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·*Case Report*·

Novel mutation in the ligand-binding domain of the androgen receptor gene (l790p) associated with complete androgen insensitivity syndrome

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Abstract

Mutations in the X-linked androgen receptor (AR) gene cause androgen insensitivity syndrome (AIS), resulting in an impaired embryonic sex differentiation in 46,XY genetic men. Complete androgen insensitivity (CAIS) produces a female external phenotype, whereas cases with partial androgen insensitivity (PAIS) have various ambiguities of the genitalia. Mild androgen insensitivity (MAIS) is characterized by undermasculinization and gynecomastia. Here we describe a 2-month-old 46,XY female patient, with all of the characteristics of CAIS. Defects in testosterone (T) and dihydrotestosterone (DHT) synthesis were excluded. Sequencing of the AR gene showed the presence in exon 6 of a T to C transition in the second base of codon 790, nucleotide position 2369, causing a novel missense Leu790Pro mutation in the ligand-binding domain of the AR protein. The identification of a novel AR mutation in a girl with CAIS provides significant information due to the importance of missense mutations in the ligand-binding domain of the AR, which are able to induce functional abnormalities in the androgen binding capability, stabilization of active conformation, or interaction with coactivators. *(Asian J Androl 2008 Jul; 10: 687–691)*

Keywords: androgen receptor; novel androgen receptor gene mutation; complete androgen insensitivity syndrome

1 Introduction

Androgens are the main steroid hormones that determine the expression of the male phenotype. Their activity is mediated by an androgen receptor (AR), alternatively known as the dihydrotestosterone (DHT) receptor, which, like all ligand-activated nuclear transcription factors, translocates to the nucleus and binds to the regulatory regions of specific chromosomal DNA sequences (androgen response elements), to activate androgen dependent genes [1]. The androgen–AR complex functions in conjunction with co-regulatory proteins [2].

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Disorders of AR function due to mutations in the AR gene cause different forms of X-linked male pseudohermaphroditism, known as androgen insensitivity syndromes (AIS; OMIM ID: 300068), affecting XY female individuals with normal androgen production and metabolisms.

AIS are estimated to be present in 1:20 000–64 000 male births. The presence of variable phenotypic expression allows the classification of AIS into complete androgen insensitivity (CAIS), partial androgen insensitivity (PAIS) and mild androgen insensitivity (MAIS).

In the most extreme form, CAIS-affected patients have normal female external genitalia, absent pubic and axillary hair ("hairless pseudofemale"), female breast development, blind vagina, absent uterus and female adnexa, and abdominal or inguinal bilateral testes producing normal or high levels of testosterone, with normal male karyotype [2, 3]. Cases affected by PAIS show various degrees of ambiguous genitalia, ranging from predominantly female external genitalia with signs of external genital masculinization (clitotomegaly and posterior labial fusion), to predominantly male genitalia (Reifenstein syndrome) with impaired spermatogenesis, hypospadias, micropenis and gynaecomastia [3–5]. MAIS is characterized by external male genitalia, undermasculinization (including sparse facial and body hair and micropenis) and gynecomastia at puberty [6–8]. A subgroup of MAIS patients is represented by phenotypically normal men with infertility as the sole clinical symptom [2, 9].

The AR is encoded by the AR gene (Xq11–12). This gene spans more than 90 kb and codes for a protein with three major functional domains. The N-terminal domain, which serves a modulatory function, is encoded by exon 1 (1586 bp); the DNA-binding domain is encoded by exons 2 and 3 (152 bp and 117 bp, respectively); and the androgen-binding domain (LBD) is encoded by exons 4–8, which vary from 131 bp to 288 bp in size [10, 11]. The C-terminus of the LBD mediates the hormone dependent transcription activation function. Functional studies demonstrate that the mutations in the AR ligand-binding domain perturb the conformation of the helix, which is unable to efficiently bind the ligand DHT and to transactivate known androgen response elements [12].

The present version of the AR database contains more than 600 AIS specific mutations (www.mcgill.ca/androgendb), with the total number of reported mutations rising from 374 to 605 in only 4 years [13]. Approximately 90% of molecular defects in the AR gene are single base mutations, mostly missense mutations. In addition to the point mutations, the AR gene contains regions of repetitive DNA sequences, trinucleotide repeat CAG and GGN, that have been associated with a number of disorders, such as androgen insensitivity, male infertility and prostate cancer [14].

Here we describe a novel mutation in the AR gene (L790P) detected in a patient affected by CAIS.

