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Complete androgen insensitivity syndrome caused by c.1769-1G > C mutation and activation of a cryptic splice acceptor site in the androgen receptor gene

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ABSTRACT

Androgen insensitivity syndrome (AIS) is the most common cause of 46,XY disorders of sex development (46,XY DSD). This syndrome is an X-linked recessive genetic disease characterized by resistance to the actions of androgens in an individual with a male karyotype and it is caused by mutations in the androgen receptor (*AR*) gene. We evaluated two siblings with primary amenorrhea, normal secondary sex characteristics, absence of uterus and ovaries, intra-abdominal testis, and elevated testosterone levels. Sequence analysis of the *AR* gene revealed a splice acceptor site mutation in intron 2 (c.1769-1G > C). The analysis of mRNA showed that this mutation resulted in the activation of a cryptic splice acceptor site located in intron 2 and in the synthesis of an aberrant mRNA transcript with 69 nucleotides insertion between exon 2 and exon 3, leading to an insertion of 23 amino acids in the AR protein instead of generating a premature termination codon. The additional 23 amino acids insertion affects AR intracellular trafficking by impairing its translocation from the cytoplasm to the nucleus after hormone stimulation. The c.1769-1G > C mutation provides new insights into the molecular mechanism involved in splicing defects and expands the spectrum of mutations associated with the androgen insensitivity syndrome.

1. Introduction

Disorders of sex development (DSD) are defined as congenital conditions in which development of chromosomal, gonadal or anatomical sex is atypical. These disorders are classified into three major categories: sex chromosome DSD, 46,XX DSD and 46,XY DSD [1]. The 46,XY DSD are characterized by ambiguous or female external genitalia, caused by incomplete intrauterine masculinization, and the presence or absence of Mullerian structures [2]. 46,XY DSD can result from impaired production of testosterone, decreased conversion of testosterone to dihydrotestosterone or impaired peripheral action of these hormones [3].

Androgen insensitivity syndrome (AIS; OMIM#300068), the most common known cause of 46,XY DSD is defined as a condition resulting from complete or partial resistance to the biological actions of androgens in an XY man or boy with normal testis determination and production of age-appropriate androgen concentrations [4]. The clinical of manifestations AIS could range from phenotypic females (complete form; CAIS) to milder degrees of undervirilization (partial form; PAIS) or the mild form (MAIS) in which males have gynecomastia and/or infertility [5]. CAIS, which has been previously termed testicular feminization syndrome, was first described by Morris in 1953 [6]. The characteristic features of CAIS are normal female phenotype, normal breast development, absent or sparse pubic and axillary hair, an absence of the uterus and ovaries, and a short blind-ending vagina. The estimated prevalence of CAIS ranges from 1:20,400 to 1:99,100 genetic males on the basis of proven molecular diagnosis [7].

CAIS is an X-linked recessive genetic disease that is a result of mutations in the Androgen Receptor gene (*AR*; OMIM#313700). The *AR* gene is located on chromosome Xq11-12 and consists of 8 exons, encoding a protein of 920 amino acids [8]. The AR protein is an intracellular transcription factor that is a member of the nuclear receptor

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superfamily. Like all nuclear receptors, the AR protein consists of four major functional domains: a N-terminal domain (NTD) encoded by exon 1, a DNA-binding domain (DBD) encoded by exons 2 and 3, a hinge region encoded by exons 3 and 4, and a ligand-binding domain (LBD) encoded by exons 4–8 [9]. To date, more than 300 different mutations have been identified in the *AR* gene (www.mcgill/androgendb/). The most common molecular defects are missense mutations leading to amino acid substitutions in the AR protein. However, splice site mutations and complete deletions, as well as small insertion and deletions leading to premature termination of the protein are also reported [10].

In the present study, we studied one Chinese family with two siblings affected with complete androgen insensitivity syndrome and identified a splice acceptor site mutation (c.1769-1G > C) in *AR* gene from patients. Moreover, the consequence of this splice site mutation was investigated by computational analysis and functional study.

2. Materials and methods

2.1. Patients

A Chinese family with two siblings clinically suspected to have CAIS was investigated in this study. The family pedigrees and generations are illustrated in Fig. 1A. Informed written consent was obtained from all analyzed individuals, and the study was approved by our institutional review board (Ethics Committee of The First Hospital of Jilin University).

The proband (III-3), a 28-year-old girl was referred to the Urology Department for left inguinal swelling and primary amenorrhea. Physical examination showed a normal female phenotype with 169 cm in height and 56 kg in weight, normal female external genitalia and breast development, with sparse pubic hair and axillary hair. Ultrasound and pelvic computed tomography (CT) scan showed absence of uterus and ovaries with a short blind vagina; presence of a dense elongated testis-like structure sized 3.0 × 2.0 cm located at the internal opening of the left inguinal canals, which was thought to be the left gonad, and a 4.0 × 2.6 cm tubular structure behind the bladder that was considered to be a Müllerian remnant. Chromosomal analysis on the peripheral blood lymphocytes revealed a 46,XY karyotype. Serum hormone measurement showed elevated levels of testosterone (31.1 nmol/L; normal male range: 6.07–27.1 nmol/L) and luteinizing hormone (LH) (40.9 mIU/mL; normal male range: 1.24–8.62mIU/mL), and a slightly increase in follicle-stimulating hormone (FSH) level (21.7 mIU/mL; normal male range: 1.27–19.26 mIU/mL).

