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Compound Heterozygous Mutations in the *SRD5A2* Gene Exon 4 in a Male Pseudohermaphrodite Patient of Chinese Origin

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ABSTRACT: The goal of this study was to perform 5-α-reductase type 2 gene (*SRD5A2*) analysis in a male pseudohermaphrodite (MPH) patient with normal testosterone (T) production and normal androgen receptor (*AR*) gene coding sequences. A patient of Chinese origin with ambiguous genitalia at 14 months, a 46,XY karyotype, and normal T secretion under human chorionic gonadotropin (hCG) stimulation underwent a gonadectomy at 20 months. Exons 1–8 of the *AR* gene and exons 1–5 of the *SRD5A2* gene were sequenced from peripheral blood DNA. *AR* gene coding sequences were normal. *SRD5A2* gene analysis revealed 2 consecutive mutations in exon 4, each located in a different allele: 1) a T nucleotide deletion, which predicts a frameshift mutation from codon 219, and 2) a missense mutation at codon 227, where the substitution of guanine (CGA) by adenine (CAA) predicts a glutamine replacement of arginine (R227Q). Testes located in the inguinal canal showed a normal morphology for age. The patient was a compound heterozygote for *SRD5A2* mutations, carrying 2 mutations in exon 4. The patient showed an R227Q mutation that has been described in an Asian population and MPH patients, along with a novel frameshift mutation, Tdel219. Testis morphology showed that, during early infancy, the 5-α-reductase enzyme deficiency may not have affected interstitial or tubular development.

Key words: 5-α-Reductase enzyme deficiency, 5-α-reductase type 2 gene mutations, male pseudohermaphroditism.

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One of the key steps during the differentiation of male genitals is the metabolism of testosterone (T) to 5-α-reduced metabolites, namely dihydrotestosterone (DHT). The primordial role of 5-α-reductase enzyme activity was first demonstrated in animal studies. Later, a cluster of male pseudohermaphrodite (MPH) patients were described in whom deficient virilization was attributable to an almost complete lack of this enzyme activity (Imperato-McGinley et al, 1974; Imperato-McGinley and Peterson, 1976). A number of deletions and point mutations in the 5-α-reductase type 2 gene (*SRD5A2*) gene that encodes the 5-α-reductase type 2 enzyme (Labrie et al, 1992) have been shown to occur in patients with various clinical and biochemical phenotypes (Thigpen et al, 1992; Wilson et al, 1993; Boudon et al, 1995a,b; Cai et al, 1996; Hochberg et al, 1996; Vilchis et al, 1997, 2000; Can et al, 1998; Nordenskjold and Ivarsson, 1998; Nordenskjold et al, 1998; Chavez et al, 2000; Hiort et al, 2002; Hafez et al, 2003; Mazen et al, 2003a,b). The phenotypes of newborn MPH patients with partial androgen insensitivity (PAIS), 5-α-reductase enzyme deficiency, or 17-β-hydroxysteroid dehydrogenase enzyme deficiency may be indistinguishable. Measurement of the T-to-androstendione (Δ_4) (T/Δ_4) ratio in peripheral blood under human chorionic gonadotropin (hCG) stimulation facilitates a biochemical diagnosis for 17-β-hydroxysteroid dehydrogenase enzyme deficiency; similarly, a serum T/DHT ratio or a 5-α/5-β urinary androgen metabolite analysis under hCG may provide evidence for the diagnosis of a 5-α-reductase enzyme deficiency. However, urinary androgen metabolites are not usually determined, and the serum T/DHT ratio may be almost normal in male adults with 5-α-reductase enzyme deficiency and, conversely, be abnormally high in adult patients with androgen insensitivity; thus, a correct differential diagnosis between PAIS and 5-α-reductase enzyme deficiency can be ensured only through the use of molecular studies, with mutations in the androgen receptor (*AR*) gene being by far more frequent than mutations in the *SRD5A2* gene. Testicular morphology has rarely been described in patients with 5-α-reductase enzyme deficiency, and reports on postpubertal

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patients' spermiograms have yielded varying results (Imperato-McGinley et al, 1980; Cai et al, 1994).

A patient with suspected PAIS, based on clinical and biochemical studies, is described in whom the testicular morphology during the neonatal period was normal and whose molecular diagnosis was heterozygous compound mutations in the *SRD5A2* gene.

