A novel mutation of the androgen receptor gene in familial complete androgen insensitivity syndrome

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Abstract. – OBJECTIVE: Androgen insensitivity syndrome (AIS) is characterized by androgen receptor (AR) dysfunction. Its main characteristic is a female phenotype in an individual with a 46, XY karyotype. The molecular basis of this disorder was investigated in two individuals with familial AIS.

PATIENTS AND METHODS: The diagnoses of the two individuals were confirmed using ultrasonography, hormonal analysis, operative findings, and a histopathological study. Blood samples were collected, and the AR genes were analyzed using PCR and direct sequencing.

RESULTS: Clinical and laboratory testing confirmed the two individuals' diagnoses of CAIS. DNA sequencing analysis of the genomes of these patients revealed a novel mutation of c.2107T > C in exon 4 of the AR gene, which results in a transformation of the protein p.S703P. The individuals' mother possesses a heterozygous allele, implying that she is a heterozygous carrier of the mutant gene.

CONCLUSIONS: These findings suggested that this previously undescribed novel mutation of the AR gene is the cause of CAIS in this family.

Key Words:
  Androgen insensitivity syndrome, Gene mutation, Androgen receptor.

Introduction

Androgens, including testosterone (T) and dihydrotestosterone (DHT), regulate the process of male sexual development. The effects of these hormones are mediated by an androgen receptor (AR), which binds T and DHT with high affinity.

The AR gene is located on the X chromosome (Xq11-13). The gene contains 8 exons and encodes a protein consisting of 919 amino acids[1]. The protein is comprised of four major functional domains: the N-terminal domain (NTD, residues 1-556) located on exon 1, the central DNA-binding domain (DBD, residues 557-627) located on exon 2 and 3, a C-terminal ligand-binding domain (LBD, residues 670-919) located on exon 4 to 8, and a hinge region connecting the LBD to the DBD (residues 628-669)[2].

Androgen insensitivity syndrome (AIS)[3] is a structural and functional disorder of the androgen receptor (AR) caused by a dynamic mutation in the AR gene, and is typically characterized by androgen unresponsiveness in the target tissue in male sexual development. This can completely or partially impair androgen bioactivity, leading to male pseudohermaphroditism. The estimated incidence of AIS varies from 1 in 99,000 to 1 in 13,000 male births[4]. These defects are transmitted as X-linked recessive genetic disorders, and the degree of insensitivity to androgens determines the mode of clinical presentation, which can either occur in its complete form (complete androgen insensitivity syndrome, CAIS), partial form (partial androgen insensitivity syndrome, PAIS), or mild form (mild androgen insensitivity syndrome, MAIS)[5].

To date, more than 1,000 AR gene mutations have been reported[6]. Here, we describe a novel mutation of the AR gene in two patients with familial CAIS, and further investigate the relationship between the phenotype and genotype of these individuals in an effort to determine the prognoses of the patients.

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Patients and Methods

Patients

The proband (A1) presented at Shanghai Children's Hospital, affiliated with Shanghai Jiaotong University (Shanghai, China). Two generations of the family confirmed the X-linked recessive mode of inheritance by providing evidence of three affected individuals. Of these, two family members were available for clinical evaluation and genetic analysis. Ultrasound examinations were obtained. Blood samples were collected from the proband, the proband's sibling (A2), and their mother. GnRH and HCG provocative tests were performed, and serum FSH, LH, T, DHT, and estrogen (E2) were detected in the blood samples. Informed written consent was obtained from each participating individual, and the study was approved by the institutional Ethics Committee.

To verify that our findings represent a novel mutation rather than an SNP, 50 healthy humans without blood lineage or a family history of AIS were collected in the control group.

