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Mutation analysis of androgen receptor gene: Multiple uses for a single test

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ABSTRACT

Androgen receptor gene mutations are one of the leading causes of disorders of sex development (DSD) exhibited by sexual ambiguity or sex reversal. In this study, 2 families with patients whom diagnosed clinically as androgen insensitivity syndrome (AIS) were physically and genetically examined. This evaluation carried out by cytogenetic and molecular analysis including karyotype and sequencing of SRY and AR genes. In family 1, two brothers and their mother were hemizygous and heterozygous respectively for c.2522G > A variant, while one of their healthy brother was a completely normal hemizygote. Family 2 assessment demonstrated the c.639G > A (rs6152) mutation in two siblings who were reared as girls. The SRY gene was intact in all of the study's participants.
Our findings in family 1 could be a further proof for the pathogenicity of the c.2522G > A variant. Given the importance of AR mutations in development of problems such as sex assignment in AIS patients, definitive diagnosis and phenotype–genotype correlation could be achieved by molecular genetic tests that in turn could have promising impacts in clinical management and also in prenatal diagnosis of prospect offspring.
In this regard, phenotype–genotype correlation could be helpful and achieved by molecular genetic tests. This could influence the clinical management of the patients as well as prenatal diagnosis for the prospective offspring.

1. Introduction

Mutations in AR gene may lead to perturbation in the uptaking of androgen and subsequent Androgen Insensitivity Syndrome (AIS) by the effect of insufficient intracellular androgenic response. There are various AIS phenotype patients' varieties i.e. from isolated infertility in less severe type of Partial Androgen Insensitivity Syndrome (PAIS) to Complete Androgen Insensitivity Syndrome (CAIS) manifested by female phenotype. The prevalence of CAIS is about 1 in 40,000–60,000 birth (Brinkmann, 2001). All AIS patients regardless of phenotype severity show 46 XY karyotype, and the absence of Mullerian derived structures including fallopian tubes, uterus and cervix with normal testicular pathology found beyond physical, paraclinical and cytogenetic examinations. Based on the Quigley et al. outline, there are 1–5 degrees of AIS in which the first degree corresponds CAIS and the 2–5 degrees are in relation with the wide diversity in clinical presentations of PAIS. CAIS is diagnosed based on the complete female appearance and breast development during puberty along with undescendent testes with or without epididymis and vas deferens. PAIS grade 1 (degree 2 of AIS) could predominantly be considered in female cases and is easily distinguished from CAIS by the presence of clitoromegaly or labial fusion. In the third degree of PAIS however, in addition to gynecomastia, clitoromegaly develops more into a penis with the length of less than

Abbreviations: AF, Activation function; AIS, Androgen insensitivity syndrome; AR, Androgen receptor; CAIS, Complete androgen insensitivity; DHT, Dihydrotestosterone; DNA, Deoxy nucleic acid; DSD, Disorders of sex development; EDTA, Ethylene diamine tetra acetic acid; FSH, Follicular stimulating hormone; LBD, Ligand binding domain; LH, Luteinizing hormone; NTD, N terminal domain; PAIS, Partial androgen insensitivity; PCOS, Polycystic ovarian syndrome; PCR, Polymerase chain reaction; PGD, Pre-implantation genetic diagnosis; POF, Premature ovarian failure; PND, Pre-natal diagnosis; SRY, Sex region on Y (chromosome); DHEA-S, Dehydroepiandrosterone sulfate; A2, Androstenedione.

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1 cm called microphallus and the testes may descend into more mature labia majora recognized as bifid scrotum. AIS patients are predominantly male in 3rd grade of PAIS phenotype and exhibit hypospadias with normal or small size of penis, descendent or undescendent testes and gynecomastia. The last but least severe type of PAIS [mild AIS (MAIS)] patients may solely indicate spermatogenesis defects and gynecomastia with mild impaired virilization (Quigley et al., 1995). Although genotype–phenotype correlation is not strong in AIS, this clinical heterogeneity is in contingency with the domain of protein that has been mutated (Balducci et al., 1996; Chu et al., 2002) and finding this correlation would help to better diagnose AIS patients. Contrary to CAIS, in molecular analysis of more than 50% PAIS patients a distinct AR gene mutation was not found (Ahmed et al., 2000; Morel et al., 2002). However, it seems AR mutation evaluation is preferable to other diagnostic tests like IHC in the evaluation of individuals with AIS phenotype because of its simplicity in sample preparation. In that AIS phenotype is apparent which is highly significant in AIS pathogenesis. Biochemical and paraclinical tests including pelvic ultra sound and genetic analysis complete the pyramid in AIS diagnosis.

Androgen receptor (AR) protein that is encoded by AR gene is located on chromosome Xq11–12 that contains 8 exons. It is the fourth member of group C classification belonged to the sub-classification of 3 nuclear steroid receptors super family and is involved in the development and differentiation of male sexual characteristics (McPhaul et al., 1991). More than 500 various types of mutations have been reported in AR gene, which is the leading cause of male sex derivation in the spectrum of diseases called disorders of sex development (DSD). Among all of the putative mutations, point mutations comprise the most common type of alterations in pathologic conditions related to AR gene (Quigley et al., 1995).

Sex dissatisfaction and other psychologically related problems in these patients demand the investigation of AR gene mutation and its role in this issue. Although the reports are controversial in both sexes.

