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Case Report

Androgen receptor gene mutations in 46, XY females

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Abstract

The androgen insensitivity syndrome is a heterogeneous disorder with a wide spectrum of phenotypic abnormalities, ranging from complete female to ambiguous forms that more closely resemble males. The primary abnormality is a defective androgen receptor protein due to a mutation of the androgen receptor gene. This prevents normal androgen action and thus leads to impaired virilization. A point mutation of the androgen receptor gene affecting two siblings with complete androgen insensitivity syndrome is described. On examination they both had normal external female genitalia.

Genomic DNA was extracted from EDTA-preserved blood samples and isolated according to standard procedures. The androgen receptor gene was screened for mutations using an automated sequence analyzer (ABI Prism 310). Both girls possess one substitutions (G>A at position 2086 in exon 4), leading to D695N mutation. Mother was found to be a heterozygous carrier for this mutation. GTG banded karyotype of the girls showed they both have male karyotype (46, XY). In addition, the SRY gene screening showed they both have intact SRY gene. The labioscrotal folds contained palpable gonads measuring 1.5 cm in largest diameter. Ultrasound examination of the pelvis revealed absence of the uterus.

Serum follicle stimulating hormone (FSH), luteinizing hormone (LH), and testosterone values were higher than normal range.

To our knowledge this is the first confirmed instance of AIS due to an AR mutation occurring in familial cases in this country. Furthermore, the phenotype has complete association with this mutation.

KEY WORDS: Androgen insensitivity syndrome, androgen receptor.

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Mutations of the androgen receptor gene in subjects with a 46, XY karyotype give rise to the androgen insensitivity syndrome (AIS). The syndrome is a heterogeneous disorder with a wide spectrum of phenotypic abnormalities ranging from complete female to ambiguous forms that more closely resemble males. The primary abnormality is a defective androgen receptor protein which prevents normal androgen action and thus leads to impaired virilization [1-3]. The androgen receptor protein is encoded by a single gene containing eight exons located on the Xq 11-12 [4]. AR can modulate gene expression directly by interacting with specific elements in the regulatory regions of target genes [5], or indirectly by activating various growth factor-signaling pathways [6]. Like other members of the nuclear receptor super family, AR has four major functional regions (figure 1): the N-terminal transactivation domain (TAD), a central DNA-binding domain (DBD), a C-terminal ligand-binding domain (LBD), and a hinge region connecting the DBD and LBD [7]. About 50% of mutations reported in the LBD of AR have been found to be associated with prostate cancer (PC) or androgen insensitivity syndrome (AIS).

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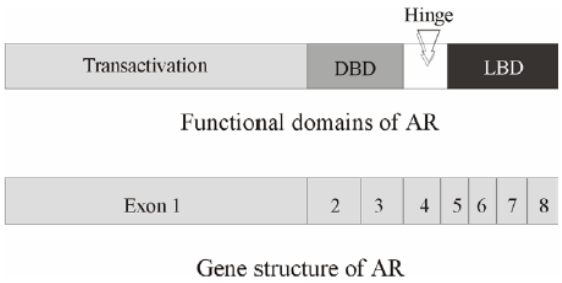

Figure 1. Schematic structure of the androgen receptor; there are eight exons encoding the receptor with a large exon 1 required for transactivation and exons 2-8 encoding a DNA-binding domain, hinge region, and hormone binding domain.

Studies of AIS to date, mainly in subjects with complete forms of the syndrome, have identified a variety of androgen receptor gene deletions and insertions and a much larger number of single base mutations that introduce premature termination codons, amino acid changes, or aberrant messenger RNA splicing [8]. Four sites in the steroid binding domain, arginine residues 774, 840, and 855, and valine 866, appear to have a particularly high frequency of mutation and together account for about one quarter of the missense mutations reported to date [2,8]. Arginine 840 is reported to be the site of substitution by either cysteine or histidine in multiple patients (three and nine patients, respectively). These substitutions are both associated with a variety of phenotypes within the partial form of AIS, highlighting the complexity of the genotype-phenotype relation in androgen insensitivity [2]. Indeed, even within a few families with partial AIS, considerable phenotypic variability has been reported [9,10], although most of these reports were before mutational analyses of the androgen receptor gene were possible. The molecular basis of this phenotypic variation has been investigated in only a few families [11] and is still not understood. We describe a point mutation of the androgen receptor gene affecting two siblings with the complete form of the AIS.

Case Report

A point mutation of the androgen receptor gene affecting two siblings with complete androgen insensitivity syndrome is described. The family consisted of 6 persons including two girls with primary amenorrhea and two boys without any clinical manifestation. The parents are non-consanguineous.