DNA Extraction and Sequencing

Genomic DNA was extracted from the peripheral blood leukocytes of the A1 and A2 individuals, their mother, and the individuals in the control group. A DNA extraction kit (TIANamp Genomic DNA Kit, TIANGEN Biotech Co, Beijing, China) was used according to the standard protocol. All 8 exons of the AR gene were amplified via a polymerase chain reaction (PCR) using the primers listed in Table I. The PCR products were purified and detected using DNA Sanger sequencing. To rule out the possibility of steroid 5-□ reductase deficiency (SRD5A2), which may affect the patients' clinical characteristics, we also performed an SRD5A2 gene analysis of the patients and the individuals in the control group, and determined the ratio of T/DHT present after the completion of an HCG provocative test. In order to confirm conformational changes within the AR gene region in which the mutation was located, the sequenced data was analyzed using Sequencher 5.1 Demo software. The abnormal sequence was subjected to repeated sequencing and searched for in HGMD, ClinVar, and the McGill International Database (http://androgendb.mcgill.ca). We assessed the clinical risks of the known and unknown gene mutations that cause amino acid changes, using PolyPhen-2 Online software.

Results

Case Report

We studied a Chinese family with a clinical diagnosis of CAIS. The proband (A1) was a phenotypically female child 10 years and 4 months old. She was brought up as female and was of normal height, weight, and intelligence.

Table I. PCR primers used to amplify the exons of the AR gene.

Primer name	Primers	Product Length (bp)	Tm (°C)
AR-E01A_F	AGCGACTACCGCATCATCAC	834	57
AR-E01A_R	ACACCGACACTGCCTTACAC		
AR-E01B_F	CTGCCCCATCCACGTTGTCC	595	60.8
AR-E01B_R	GCTGCCTCGTCCAGTGCTCC		
AR-E01C_F	AGGCAAGAGCACTGAAGATAC	793	57
AR-E01C_R	CGCTAGATACCCCAGAACAC		
AR-E02-F	CACCCTACAACCATCATCTT	513	53.1
AR-E02-R	TGCTGAAACCAGGGAACTAG		
AR-E03-F	TGCCATACTCTGTCCACTTT	451	51.2
AR-E03-R	TGGGGATTGGTATAGATAAG		
AR-E04-F	ATTTGGGTTTAGCAGGTATT	616	51.2
AR-E04-R	CCACAGGGTTATGATGAAGA		
AR-E05-F	AGGGAAGTAGGGAGGATAAG	736	57
AR-E05-R	GGTGACTAGAAGTAGCCTTTG		
AR-E06-F	TTCCCTCTGGGCTTATTGTA	421	53.1
AR-E06-R	AATCTGATGGAGGTTGACTT		
AR-E07-F	GCTTTGTCTAATGCTCCTTC	437	53.1
AR-E07-R	TCTGTTCTCAACTCCTCCAC		
AR-E08-F	AGAGCACAAGCTGGAGAAGT	723	51.2
AR-E08-R	AAGGCATACAACACCATTCA		

Figure 1. The external genitalia of patient A1.

She has possessed female external genitalia with labia majora for over 10 years (Figure 1). Her karyotype was determined to be 46, XY, SRY (+). Hormonal analysis revealed elevated serum testosterone levels of 4.4 ng/mL, and normal serum estradiol/estrogen E2 levels below 73.4 pmol/L. Sonography revealed the absence of a uterus and the presence of intra-abdominal masses resembling testes. A histopathological study of sections taken from these masses revealed the presence of seminiferous tubules. Clinical and laboratory testing confirmed the CAIS diagnosis for this patient.

In this family, the proband's sibling (A2, age 3 years and 7 months old) was also diagnosed with CAIS (Figure 2), and a chromosomal analysis revealed a 46, XY male karyotype. As in A1, ultrasound examination failed to detect a uterus or ovaries, but revealed bilateral inguinal masses. The results of the two individuals' laboratory tests are shown in Table II.

Mutation Analysis of the AR Gene

During the analysis of all 8 exons of the AR gene, a novel mutation (c.2107T>C) was detect-

Figure 2. The external genitalia of patient A2.

ed in exon 4 of the LBD region of the AR gene. This missense mutation results in a transformation of protein p.S703P (Figure 3A), with a PolyPhen-2 score of 1.000 (Figure 4), indicating that it is a hazardous mutation. Sequencing analysis demonstrated that A2 possesses the same missense mutation. Moreover, the mother possesses a heterozygous allele in exon 4 (Figure 3B), which indicates that she is a heterozygous carrier of the mutant gene. Together with the fact that their father possesses the normal AR gene (Figure 3A), this demonstrates that the mutation originated from the maternal side.

