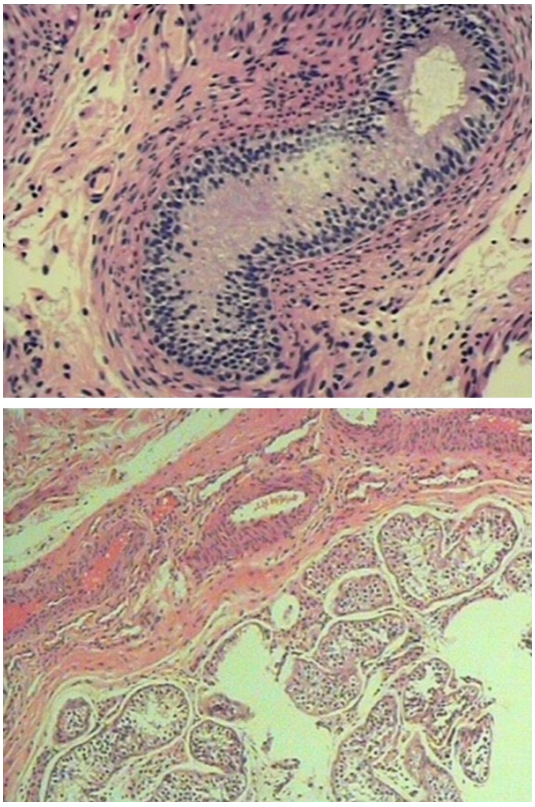

Figure 3. Histological analysis of excised tissue from the proband in the family 2. Plenty of tubuli contorti and ductus epididymidis could be observed. The excised tissue was confirmed to be testicular tissue. Top, × 200; bottom, × 40.

which is required for normal development. Thus, further study needs to address how gonadectomy impacts the patients and when hormone-replacement treatment should start after testisectomy. Additionally, a number of patients highly suspected of CAIS did not show detectable mutations in *AR* gene, which may be ascribed to the sequencing of highly susceptible regions instead of full-length gene sequencing.

In conclusion, we show 2 novel mutations in *AR* gene (i.e., c.2521C>A and c.946T>V) in 2 families, which are associated with the development of complete AIS. Genetic analysis of pathogenic SNPs in *AR* gene is valuable for final diagnosis in clinically suspected AIS patients.

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Disclosure of conflict of interest

None.

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