On examination, both girls had normal external female genitalia with inguinal masses suggestive of testes. In the elder sister, secondary sexual features development such as breast growth was normal but pubic hair was absent. The uterus was absent in ultrasound examination and no ovaries were noticed.

Both girls possess one substitutions (G>A at position 2086 in exon 4), leading to D695N mutation. Mother was found to be a heterozygous carrier for this mutation (figure 2). GTG-banded karyotype of the girls showed they both had male karyotype (46, XY). In addition, screening the SRY gene showed they both had intact SRY gene. The labioscrotal folds contained palpable gonads measuring 1.5 cm in largest diameter.

Serum follicle stimulating hormone (FSH), luteinizing hormone (LH), and testosterone values were higher than normal range. In the elder sister, puberty began at age 13 with mammary gland development but no hair growth (pubic or axillary) and no virilization. Castration was performed at age 17, and histological examination of the gonads confirmed the presence of testes. The younger sister presented with a very similar picture. But no surgical intervention has been performed on her yet.

Method of mutation identification

Informed consent was obtained from both subjects and their parents, according to the protocol of the Ethical Review Board of Uremia University.

PCR amplification

Genomic DNA was extracted from EDTA-preserved blood samples and isolated according to standard procedures [12]. Samples used for mutation screening were amplified in 50μl

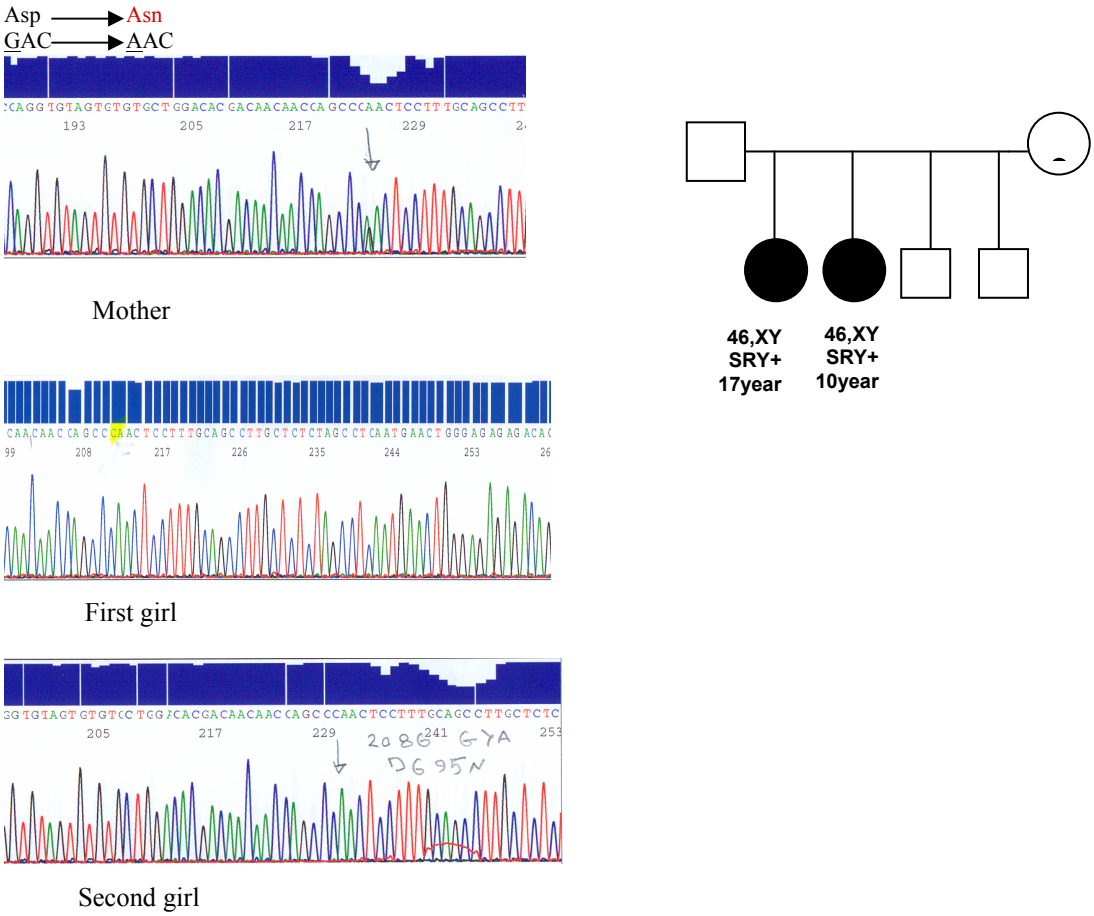

Figure 2. Partial DNA sequence chromatogram of exon 4 of the AR gene of the two affected siblings and their mother (left), family pedigree is shown on the right side. Both girls possess one substitutions (G>A at position 2086 in exon 4), leading to D695N mutation. Mother was found to be a heterozygous carrier for this mutation.