No other mutations were found in the AR and SRD5A2 genes, and no mutations were found in the AR genes of the control group.

Table II. Laboratory tests of the two patients.

| | HCG provocative test | | | | GnRH provocative test | | | | |
| | T | | DHT | | LH | | FSH | | E2 |
	Basis	After provocation	Basis	After provocation	Basis	Peak	Basis	Peak	Basis
A1	4.4	11.8	255.11	443.5	2.42	47	6.35	15.2	< 73.4
A2	< 0.69	8.74	83.59	276.98	0.46	16.30	4.35	16.10	79.30

Abbreviation: HCG: human chorionic gonadotropin; GnRH: gonadotropin-releasing hormone; T: testosterone (nmol/L); DHT: dihydrotestosterone (pg/mL); LH: luteinizing hormone (IU/L); FSH: follicle-stimulating hormone (IU/L); E2: estrogen (pmol/L).
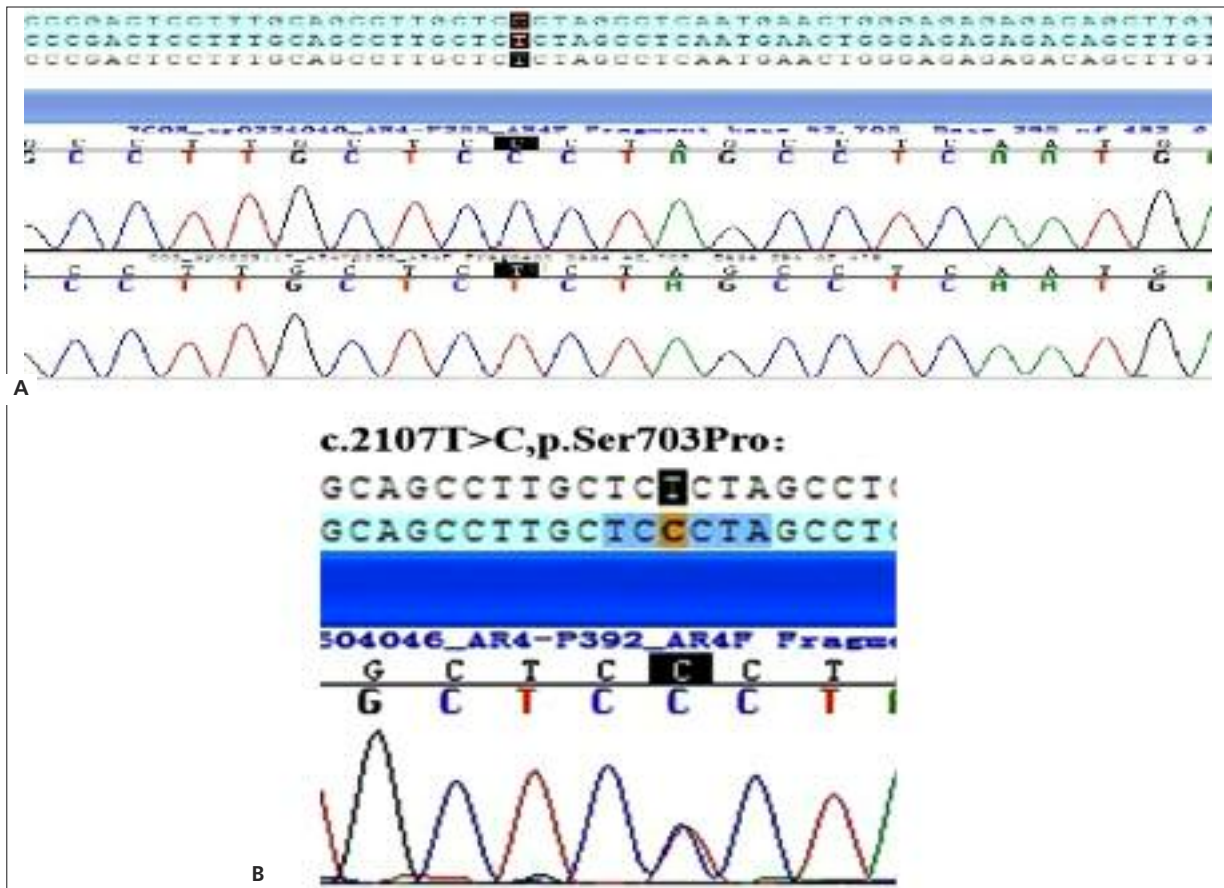
Figure 3. *A,* From top to the bottom, respectively: the homozygous mutation c.2107T>C, p.Ser703Pro of the AR gene in the proband, the proband's father, and healthy (*control*) individuals. The localization of the missense mutation is indicated by a solid black box. *B,* The proband's mother possessed the heterozygous mutation c.2107T>C in exon 4, which indicates that she is a heterozygous carrier of the mutant gene.

