

·Case Report·

Molecular diagnosis of 5 α -reductase-2 gene mutation in two Indian families with male pseudohermaphroditism

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Abstract

Aim: To identify the genotype of two Indians with male pseudohermaphroditism. **Methods:** Standard radioimmunoassay procedure was used for estimating hormonal levels. Conventional cytogenetic analysis was carried out for diagnosing the genetic sex in these subjects with genital ambiguity. Molecular analysis was carried out by standard polymerase chain reaction procedure using different sets of primers and reaction conditions specific for the 5 α -reductase type 2 gene (SRD5A2) gene. Direct sequencing was carried out using the ABI Prism dye terminator sequencing kit and the ABI 310 sequencing apparatus. **Results:** We found an SRD5A2 gene mutation in exon 5, where arginine is substituted with glutamine (R246Q), in two males with pseudohermaphroditism and ambiguous genitalia from unrelated families. This is the first time this mutation has been reported in individuals from India. **Conclusion:** Identification of the R246Q mutation of the SRD5A2 gene from two unrelated Indian families possibly extends the founder gene effect. (*Asian J Androl* 2008 Sep; 10: 815–818)

Keywords: male pseudohermaphroditism; 5 α RD-2 deficiency; dihydrotestosterone; SRD5A2 gene mutation; perineoscrotal hypospadias

1 Introduction

Deficiency in 5 α -reductase-2 (5 α RD-2) is an inborn error of metabolism inherited as an autosomal recessive trait. Individuals with this disorder have an impaired con-

version of testosterone (T) to dihydrotestosterone (DHT). Mutations in the 5 α -reductase type 2 (SRD5A2) gene results in the decreased synthesis of DHT, a key hormone in the virilization of male external genitalia, which then leads to male pseudohermaphroditism. Males with 5 α RD-2 deficiency have striking ambiguity of the genitalia with a clitoral-like phallus, severely bifid scrotum, pseudovaginal perineoscrotal hypospadias, and a rudimentary prostate [1, 2].

The molecular genetic analysis of individuals with 5 α RD-2 deficiency indicates a region-specific predominance of certain mutations, reflecting the influence of a

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founder gene effect in some populations. Although there are few data on the genotyping of intersex patients from India, two families from Pakistan with an R246Q mutation in *SRD5A2* have been described in published reports [3-5]. In the present study, we report an R246Q mutation in exon 5 of *SRD5A2* for the first time in two Indian males with ambiguous genitalia.

2 Materials and methods

2.1 Subjects

Two males with pseudohermaphroditism and ambiguous genitalia from unrelated Indian families were studied.

2.1.1 Case 1

This child was born with a 46,XY chromosomal pattern to nonconsanguineous parents and was reared as a girl, attending a girls' school until the age of 10 years. At 11 years, medical attention was sought because the child considered himself a boy and lacked any signs of female pubertal development. Physical examination revealed genital ambiguity with perineoscrotal hypospadias. The left testis was palpated in the labial fold and the right was in the inguinal canal. After several interviews with a psychiatrist and an endocrinologist, it was decided that the child should be reared as a boy. He subsequently changed to attend a boys' school.

2.1.2 Case 2

This child was born to consanguineous parents and was seen in our clinic at the age of 18 months. On examination, he was found to have bilateral testes of 1 mL each in the scrotum with a perineoscrotal hypospadias. Karyotype revealed a 46,XY chromosomal pattern.

2.2 Methods

2.2.1 Hormonal analysis

Serum samples were taken at baseline and after three doses of intramuscular injection of human chorionic gonadotropin (hCG) 2 000 IU (1 000 IU for Case 2) daily. Both T and DHT were measured in each sample. Diagnosis of 5 α -reductase deficiency was made when the T:DHT ratio was > 25 after hCG stimulation. Radioimmunoassay (RIA) for luteinizing hormone (LH), follicle-stimulating hormone (FSH), T and DHT were carried out with commercial RIA kits. Total T was measured by an RIA kit (Diagnostics systems laboratories, Inc., Webster, TX, USA) supplied by Immunotech (Marseille, France). In-

tra-assay and inter-assay precision were < 12% and < 16%, respectively. DHT was measured by an RIA kit supplied by Immunotech. DHT was separated from other hormones by diethyl ether extraction and celite column chromatography with minor modifications [2]. The recovery measured was approximately 85%–90%. The cross-reactivity of the antibody for T was 29% and other steroids was extremely low (< 0.01%). The intra-assay and inter-assay precision was < 13% and < 16%, respectively.

2.2.2 Cytogenetic analysis

Conventional cytogenetic analysis was carried out on peripheral blood using standard techniques. Karyotyping was carried out on G-banded metaphases obtained from 72-h cultures.

2.2.3 Genomic DNA isolation

Informed consent to carry out molecular genetic analysis was obtained from these two families. High molecular weight DNA was prepared from peripheral white blood cells by phenol-chloroform extraction and ethanol precipitation [6, 7]. DNA was resuspended in 10 mmol/L Tris-HCl, pH 8.0, and 1 mmol/L ethylenediaminetetraacetic acid (EDTA).

2.2.4 Enzymatic amplification and direct DNA sequencing

All five exons of *SRD5A2* were amplified by polymerase chain reaction (PCR) using different sets of primers and reaction conditions previously described [6, 7]. PCR products were verified for correct length on agarose gel, purified using QIAquick PCR columns (Qiagen, Courtaboeuf, France) and sequenced using the ABI Prism dye terminator sequencing kit and the ABI 310 sequencing apparatus (Applied Biosystems, Courtaboeuf, France).