reaction volumes containing approximately 200 ng DNA, $MgCl_2$ (25 mM) 3 µl, dNTP mixture (1.25 Mm) 4 µl, AmpliTaq GoldTM (5 U/µl) 0.5 µl, and 50 pmol of each primer. Cycle conditions used for amplification of the AR exons were 15 minutes at 95°c for denaturation, 35 cycles of 30 seconds at 95°c, 30 seconds at 56°c and 55 seconds at 72°c. All amplifications were finished by a prolonged extension step of 4 minutes at 72°c.

The PCR program used for amplification of SRY gene was as follows:

96°c 15 minutes, (96°c 30 seconds, 64°c 30 seconds, 72°c 45 seconds) 30 cycle, 72°c 2 minutes.

Primers for all the exons of *AR* and *SRY* genes were designed using the web-based *Primer 3* program using standard selection criteria; the average size of fragments was between 180 and 350 bp (tables 1 and 2).

PCR amplicons were purified using the QIAquick PCR Purification Kit (Qiagen, Valencia, Ca.). DNA concentration was determined by spectrophotometry. Cycle sequencing extension products were created in a final volume 20 µl reaction using 12.5 µl H2O, 0.5 µl forward or reverse primer at 10 µg/ml, 0.5 µl template DNA 4 µl Big Dye Terminator Ready Reaction mix V1.1 (PE-ABI, Foster City, Ca.) and 2 µl 5X Big dye buffer. Cycle sequencing conditions consisted of an initial denaturation step at 96°C for 1 minute followed by 25 cycles of 96°c for 10 seconds, 50°c for 5 seconds, and 60°c for 4 minutes. Unincorporated dye and other contaminants were removed with ethanol precipitation

Table1. Primers, for each of the 8 exons of the AR gene

Exon	Sequence 5'→3'	Tm
Ex1AF	AGGGAAGTAGGTGGAAGATTCAG	56°C
Ex1AR	ACGATGGGCTTGGGGAGAACC	56°C
Ex1BF	CAGCAAGAGACTAGCCCCAGG	56°C
Ex1BR	CCTCGCTCAGGATGTCTTTAAG	56°C
Ex1CF	CCCCACTTTCCCCGGCTTAAG	56°C
Ex1CR	TCCCAAAAGTGGGGCGTACATG	56°C
Ex1DF	TTGGAGCATCTGAGTCCAGGG	56°C
Ex1DR	CGCAGCTGCCTCGTCCAGTG	56°C
Ex1GF	TCCGGGACACTTGAACTGCCG	56°C
Ex1GR	TGTGAAGAGAGTGTGCCAGG	56°C
Ex1FF	GCGGCGAGGCGGGAGCTG	56°C
Ex1FR	TGCCCTGGGCCGAAAGGCG	56°C
Ex2F	CAGTGACATGTGTTGCATTGG	56°C
Ex2R	TGAAAGGTTAGTGTCTCTCTCT	56°C
Ex3F	TCTGGAAACTCATTATCAGGTC	56°C
Ex3R	TAGAAAATGAGGGAGAAGGGG	56°C
Ex4F	TTCAAGTCTCTCTTCCTTCCC	56°C
Ex4R	GCTCCCACTTCCCTTTTCC	56°C
Ex5F	GACCACTGCCTCTGCCTC	56°C
Ex5R	CCAACCAGGTCTGGCCAAG	56°C
Ex6F	CTTTTTCCTCTGTGTATCTCCT	56°C
Ex6R	GGTCCTCTCTGAATCTCTGTG	56°C
Ex7F	AACTTGGTGCTTTGTCTAATGC	56°C
Ex7R	CTGATAAAGCACCCTCCATCG	56°C
Ex8F	TTTTCTCCCTCTTATTGTTCCC	56°C
Ex8R	GGGCATGAGCTGGGGTGG	56°C

Table2. Primers used for amplification of the SRY gene.