Discussion

Androgens and androgen receptors are involved in the development of male-specific phe-

Figure 4. TThe PolyPhen-2 score of the missense mutation is 1.000; this indicates that the mutation is hazardous.

notypes during embryogenesis, spermatogenesis, sexual behavior, and fertility[7]. As one of the nuclear receptors of this hormone superfamily, AR is located in the cytoplasm, where it can bind to an androgen, pass through the nuclear membrane, and identify the hormonally responsive target genes within corresponding tissues, which subsequently leads to AR transactivation that in turn results in the modulation of AR downstream gene expression[8]. Thus, mutations in the AR gene can result in a disordered responsiveness to androgens, which is the cause of AIS. AIS is fully defined by its clinical features. Therefore, CAIS is characterized as the presentation of a female phenotype with an XY karyotype and testes that produce androgens normally. This form of AIS features the highest degree of androgen insensitivity and feminization. Partial androgen insensitivity syndrome refers to a phenotype in which the external gen-
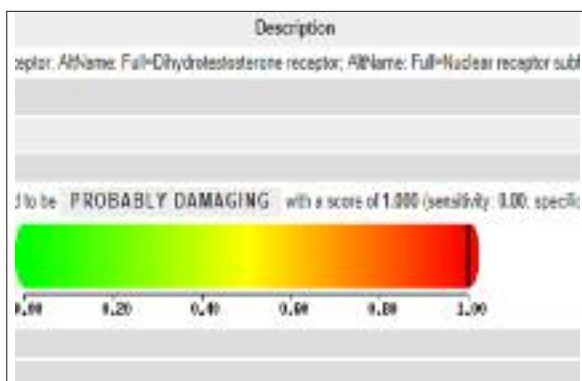
italia are masculinized to varying degrees because of partial androgen responsiveness. The clinical presentation of PAIS varies from a phenotype similar to that of females to an approximately male phenotype, including anatomy such as a "clitoridauxe", hypospadias, and micropenis. MAIS presents as the phenotype of a male with normal development, but presenting with gynaecomastia and infertility in later life[5].

AIS is recognized as one of the causes of pseudohermaphroditism, and must be differentiated from numerous diseases with similar phenotypes. The following are characteristics that can help in this differentiation: (1) The LH/hCG receptor gene mutation is characterized by an obvious increase in LH/hCG and a decrease in peripheral hormones[9]. (2) Disorders in the generation of androgens: congenital adrenal hyperplasia, including 17α-hydroxylase/17, 20-lyase deficiency (17OHD), and 3β-hydroxysteroid dehydrogenase deficiency, present mainly with adrenal insufficiency and imaging findings of adrenal hyperplasia[10]. (3) Defects in androgen action: SRD5A2 presents clinical characteristics similar to those of PAIS. Genetic analysis is the best way to differentiate between the two diseases; in addition, the ratio of T/DHT present after a HCG provocative test is needed to make an accurate diagnosis[11]. (4) Disorders of chromosomes and some transcription factors, such as the mutation of SOX9 and SF1 genes, may lead to pseudohermaphroditism in which multiple systems may be involved[12,13].