Materials and Methods

Patient, Hormone, and Morphologic Studies

The patient was born in China to Chinese parents and was adopted as a girl by prospective Spanish parents. Ambiguous genitalia consisting of a urogenital sinus with a single urethral meatus, a hypertrophic clitoris (1 cm), and bilateral labioscrotal pouches with palpable gonads (1.5 cm) were observed by the pediatrician. The karyotype was 46,XY. A urethrography showed a female urethra, though longer than normal, with a prostatic utricle being replenished and no apparent vaginal pouch. A cystoscopy showed a male urethra with a veru montanum and prostatic utricle. A baseline hormone study was performed at 14 months, and an hCG test (1000 IU/48 h × 3) was performed at 17 months, prior to surgery. Gonadectomy and genitoplasty were performed at 20 months. Left and right testes measured 2 × 1 × 1 cm and 1.6 × 1 × 1 cm, respectively, and had normal epididymes and ductus deferens. Testis morphology was determined by referencing Nistal and Paniagua (1999).

The patient was classified as having PAIS.

Molecular Study

Genomic DNA was isolated from blood leukocytes by standard procedures. *AR* gene exons 1–8 and *SRD5A2* gene exons 1–5 were amplified by polymerase chain reaction (PCR). The primers used in the amplification of the *SRD5A2* gene have been described by Vilchis et al (1997). PCR amplification of exon 1 was carried out in a volume of 12.5 μL containing 50 ng of genomic DNA, 0.5 mM of each primer, 0.375 U of FailSafe PCR Enzyme Mix, and Premix D (EPICENTRE, Madison, Wis). PCR amplification of exons 2–5 was carried out in a volume of 12.5 μL containing 50 ng of genomic DNA, 0.3 mM of each primer, 0.05 mM of each deoxynucleotide triphosphate (dNTP), 1.5 mM $MgCL_2$, and 0.25 U *Taq* polymerase (ECOGEN, Barcelona, Spain). Reactions were first denatured at 94°C for 5 minutes and then subjected to 40 cycles of amplification with denaturation at 94°C for 1 minute, annealing at 60°C for 30 seconds (65°C for 1 minute for exon 1 amplification), and elongation at 72°C for 1 minute. A final extension at 72°C for 7 minutes was employed. After PCR, the products were analyzed in ethidium bromide–stained agarose gels and were found to be single band with the expected size. These products were sequenced in an automated sequencer (ABI PRISM 3100 Genetic Analyzer; Applied Biosystems, Foster City, Calif) using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) according to the directions provided by the manufacturer. Primers used in the sequencing were the same as those used in the PCR amplification.

Results

Hormone Study

Baseline steroid hormone concentrations at 14 months of age were normal for prepubertal patients (cortisol, 6.8 μg/dL; DHEAS, 30 μg/dL; 17-OH-P, 13 ng/dL; androstendione, 24 ng/dL; T, 9 ng/dL; and DHT, 19 ng/dL), as were gonadotrophins (luteinizing hormone, 0.2 mIU/mL; and follicle-stimulating hormone, 0.7 mIU/mL). hCG stimulation increased T concentration from 40 to 327 ng/dL, which was considered normal; the DHT serum concentration was not measured.

Testis Pathology

The left and right testis morphologies were similar: the albuginea were normal, with 325 and 330 μm of width, respectively; the mean seminiferous tubule diameters were 68 and 61.2 μm, respectively; the tubular fertility indices were 64% and 70%, respectively; the mean germinal cells per tubular section were 1.3 and 1.4, respectively, with abundant hypertrophic and multinucleated spermatogonia in the left testis; and the mean Sertoli cell numbers per cross-sectioned tubule were 25.5 and 24.5, respectively (Figure 1). All quantitative parameters were normal for the age of this patient. The interstitial tissue showed undifferentiated fibroblast cells. The epidydimides and initial segment of ductus deferens were normal for the patient's age.

Molecular Study

AR gene analysis showed no abnormality in the entire coding region. *SRD5A2* gene analysis revealed 2 consecutive mutations in exon 4 (Figure 2), each located in a different allele as follows: 1) a T nucleotide deletion at position 1998 (GenBank: 338466, L03843), which predicts a frameshift mutation from codon 219 and a longer protein with 277 amino acids and an anomalous sequence from position 219; and 2) a single base substitution of guanine (CGA) by adenine (CAA) at codon 227, predicting a glutamine replacement of arginine at amino acid 227 (R227Q). The patient was a compound heterozygote for 2 mutations in the *SRD5A2* gene, each located in a different allele, as shown by sense and antisense sequences (Figure 2). The biologic parents were not available for study.