2 Patients and methods

A 2-month-old girl was referred to us because of the presence of a bilateral mass in the inguinal canal associated with inguinal herniae. Physical examination revealed a short and blind-ending vagina with female external genitalia. Testosterone (T) and DHT synthesis defects were excluded given the normal rise of T and DHT after HCG stimulation (basal T: 1.3 nmol/L; basal DHT: 0.7 nmol/L, T/DHT ratio after human chorionic gonadotropin (HCG) stimulation: 7:9). Gonadotropin levels were normal (follicle stimulating hormone: 1.1 UI/L; luteinizing hormone: 1.2 UI/L). Chromosomal analysis showed diploid 46, XY male karyotype. During the intervention the presence of testes was confirmed and gonadal histology was consistent with AIS. After clinical, hormonal and cytogenetic examinations the girl was diagnosed as affected by CAIS.

Peripheral blood samples were obtained from the girl and her mother for molecular analysis. The subjects gave informed consent for molecular analysis of their blood samples and the study was approved by a local ethical committee. Genomic DNA was extracted with the use of the High Pure PCR Template Preparation kit (Roche Applied Science, Penzberg, Germany). PCR amplification of AR exonic fragments 1–8 (including the intron exon boundaries) was carried out using the primers described by Ishii *et al.* [15], except for exons 1 and 5, which were amplified using the primers reported in Table 1.

Direct sequencing of the PCR products was performed using the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction kit and analyzed using an ABI PRISM 3100 Genetic Analyzer (PE Applied Biosystems, Monza, Italy).

3 Results and discussion

Table 1. Oligonucleotide primers used for genomic DNA amplification of human androgen receptor (AR) gene exons 1 and 5.

Primer number	Primer symbol	Sequence	Annealing temperature
1	Exon 1 A F	5'-AGA GAA GGG GAG GCG GGG TAA-3'	59ºC
2	Exon 1 A R	5'-CGA CTG CGG CTG TGT GAA GGT TG-3'	59ºC
3	Exon 1 B F	5'-CCC CAA GCC CAT CGT AGA-3'	58ºC
4	Exon 1 B R	5'-AAG TCC TTG GCG TTG TCA GAA-3'	58ºC
5	Exon 1 C F	5'-GGC ACT TCG ACC ATT TCT GA-3'	60ºC
6	Exon 1 C R	5'-CAG TCG TCC GGA CTT GTA GAG-3'	60ºC
7	Exon 1 D F	5'-CAG TTG AAC TGC CGT CTA CCC-3'	58ºC
8	Exon 1 D R	5'-CCA CCC CCA CCA CCA-3'	58ºC
9	Exon 1 E F	5'-GGA CCG TGT GGT GGT GG-3'	60ºC
10	Exon 1 E R	5'-AAG CGA CAT TTC TGG AAG GAA-3'	60ºC
11	Exon 5 F	5'-GGG AGT CAG ACT TAG CTC AAC-3'	59ºC
12	Exon 5 R	5'-TGG CCA AGC TGC TGT A-3'	59ºC

Direct sequencing analysis of PCR products revealed in the patient the presence of a T to C transition in exon 6 resulting in the previously unreported leucine 790 proline substitution (Leu790Pro) (Figure 1). The same mutation was detected in heterozygous form in the mother of the patient. The mutation was confirmed in both the patient and her mother in two independent experiments. Leucine 790 is located in the LBD of the AR protein (Figure 2). Mutations of a single amino acid in the LBD of the AR can induce functional abnormalities in androgen binding, stabilization of active conformation, or interaction with coactivators. In the AR C-terminal LBD, there is a clear predominance of missense mutations, with a significantly greater number of CAIS and PAIS. Out of 193 mutations, 103 causing CAIS are located in the LBD, 51 in the N-terminal and 24 in the DNA binding domain [13]. Substitutions resulting in CAIS have been reported also in the N-terminal region of the AR LBD, while only one mutation at position 790 has been previously reported (Leu790Phe), but was associated with MAIS [8].

Given the highly conserved nature of these residues, they likely play a critical role in creating the correct structural architecture of the AR LBD. Although residues in this region of the AR do not interact directly with the hormone, each residue probably has an important role in ordering the structural domains containing the residues that contribute to the ligand-binding pocket. This is consistent with the observation that substitution mutations in this region result most frequently in the complete form of androgen insensitivity. Therefore, the different phe-