3 Results

3.1 Hormonal profile

Hormonal profile of Case 1 revealed basal serum T, LH, FSH and DHT levels as 660 ng/mL, 7.3 U/L, 9.0 U/L, and 17.0 ng/dL, respectively. T and DHT levels rose to 800 ng/mL and 22 ng/dL, respectively, following hCG stimulation. The T:DHT ratio before and after hCG stimulation was 37.5 and 36.4, respectively.

In Case 2 the hormonal analysis revealed normal LH, FSH and T levels. Basal serum T, LH, FSH and DHT

levels were 273 ng/mL, 0.1 U/L, 1.5 U/L and 24.0 ng/dL, respectively. T levels rose to 473 ng/dL after hCG stimulation. The basal T:DHT ratio was 11.3.

3.2 Molecular analysis

The genomic DNA samples of these two families were subjected to molecular analysis of the five coding exons and flanking regions of *SRD5A2* by means of PCR and direct sequencing. Direct sequencing of the PCR fragments led to identification of an R246Q mutation at amino acid position 246 in exon 5 of *SRD5A2* in both cases (Figure 1). This homozygous missense mutation presented with a G→A transition at codon 246 in exon 5, which was responsible for an arginine (CGG) to glutamine (CAG) substitution. Both parents of Case 2 were heterozygous for this mutation (R246Q).

4 Discussion

DHT, a potent androgen, is converted from T by two 5 α -reductase isozymes encoded by separate loci. These two isozymes have differential biochemical and molecular features. Inherited defects in the 5 α RD-2 isozyme lead to male pseudohermaphroditism with ambiguous genitalia. Both T and DHT play a critical role in binding to the same intracellular androgen receptor [8]. T acts on the Wolffian ducts to cause differentiation of the vas deferens, epididymis and seminal vesicles. The differentiation of external genitalia and prostate is governed by DHT, which causes elongation and enlargement of the urogenital tubercle and fusion of urogenital swellings [9, 10].

Molecular genetic studies of *SRD5A2* in a world-wide cohort of subjects with male pseudohermaphroditism have revealed more than 40 gene mutations [11-14]. More than half of these affected individuals were true homozygotes, whereas 40% were either compound heterozygotes or inferred compound heterozygotes. Identical mutations have been found in individuals from widely differing geographical and ethnic backgrounds, suggesting the presence of mutational hotspots in the *SRD5A2* gene [15].

Our two subjects were found to have homozygous mutations of *SRD5A2* at exon 5, with arginine substituted by glutamine at amino acid position 246. In Case 2, this mutation was inherited from both parents, who were heterozygous for the same mutation (R246Q). Previous functional studies have revealed that the R246Q mutation decreases the affinity for the nicotinamide adenine dinucleotide phosphate (NADPH) cofactor [13]. This mutation not only affects cofactor binding directly by disrupting this enzyme domain, but also diminishes the enzyme activity and changes the optimal pH.

The R246Q mutation of *SRD5A2* at exon 5 has been described in African-American, Pakistani, Italian, Austrian, Dominican, Brazilian and Egyptian ethnic groups [3, 12, 16-20]. Two Pakistani families, one consanguineous and the other nonconsanguineous, were reported to share the same mutation. It was further proposed [3-5] that these mutations within the same ethnic group possibly had common ancestral origins. Identification of the R246Q mutation of *SRD5A2* in two unrelated Indian families possibly extends the founder gene effect. More studies are required to confirm this postulation.

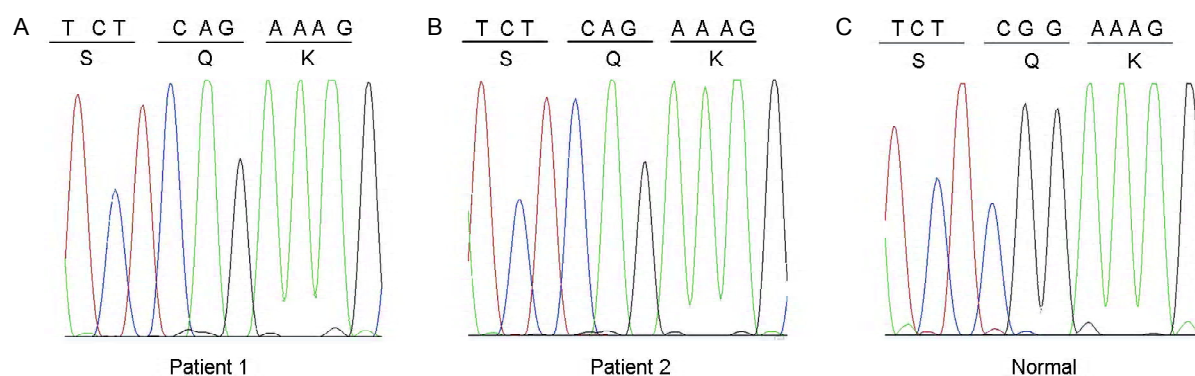


Figure 1. (A): Electropherogram showing homozygous missense mutation with a G→A transition at codon 246 in exon 5 (Case 1). (B): Electropherogram showing homozygous missense mutation with a G→A transition at codon 246 in exon 5 (Case 2). (C): Normal electropherogram showing CGG codon at position 246 in exon 5.

To conclude, we identified for the first time an R246Q mutation in exon 5 of the SRD5A2 gene in two unrelated families from India. Case 1 was reared as a girl and changed to male gender identity at peripuberty. In this case, molecular genetic analysis confirmed the diagnosis of male pseudohermaphroditism. In Case 2, the molecular genetic analysis paved the way for the diagnosis of the proband in infancy, as well as in both phenotypically normal parents. To the best of our knowledge, this mutation is being reported for the first time in individuals from India.

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