Discussion

Mutation of the *SRD5A2* gene in codon 227 has been described in Creole Brazilians and Mexican Americans who presented with a premature termination codon (Thigpen et al, 1992; Vilchis et al, 1997). In those patients, the testes were extra-abdominal, located in the inguinal ca-

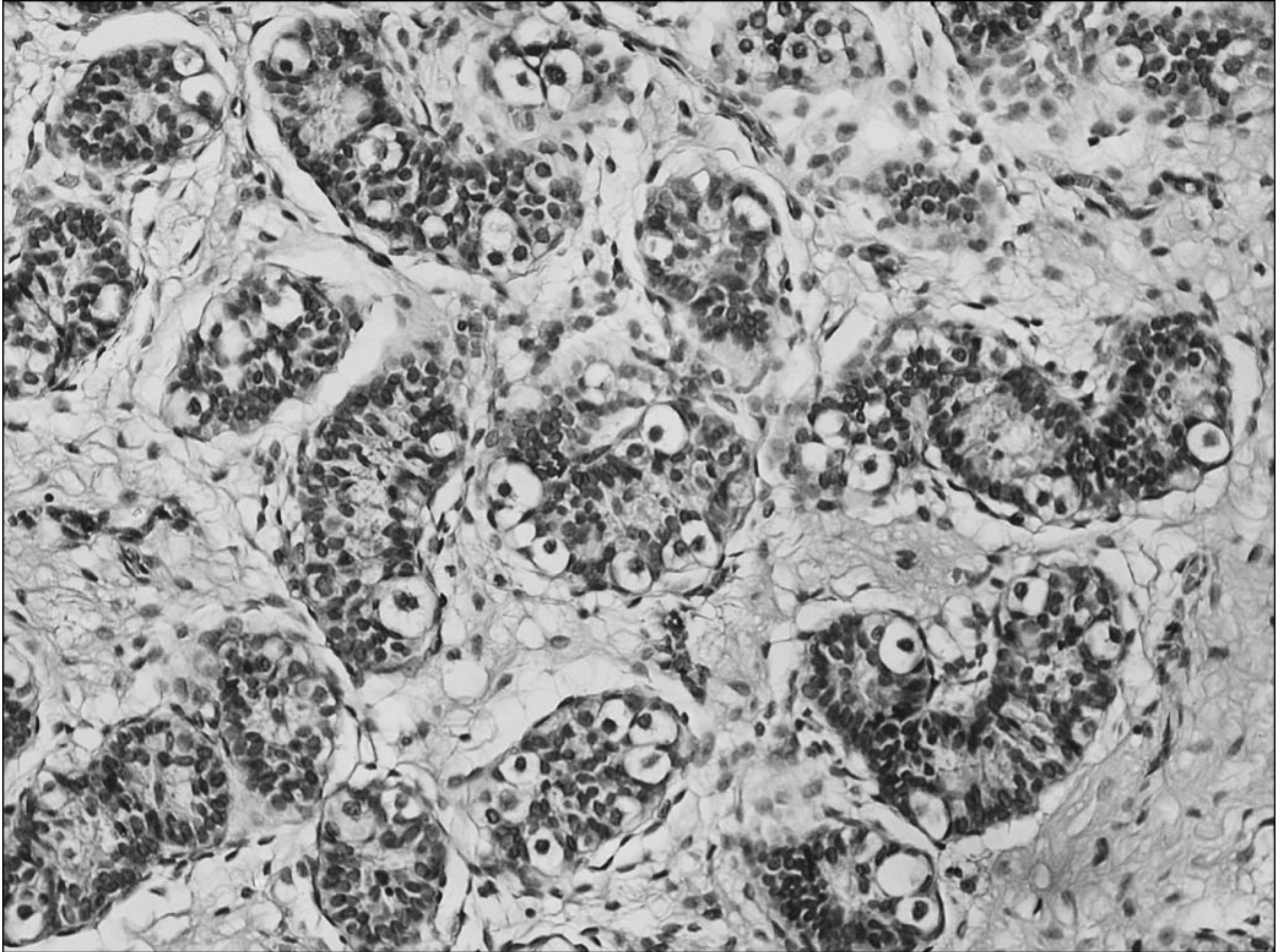

Figure 1. Left testis section. The epithelium of the seminiferous tubules is composed of immature Sertoli cells and a normal number of germinal cells. Hematoxylin-eosin (400× magnification).

nals, labia majora, or scrotum, but spermatogenesis was absent or was profoundly impaired in the 9 studied subjects. Whether the abnormality was a direct effect of the mutation or the secondary consequence of incomplete testis descent was uncertain (Johnson et al, 1986).