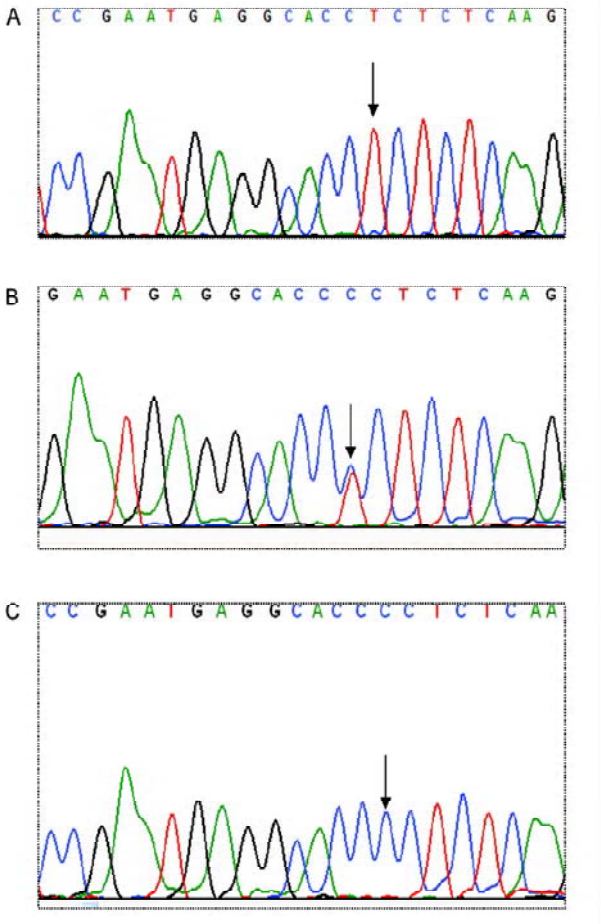

Figure 1. DNA sequence in exon 6 of AR gene exon 6 gene showing the normal sequence (A) and the presence of the T to C substitution in the patient (C) and her mother (B).

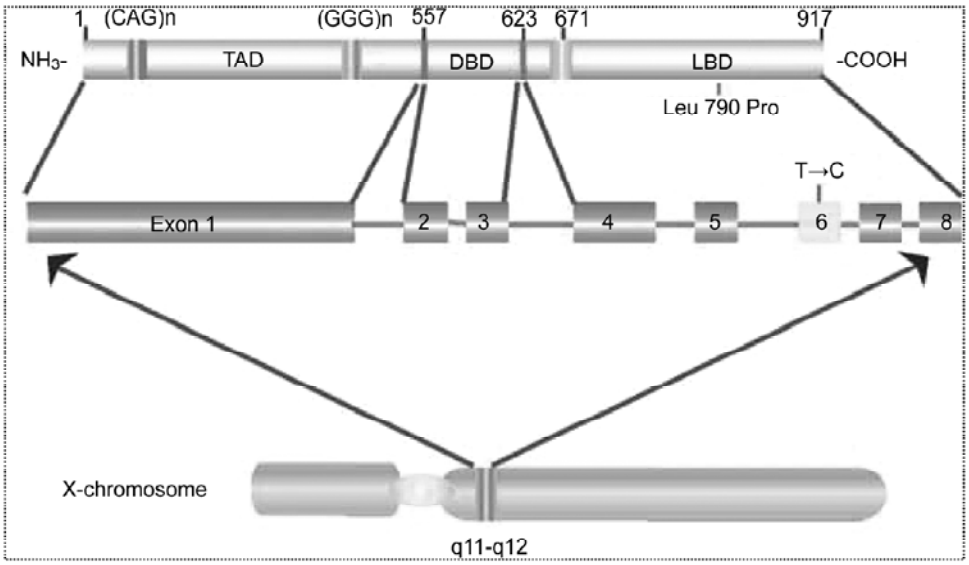

Figure 2. Localization of the T to C transition within the genomic structure of the androgen receptor (AR) gene and of the Leu790Pro substitution in the LBD. NTD, N-terminal domain; DBD, DNA-binding domain; LBD, androgen-binding domain; TAD, transactivation domain.

notypes showed by our patient and the one described by Tsukada *et al*. [8], both carriers of a mutation involving codon 790, can be explained by the different amino acid substitution. In fact, the insertion of a proline instead of a leucin in the amino acidic chain could act as a structural disruptor in the middle of the regular secondary structural elements, because of the exceptional conformational rigidity of proline cyclic structure compared to other amino acids. In contrast, the substitution of the leucin with a phenylalanine, as in the case reported by Tsukada *et al*. [8], would lead to a less severe structural disruption in the conformational status of the AR protein, causing a milder phenotype.

In conclusion, the characterization of mutations in the AR gene serves as a reliable tool for the diagnosis and molecular subclassification of AIS. The present case demonstrates that, in addition to the localization of the mutation within the gene sequence, the kind of amino acid substitution in a case of missense mutation strongly affects the resulting phenotype. Knowledge of the mutation in the AR and its functional consequences can provide useful information about the genotype-phenotype correlation, improving the management of cases of male pseudohermaphroditism with regard to gender assignment, genital surgery and gonadectomy.

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