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Complete androgen insensitivity syndrome caused by a novel splice donor site mutation and activation of a cryptic splice donor site in the androgen receptor gene

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ABSTRACT

The androgen insensitivity syndrome is an X-linked recessive genetic disorder characterized by resistance to the actions of androgens in an individual with a male karyotype. We evaluated a 34-year-old female with primary amenorrhea and a 46,XY karyotype, with normal secondary sex characteristics, absence of uterus and ovaries, intra-abdominal testis, and elevated testosterone levels. Sequence analysis of the androgen receptor (*AR*) gene revealed a novel splice donor site mutation in intron 4 (c.2173 + 2T > C). RT-PCR analysis showed that this mutation resulted in the activation of a cryptic splice donor site located in the second half of exon 4 and in the synthesis of a shorter mRNA transcript and an in-frame deletion of 41 amino acids. This novel mutation associated with a rare mechanism of abnormal splicing further expands the spectrum of mutations associated with the androgen insensitivity syndrome and may contribute to the understanding of the molecular mechanisms involved in splicing defects.

1. Introduction

The androgen insensitivity syndrome is a disorder of sex development resulting from complete or partial resistance to the biological actions of androgens in an individual with an XY male karyotype, with normal testis determination and production of age-appropriate androgen concentrations [1]. According to the degree of androgen insensitivity, the clinical phenotype can vary between complete, partial or mild androgen insensitivity syndromes. Complete androgen insensitivity syndrome is the classical phenotype and is characterized by a normal female appearance, development of breasts and other secondary sex characteristics at puberty, absent or scarce axillary and pubic hair, female external genitalia, shortened vagina, no uterus or ovaries, and bilateral undescended testes that produce androgens [1]. These features result from the combination of lack of masculinization due to the androgen insensitivity, unopposed effects of estrogens that result from excess aromatization of androgens, and action of antimullerian hormone produced by the testes. The diagnosis is usually made during the investigation of primary amenorrhea in adolescence, or of an inguinal hernia or labial swelling containing a testis in an apparently normal female infant [2].

Mutations in the Androgen Receptor gene (*AR*; OMIM# 313700), which encodes the androgen receptor (AR) protein, cause androgen insensitivity with X-linked recessive inheritance [3]. The *AR* gene is located on chromosome Xq12 and contains 8 exons that encode a 919 amino acid protein [4]. The AR protein belongs to the steroid receptor transcription factor family and regulates the transcription of AR-responsive genes after binding of its respective hormone [3]. Masculinization and virilization can only occur if androgens are able to act on their target tissues, and this requires the presence of a functioning AR.

Over 800 germline mutations of the *AR* gene have been reported in the literature, which completely or partially inactivate the AR protein and cause variable degrees of androgen insensitivity [5]. The most frequent of these are missense mutations, followed by nonsense mutations and small deletions, or insertions which all lead to a premature stop codon [5].

Abbreviations: AR, androgen receptor; bp, base-pairs; cDNA, complementary DNA; DNA, deoxyribonucleic acid; mRNA, messenger RNA; PCR, polymerase chain reaction; RNA, ribonucleic acid; RT-PCR, reverse transcription PCR; snRNA, small nuclear RNA.

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The aim of this study was to identify the genetic defect and its functional consequences in a patient with a clinical diagnosis of complete androgen insensitivity syndrome.

2. Material and methods

2.1. Patient

A 34-year-old Portuguese woman was evaluated due to primary amenorrhea. She had undergone normal breast development at the age of 12 years but had scarce pubic and axillary hair growth. Her past medical history was unremarkable except for the surgical removal of a right inguinal indeterminate mass in her childhood. Her family history included a younger sister, a maternal aunt, and a maternal cousin with primary amenorrhea (Fig. 1A). These family members were not available for evaluation. Physical examination revealed an apparently normal female phenotype, a height of 168 cm, a weight of 84 kg, normal adult breast development, scarce pubic and axillary hair (Tanner stage 2), normal external genitalia, but a blind-ending vagina measuring 7 cm in length with no observable cervix. A gynecological ultrasound examination revealed absence of a uterus, and presence of a dense elongated structure measuring 27 × 8 mm, which was thought to be the left gonad. Serum hormone measurements showed elevated total and free testosterone levels (total testosterone 2.9 ng/dL; reference range for females 0.1–0.9; reference range for males 2.7–11.0) (free testosterone 10.0 pg/dL; reference range for females <3.9; reference range for males 13.0–40.0). No other hormone abnormalities were detected. Chromosomal analysis of peripheral blood revealed a male 46,XY karyotype. Laparoscopy was performed to remove the intra-abdominal gonad, and histological examination revealed that it consisted of testicular tissue. She was placed under hormone replacement therapy with oral 17-beta estradiol (2 mg/day).

The patient gave written informed consent for the genetic studies and these were approved by the institutional ethics committee (Faculty of Health Sciences, University of Beira Interior, Ref: CE-FCS-2013-017).