2. Patients and methods

2.1. Patients

This study was carried out on two Iranian families affected by AIS diagnosed on the basis of clinical presentations, biochemical and screening discoveries. The informed consent forms were filled up by every family member. Progesterone, 17-OH progesterone, DHEA-S, androstenedione (A2), testosterone (T), dihydrotestosterone (DHT) and estradiol were measured at 08:00 am in patients whose FSH and LH levels were high and subsequently after hCG test in case the patients were prepubertal. The ratios of T/DHT and T/A2 were calculated. The ratios of T/DHT > 20 and T/A2 < 0.8 were considered as 5α reductase and 17β-hydroxysteroid dehydrogenase type 3 deficiency, respectively (Dattani et al., 2011).

2.2. DNA extraction and karyotype

Six milliliters of whole blood was taken from every family member and 4 mL of it was transferred into tubes containing 200 μL EDTA for DNA isolation. The remaining 2 mL of whole blood was preserved in tubes containing heparin to be used for karyotype examination. DNA was isolated from peripheral blood of all samples by standard salting out method. The quality and concentration of the genomic DNA were determined through PCR products in (1.5%) gel electrophoresis and spectrophotometer respectively. The karyotype of all the patients enrolled was determined through cell culture and subsequent preparation of chromosome ideogram according to the common method of G-banding. It is comprised of cell culturing, harvesting and slide preparation. The slides were GTG banded and examined under light microscope and 20 chromosome spreads were examined.

2.3. Polymerase chain reaction (PCR) and sequencing

The exons 1–8 of AR gene residing on chromosome Xq12 were amplified using 10 primer pairs designed via Primer 3 software (http://frodo.wi.mit.edu/primer) (Table 1). PCR reaction was incurred in a 25 μL reaction mixture including 50 ng of each genomic DNA template. The mixture consisted of 20 mmol/L Tris–HCl pH 8.4, 50 mmol/L KCl, 1 mmol/L MgCl2, 10 pmol for each primer, 0.2 mmol/L for each dNTP mixture and also 1.25 units of Smart Taq DNA polymerase Fermentas, (Canada). After initial denaturation at 94 °C for 5 min, 37 PCR cycles was performed using Biorad thermocycler (Bio-Rad Laboratories, Hercules, CA, USA); each cycle included denaturation at 95 °C in 30 s, annealing at 60 °C in 30 s, extension at 72 °C for 30 s and final extension at 72 °C in 5 min. However, the mentioned annealing temperature was changed to 55 °C, 58 °C and 63 °C for exons 3, 4 and 7 respectively. The single exon of SRY gene on chromosome Yp11.3 was amplified using one pair of forward and reverse SRY specific primer for sequence analysis (Table 2). Each 25 μL PCR reaction contained 20 mmol/L Tris–HCl pH 8.4, 50 mmol/L KCl, 2 mmol/L MgCl2, 10 pmol of each primers, 0.2 mmol/L of each dNTP mix, 1.25 units of Smart Taq DNA polymerase (Fermentas) and 50 ng of genomic DNA. Amplification was also carried out on using thermal cycler (Corbet, USA) with an initial 7-minute denaturation at 96 °C, followed by 35 PCR amplification cycles of denaturation at 95 °C for 30 s, annealing at 56 °C for 60 s, elongation at 72 °C for 45 s, and extension at 72 °C for 10 min respectively. PCR products were subjected to electrophoresis on agarose gel (1.5%) followed by staining with ethidium bromide (1 μg/mL) to verify anticipated length of amplified fragments. Direct sequencing was used with primers (Table 2) to observe AR and SRY gene sequences alterations in each patient. Sequencing was carried out by MACROGEN company in South Korea using classic Sanger method with ABI. The analyzing of raw data was performed by Gene Marker software.

2.4. Immunohistochemistry (IHC)

The genital skin biopsies of IV1 and IV2 for the first family were embedded in paraffin. IHC for AR protein was performed on 5 μm sections using Pharm Dx immunohistochemistry kit (Dako, Cambridge, UK).

3. Results

Genitalia phenotype in siblings (two brothers) for the first family indicated microphallus, hypospadiasis, bilateral cryptorchidism and gynecomastia. Additional examinations by ultrasound also manifested

Table 1
Primer list used fro AR gene mutation detection.

Primer	Sequence
Ex1a-F	5′-AGG GAA GTA GGT GGA AGA TTC AG-3′
Ex1a-R	5′-CCT CGC TCA GGA TGT CTT TAA G-3′
Ex1b-F	5′-CCC CAC TTT CCC CGG CTT AAG-3′
Ex1b-R	5′-GCA CTG GAC GAG GCA GCT GCG-3′
Ex1c-F	5′-TCC GGG ACA CTT GAA CTG CCG-3′
Ex1c-R	5′-TGC CCT GGG CCG AAA GGC G-3′
Ex2-F	5′-AAT GCT GAA GAC CTG AGA CT-3′
Ex2-R	5′-AAA ATC CTG GGC CCT GAA AG-3′
Ex3-F	5′-CTA GAA ATA CCC GAA GAA AG-3′
Ex3-R	5′- GAG AGA CTA GAA AAT GAG GG-3′
Ex4-F	5′-GTG ATT TTC TTA GCT AGG GC-3′
Ex4-R	5′-ATC CCC CTT ATC TCA TGC TC-3′
Ex5-F	5′-GAC TGA CCA CTG CCT CTG CC-3′
Ex5-R	5′-TCA CCC CAT CAC CAT CAC CA-3′
Ex6-F	5′-TGT AAA CTT CCC CTC ATT C-3′
Ex6-R	5′-TAA TGG CAA AAG TGG TCC TC-3′
Ex7-F	5′-TGT GGT CAG AAA ACT TGG TG-3′
Ex7-R	5′-CTC TAT CAG GCT GTT CTC CC-3′
Ex8-F	5′-GCC ACC TCC TTG TCA ACC CT-3′
Ex8-R	5′-AGA GGA GTA GTG CAG AGT TA-3′