The second patient (III-4), younger sister of the proband was a 26-year-old girl and referred to the Urology Department due to bilateral inguinal swelling and primary amenorrhea. She was 175 cm in height and weighed 62 kg. The clinical presentations, pelvic CT scan and karyotype were the same as that of the proband, except that the testes were found in both inguinal regions. At the time of examination, serum hormone measurement showed elevated levels of testosterone (34.9 nmol/L; normal male range: 6.07–27.1 nmol/L) and luteinizing hormone (LH) (30.7 mIU/mL; normal male range: 1.24–8.62 mIU/mL).

Laparoscopy was performed to remove the gonads due to the risk of malignancy, and histological analysis of the excised gonads revealed that it consisted of testicular tissue characterized by immature seminiferous tubules with Sertoli cells and spermatocytes, but no sperm differentiation. The patients were treated with estrogen replacement therapy with oral estradiol valerate (1 mg/day).

2.2. Deoxyribonucleic acid (DNA) sequence analysis

Genomic DNA was extracted from peripheral blood leukocytes using the QIAamp DNA Blood Mini kit (Qiagen GmbH, Hilden, Germany), according to the manufacturer's instructions. All eight exons and flanking intronic regions of the *AR* gene were amplified by polymerase chain reaction (PCR) using the sets of primers described in our previously study [11].A 5-μl aliquot of each PCR was loaded on a 2% agarose gel and visualized by ethidium bromide (Sigma-Aldrich, Beijing, China) staining to confirm the presence of an appropriate sized product. Bi-directional sequencing of the PCR products was performed using BigDye® Terminator v3.1 (Applied Biosystems, Foster City, CA, USA) with the ABI 3730 automated sequencer. Sequences generated from patients were compared with the published *AR* complementary DNA (cDNA) reference sequences from the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/; GenBank accession number: NM_000044.3). The mutation was annotated according

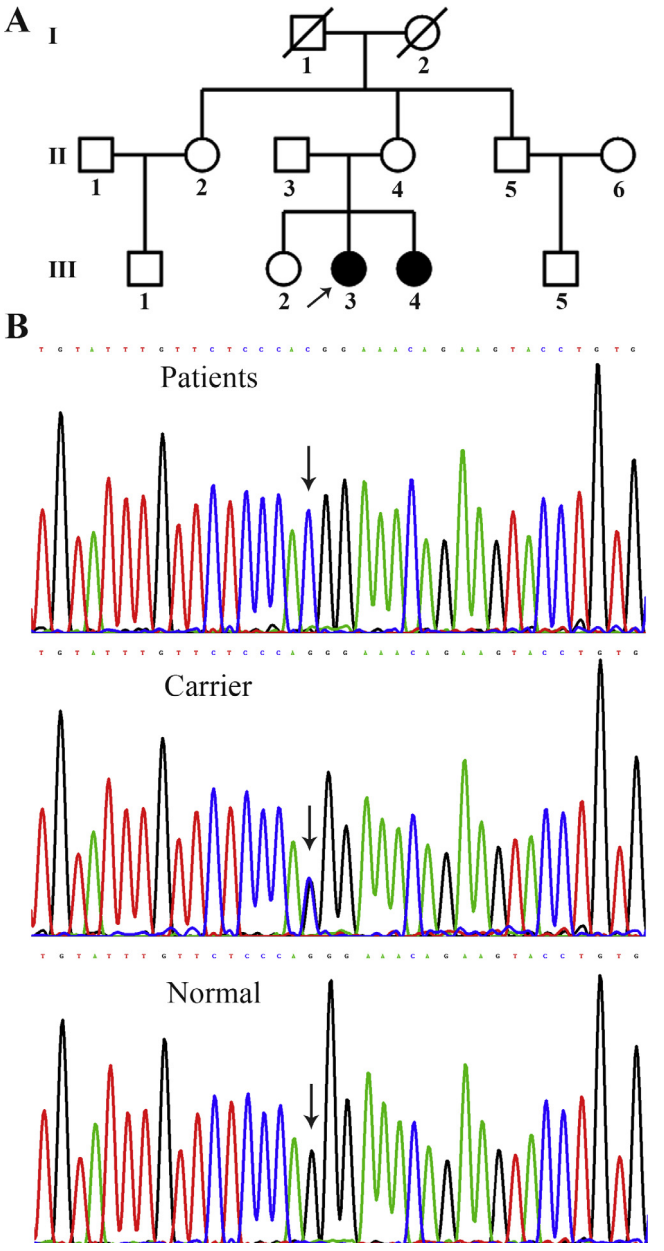

Fig. 1. Identification of a splice acceptor site mutation (c.1769-1G > C) in the *AR* gene from patients with complete androgen insensitivity syndrome. (A) Pedigree of the family with the proband (III-3) indicated by an arrow. Individuals are represented as males (squares), females (circles), unaffected (open symbols), and affected (filled symbols). Boxes or circles with an oblique line mean that the individual is deceased. (B) DNA sequence analysis of the *AR* gene in the patients and their relatives. The location of the mutation site is indicated by the black arrow. The patients harbored the homozygous G-to-C substitution at the splice acceptor site of intron 2 (top). The mother (II-3) of the proband was a heterozygous carrier at the splice site (middle). The father (II-4) of the proband was normal at this site (bottom).