In this study, the testosterone levels of A1 and A2 were evaluated after a GnRH provocative test. The two patients exhibited elevated testosterone and dihydrotestosterone levels following the test, which is indicative of the presence of normal testes. Considering these results, we performed an HCG provocative test in order to rule out the possibility of SRD5A2, because the T/DHT ratio was below 30[11]. Moreover, their adrenal function was normal, and chromosome analysis confirmed that they possess a 46, XY karyotype. Therefore, the two patients in our study were both clinically diagnosed with CAIS. Postoperative histological examination of the masses was consistent with the diagnosis of CAIS, as no signs of Wolffian derivatives or male gonad texture were found.

Genetic analyses were performed to verify the clinical diagnosis of CAIS, and revealed abnormal banding patterns in the genes of both A1 and A2, suggesting the possible presence of a muta-

tion. Direct sequencing of this fragment revealed a novel homozygous mutation (c.2107T>C), which alters the codon UCU in exon 4 to a CCU codon. This novel mutation causes Ser703 to change to Pro in a major section of the LBD. Moreover, the PolyPhen-2 score is 1.000, which shows the mutation might be the cause of the inability of ligand to bind to AR and, thus, may be responsible for the clinical symptoms of CAIS. The same mutation was also found in the mother of the two patients. As the asymptomatic carrier, the mother was proved to possess a karyotype of 46, XX. The fact that the mutation originates from the maternal side also indicates that AIS is an X-linked disorder. This is the first report of this mutation of c.2107T>C (p.S703P). However, a case previously entered in the McGill International Database (http://androgendb.mcgill.ca) revealed a mutation located at the same position. A mutation of c.2107T>G leads to the transformation of p.S703A and a completely feminine phenotype. Three individuals in the family were affected by the mutation to varying degrees, and possessed vague external genitals. Two of the three were brought up as girls, while the other was brought up as a boy[14].

The crystallographic structure of the AR has been described. It is comprised of 12 helices and a small β-sheet arranged in a so-called helical sandwich; this is a fold typical of nuclear receptors that is visible in its three-dimensional structure[15]. When binding to the AR, androgens lie in the hydrophobic ligand-binding pocket, which induces a repositioning of helix 12 that closes the pocket after binding. Then, the activation function 2 (AF2) surface is formed, and interacts with P160 co-regulators[16]. The S703 mutation currently known to be associated with CAIS, is in an α-helix[15] and, thus, might alter the spatial structure of the hydrophobic pocket, thereby preventing androgens from binding to AR. To date, four different types of AIS mutations have been reported: single point mutations, nucleotide insertions or deletions, complete or partial gene deletions and intronic mutations in either splice donor or acceptor sites[6,17]. Most mutations are missense substitutions located in the ligand binding domain of the receptor[18]. However, in addition to the point mutations noted above, when systematically screened, regions of repetitive DNA sequences, the trinucleotide repeat of CAG and GGN within exon 1 have all been associated with a number of disorders, including androgen insensitivity[18,19]. Although their role in androgen in-

sensitivity is somewhat unclear, some reports have indicated that a mutation the CAG repeat might decrease AR activity[18].

The two individuals underwent an orchiectomy and were brought up as girls in their later lives; this was approved by the Institutional Ethics Committee. In addition to AIS, AR mutations are a major cause of gonad cancer[20]. Therefore, gonadectomy is advisable in such patients, as undescended testes undergo neoplastic changes in 30% of cases[21]. The surgical management of AIS aims for an outcome in which the genitalia have a pleasant cosmetic appearance and a degree of functionality that provides satisfactory sexual sensitivity and responsiveness, while reducing the risk of malignant gonadal tumors. In addition to surgery, patients with CAIS who have had their gonads removed require hormone replacement therapy such as androgen supplementation, and psychological counseling.

Conclusions

This report describes a novel causal mutation of familial CAIS. In addition to the detection of the affected AR protein, our findings may imply that this mutation of c.2107T>C in exon 4 of the AR gene is the cause of CAIS in this particular family. Information about this mutation will be useful for conducting prenatal diagnoses and recommending counseling appropriate for this family.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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