Exon	Sequence 5'→3'	Tm
SRY1F	TTTCAATTTTGTCGCACTCTCC	64°C
SRY1R	TGGAAGAATGGCCATTTTTCGG	64°C
SRY2F	CCCAGAATGCGAAACTCAGAG	64°C
SRY2R	AAAGTGAGGGCTGTAAGTTATC	64°C

procedure. Data was analyzed with the Sequencers software (Genecodes Inc. Ann Arbor, Michigan).

Gene symbols used in this article follow the recommendation of the HUGO Gene Nomenclature Committee [13]. Mutations are described according to recommendations by den Dunnen and Antonarakis [14].

Discussion

We identified a single base substitution (G>A) in the DNA-binding domain of the AR gene resulting in the replacement of a conserved Aspartic acid residue by an asparagine at position 695 in two siblings. This mutation results in a nonfunctional receptor with decreased DNA-binding ability and could, therefore, be responsible for the CAIS in both patients.

This study highlights the importance of Aspartic acid-695 in the DNA-binding domain of the AR in the protein-DNA interaction. This Aspartic acid residue is highly conserved within the steroid receptor superfamily, suggesting that it may play a crucial role in androgen action.

In addition, this residue is located next to a conserved proline residue involved in the formation of the kink in the DNA chain. Aspartic acid, with its free carboxyl group has acidic and hydrophilic character but asparagine has

basic side chains with similar polarity and charge in the amino acid residues and carbohydrate can be covalently linked ("N-linked) to its -NH. However, there are differences in the structure of the side chains. To address this alteration of the specific residue involved in either protein-protein or protein-DNA interaction, we have to carry out further experiments such as comparing data from the known crystallographic structure of the AR with the mutated one. In an experiment by Mowszowicz and his collogues they showed that Arginine-614 is the only residue in the carboxy-terminal a-helix in the DNA-binding domain of the AR involved in both specific and nonspecific phosphate contacts with DNA. Thus, they hypothesize that mutation in this residue may decrease or even abolish the interaction between AR and DNA. These data indicate that the mutated residue at position 614 markedly influences protein architecture during protein-DNA interaction. Therefore, the native arginine at position 614 may be structurally and functionally important [15].

Like the mutation we found, Ris-Stalpers and his collogues identified a single nucleotide alteration in codon 686 (GAC; aspartic acid) in exon 4 of the human androgen receptor gene in three unrelated families with the complete form of androgen insensitivity [16]. This mutation (G----A) leads to an aspartic acid----asparagine substitution with normal androgen-binding capacity, but a rapidly dissociating ligand-receptor complex. Both cases were raised as females and had normal external genitalia. The androgen binding capacity was in the normal range. Thus, aspartic acid-686 appears essential for normal androgen receptor function. Substitution of this amino acid residue by asparagine results in androgen insensitivity and lack of androgen-dependent male sexual differentiation [15]. According to their findings it is possible to say in our cases that the same mechanism is responsible for the disease. Hiort, et al [17] reported this mutation as a de novo mutation. Their patient was a female with ambiguous genitalia. But in our study we showed the mutation is a familial one and it is inherited through mother. In conclusion it should be noted that because of the importance of prophylactic gonadectomy, detection of this syndrome demands careful investigation for other affected family members. This syndrome follows an X-linked recessive pattern of inheritance. Apparent sisters of affected individual have 1 in 6 chance of being XY. Female offspring of a normal sister of an affected individual has a 1 in 6 chance of being XY. About a third of the patients have negative family histories and presumably represent new mutations [18].

It is relatively easy to imagine a mechanism through which a defective DNA-binding domain could prevent the receptor from normally trans-activating the transcription of androgen-regulated genes in spite of normal hormone binding. A mutation in the ligand-binding domain resulting in an inactive AR may further indicate that the ligand-binding domain contains a transcriptional activation function in addition to its ligand-binding function [7,19]. In order to check the binding capacity in our patients we need to carry out the experiment in genital skin fibroblasts and measure the AR mRNA expression level. But it should be considered that the assessment of AR mRNA in cultured genital skin fibroblasts is difficult and not accurate enough for conclusions to be drawn from the difference between the patients. Nevertheless, variable and most often decreased levels of mRNA have been reported in patients with androgen insensitivity [20]. The possibility that additional mutations in the promoter region of the AR gene result in the alteration of AR mRNA levels cannot be excluded. However, variation in AR mRNA levels could in itself modulate the degree of androgen resistance, regardless of the cause. It is of interest to note that a marked decrease of steady state level of vitamin D receptor mRNA has been reported in patients with mutations within the vitamin D receptor coding segment [21]. It is, therefore, possible that mutations of the AR alter the turnover of the AR mRNA or protein, thus modulating the degree of androgen resistance.

Acknowledgments

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