Short communication

Transition C2718T in the AR gene, resulting in generation of a termination codon and truncated form of the androgen receptor, causes complete androgen insensitivity syndrome

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Abstract. The action of testosterone and 5α-dihydrotestosterone are essential to the development of the male phenotype. Patients with karyotype 46,XY, resistant to these hormones, exhibit a wide spectrum of phenotypes: from phenotypic female, through a range of incomplete masculinization, to under-virilized, infertile man. These disturbances are caused by mutations in the androgen receptor gene (AR). We studied a 46,XY phenotypic female with typical symptoms of Complete Androgen Insensitivity Syndrome (CAIS). Multiple temperature single-stranded conformation polymorphism (MSSCP) and sequence analysis of exon 6 of the AR gene in a patient revealed a C2718T transition causing R786X mutation in the loop between helices VII and VIII of the LBD of the androgen receptor. The R786X mutation has been described in a patient with CAIS only once and no such mutations have been described in Eastern Europe.

Key words: AR gene, C2718T transition, CAIS, R786X mutation, truncated receptor.

Introduction

The action of testosterone and 5α-dihydrotestosterone during embryogenesis is of crucial importance in the development of male phenotype (WILSON et al., 1981). Individuals with 46,XY karyotype, resistant to androgens, exhibit a wide...
spectrum of phenotypes, ranging from female (Complete Androgen Insensitivity Syndrome, CAIS), through incomplete masculinisation (Partial Androgen Insensitivity Syndrome, PAIS) to under-virilised, infertile man (Mild Androgen Insensitivity Syndrome, MAIS). These diseases are caused by mutations in the androgen receptor gene (AR) localised on the X-chromosome (Xq11-q12) (QUIGLEY et al. 1995). The protein product of the AR gene acts as a transcription factor involved in expression of genes responsible for the development of male sexual characteristics (EVANS 1988). To date over 400 point mutations in the AR gene were described. However, few of them result in a truncated form of the androgen receptor (ANDROGEN RECEPTOR GENE MUTATIONS DATABASE, 2001). On the basis of crystal structure of progesterone and glucocorticoid receptors (WILLIAMS, SIGLER 1998), it has been postulated that the ligand-binding domain (LBD) of AR consists of twelve helices. The hydrophobic ligand-binding pocket is formed by helices III, V, VII, IX, XII and the region of β-pleated sheet (WURTZ et al. 1996).

The mutation described herein results in a truncated form of the androgen receptor devoid of the major part of ligand-binding domain (LBD) including part of helix VII, helices IX, XII and the region of β-pleated sheet.

Description of the case

An 18-year-old girl (I. L.) was admitted to the Department of Paediatric Endocrinology and Diabetes, University of Medical Sciences, Poznań, because of primary amenorrhoea. First signs of puberty were present at the age of 14 years. Five years ago she was operated upon because of bilateral inguinal hernia, but no histopathological data were available.

At the time of admission her height was 167 cm and her weight was 57.0 kg. The advancement of puberty based on Tanner scale was as follows: axilarche I, pubarche II and thelarche V.

Abdominal and retroperitoneal ultrasound did not show any abnormalities, except that no ovaries and uterus were detected. A round cyst (diameter 1.5 cm) between the vascular fascicle and left lateral wall of the urinary bladder was noted. Adjacent to the cyst a round solid lesion of a similar diameter was found. In the left inguinal region a cyst of 1.8 cm in diameter was detected. No solid or cystic lesions were visible in the right inguinal region.

Gynaecological inspection: external genitalia normal with no signs of virilisation, lack of uterus. Breast development normal for this age.

Hormonal profile: LH 18.7 mIU/mL, FSH 7.2 mIU/mL, oestradiol 44 pg/mL, testosterone 30.0 nmol/L ( ), 17a-hydroxyprogesterone 2.7 ng/mL, cortisol 8:00 44 ng/ml, 20:00 100 ng/ml).

Genetic investigation revealed 46,XY karyotype

Gonadectomy was performed and post-operative histopathology showed no signs of Wolfian derivatives. On the basis of physical examination and laboratory findings complete androgen insensitivity syndrome (CAIS) type 5b was diagnosed
(Sinnecker et al. 1987). The girl was subsequently treated with oestrogen replacement therapy (2mg oestradiol/day) and genetic counselling was recommended.

**Materials and methods**

Genomic DNA was extracted (Miller, Dykes 1988) from peripheral blood lymphocytes of the patient and was used as a template to amplify exons two through eight of the AR gene by PCR (Ignacak et al. 2000) using Taq Polymerase (Fermentas, Lithuania) and specific primers (Table 1). The amplified fragments were screened for sequence-dependent differences in electrophoretic mobility by multiple temperature single-stranded conformation polymorphism analysis (MSSCP). The amplified fragments were purified on DNA GEL-out columns (AA Biotechnology, Poland) and were sequenced in both directions (Ruano, Kidd 1991) using Cy5-labelled primers and DNA sequencer ALFexpress (Pharmacia-LKB, Sweden). The study obtained the approval of a local Ethical Committee and a written consent of the patient was obtained.

**Table 1. Sequences of primers used for amplification**

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<tr>
<th>Exon number</th>
<th>Sequence</th>
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<td>230</td>
</tr>
<tr>
<td>AR2R</td>
<td>tgcatttgcaggcacttta</td>
<td></td>
</tr>
<tr>
<td>AR3F</td>
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<td>339</td>
</tr>
<tr>
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Results and discussion

The amplified fragments of all exons of the entire AR gene of the patient were analysed by MSSCP. The analysis revealed no changes in the electrophoretic mobility of the amplification products of exons 2 through 5 and exons 7 and 8 (not shown). However, the amplification product of exon 6 showed abnormal mobility, sug-

Figure 1. Multiple temperature single-stranded conformation polymorphism (A) and sequence analysis (B) of exon 6 of the AR gene in the patient with complete androgen insensitivity syndrome. P = patient; H = healthy individual. The position of the C2718T mutation is indicated by an arrow.
gesting a possible mutation (Figure 1A.). Sequence analysis revealed a C2718T transition (Figure 1B.). This mutation created a termination codon in place of Arg786 and resulted in a truncated form of the receptor, devoid of 135 carboxy-terminal amino acids, including a major part of the ligand-binding domain and the region responsible for dimerisation and ligand-dependent transactivation.

The complete form of androgen insensitivity syndrome is caused by a wide spectrum of mutations in the androgen receptor gene (AR). Most of these mutations, however, cause amino acid substitutions in the protein product of the gene. To date only 24 mutations causing premature termination of translation of the receptor protein were described (8). The mutation described herein was previously reported only once by PINSKY et. al. (1992), who showed that such a truncated receptor cannot bind the ligand and that this mutation resulted in CAIS.

On the basis of DNA analysis we conclude that the R796X mutation in the androgen receptor is responsible for the symptoms of CAIS in our patient and this finding is consistent with the clinical diagnosis. This mutation adds to the 24 mutations in the AR gene, causing premature termination of translation described to date, and is described in Eastern Europe for the first time.

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REFERENCES