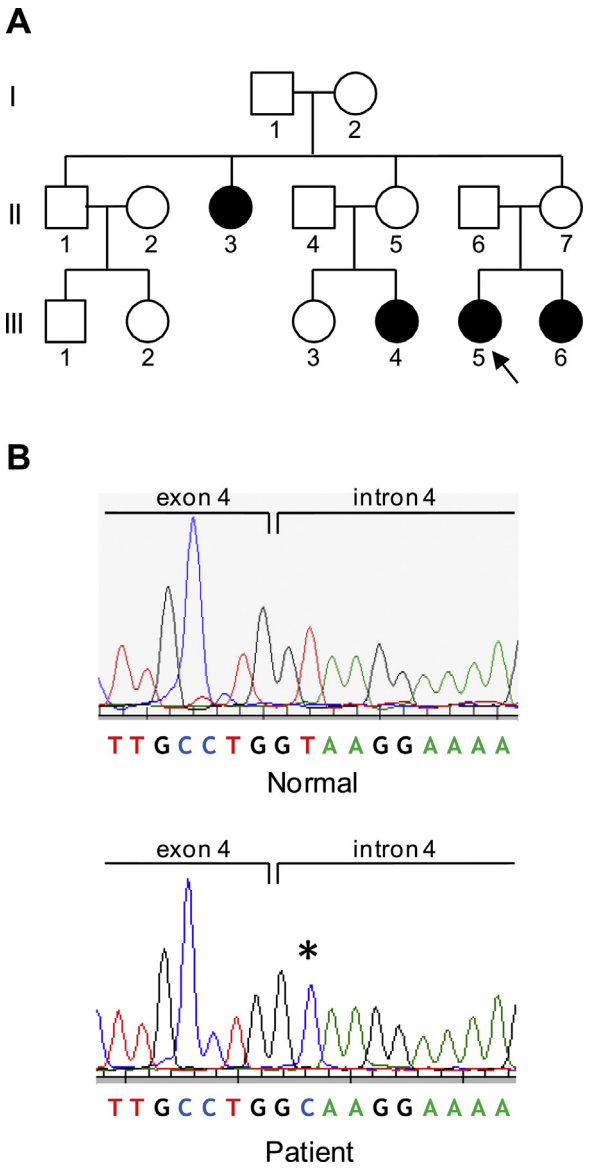

Fig. 1. Identification of a splice donor site mutation (c.2173 + 2T > C) of the *AR* gene in a patient with complete androgen insensitivity syndrome. (A) Pedigree of the family with the proband (III-5) indicated by an arrow. Individuals are represented as males (squares), females (circles), unaffected (open symbols), and affected (filled symbols). (B) DNA sequence analysis of a normal individual (above) and the patient (below). The position of the mutation (substitution of a thymine by a cytosine) is indicated by an asterisk. The patient is hemizygous for the mutation, as she has an XY karyotype and the *AR* locus is located on the X chromosome.

2.2. Deoxyribonucleic acid (DNA) sequence analysis

DNA was extracted from the peripheral blood of the patient and used with specific polymerase chain reaction (PCR) primers [6] to amplify all coding exons (exons 1 to 8) and respective exon-intron boundaries of the *AR* gene. Bi-directional sequencing of the PCR products was performed using the same PCR primers and CEQ DTCS sequencing kit (Beckman Coulter, Fullerton, CA, USA) and an automated capillary DNA sequencer (GenomeLab TM GeXP, Genetic Analysis System; Beckman Coulter, Fullerton, CA, USA), following the manufacturer's instructions. Sequence variations were described in relation to the *AR* complementary DNA (cDNA) reference sequence (GenBank accession number NM_000044.2), whereby nucleotide c.1 was the A of the ATG-translation initiation codon. The Human Splicing Finder bioinformatics tool [7] was used to predict the functional consequences of the mutation.

2.3. Reverse Transcription PCR (RT-PCR) analysis

Ribonucleic acid (RNA) was extracted from peripheral blood leukocytes using TRI Reagent (Sigma–Aldrich, St. Louis, MO, USA), and from formalin-fixed paraffin-embedded gonadal tissue using the InviTrap Spin Tissue RNA Mini Kit (Stratec Biomedical, Birkenfeld, Germany), following the manufacturer's instructions. Reverse Transcription was performed from 1 μg of RNA using oligo (dT) primers from the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, Waltham, MA, USA). The *AR* cDNA was PCR-amplified using forward primer 5′-GGGGACATGCGTTTGGA-GACTG-3′ (spanning the junction of exons 1 and 2) and reverse primer 5′-GTGCATGCGGTACTCATTGAAA-3′ (spanning the junction of exons 5 and 6). The RT-PCR products were then sequenced as described above.

3. Results

Sequence analysis of the *AR* gene identified a novel splice donor site mutation that resulted from a substitution of a thymine by a cytosine in intron 4 (c.2173 + 2T > C) (Fig. 1B). Bioinformatics analysis using the Human Splicing Finder software predicted that the mutation was likely to have a damaging effect on splicing, but no other effect was predicted. The functional consequences of this mutation were determined by the analysis of the resultant messenger RNA (mRNA) transcript. The RT-PCR analysis showed