The R227Q variant has been observed only among Far East Asians, and these patients have presented with significantly reduced in vitro 5-α-reductase activity (3.2% of normal activity) (Makridakis et al, 2000). In a population-based case-control study conducted in China that focused on polymorphic markers in the *SRD5A2* gene and prostate cancer risk, the allele R227Q was considered a polymorphism, even though the heterozygous form was present only in 1 of 304 controls and in 2 of 191 patients (Hsing et al, 2001). The latter report confirmed the presence of the mutated allele in the Chinese population. Two brothers of Vietnamese origin, both of whom were homozygous for the Q allele, have been described by Hiort et al (1996) and Sinnecker et al (1996): one presented with scrotal hypospadias with a bifid scrotum and a small penis and thus could be considered an MPH patient, whereas the other presented with a small penis but otherwise normal male genitalia; in both patients, the T/DHT ratio was abnormally high after prolonged hCG stimulation. The mutated allele has also been described in the Japanese population: in 2% of normal male controls (1 of 50 normal boys and 1 of 50 fertile male adults) and in 3 of 81 males with a micropenis but otherwise normal genitalia (1 was homozygous for R227Q and the 2 others were compound heterozygotes with another mutation in the *SRD5A2* gene) (Sasaki et al, 2003b). This would mean that *SRD5A2* mutations might present a prevalence of approximately 3.7% in Japanese patients with a micropenis (penile length, <-2 SDs below the mean).

In addition to the mutated R227Q allele that has been described to date only in Asian populations, the patient of our study presented with a single nucleotide deletion in the other allele. Because the 2 mutations are located

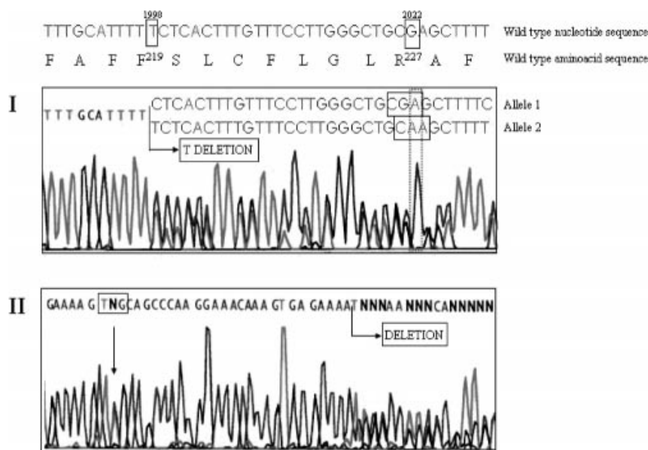

Figure 2. Electropherograms of the patient's 5-α-reductase type 2 gene (*SRD5A2*) exon 4: 1) sense sequence showing thymine (T) heterozygous deletion at position 1998 (GenBank: 338466, L03843) in one allele (allele 1), followed by a heterozygous sequence and a G substitution by A at position 2022 in the other allele (allele 2) compared to a wild-type sequence; and 2) the antisense sequence showing the 2 consecutive mutations.

very near to each other, this could be clearly demonstrated by analysis of the sense and antisense exon 4 sequences. The deletion affects codon 219 and predicts an anomalous protein with an abnormal sequence from amino acid 219 as well as a longer sequence of 277 amino acids. Unfortunately, no genital skin fibroblasts were available from this patient; hence, a determination of the *SRD5A2* messenger RNA (mRNA) sequence could not be conducted.

The degree of external genitalia virilization at birth in *SRD5A2* mutations has been described as extremely variable, ranging from a simple micropenis to an enlarged clitoris, labioscrotal fusion, single urethral meatus, and palpable gonads in the more highly affected cases. The less severe forms seem to carry mutations such as R227Q, for which residual enzyme activity has been detected (Hiort et al, 1996; Sasaki et al, 2003b). Our patient of Chinese origin presented with a severely affected phenotype, although a small prostatic utricle was detected by cystoscopy. This R227Q mutation was combined with a novel mutation in the other allele, which predicted that an abnormal protein would be produced. Female gender was assigned in China and was maintained in Spain for 3 reasons: 1) because of legal adoption requirements, 2) because of the severely affected phenotype, and 3) because of the molecular diagnosis, which had been performed only after an orchidectomy and a genitoplasty.

Testicular morphology has rarely been described in patients with 5-α-reductase enzyme deficiency, because a considerable percentage of patients were not diagnosed until puberty and male gender assignment changed at that age. Various reports on spermiogram and fertility analyses have yielded varying results and, although the volume was very low in all reports, one patient from an initial Dominican pedigree presented with a normal total sperm concentration count, motility, and morphology, but others from the same pedigree presented with oligospermia and azoospermia (Cai et al, 1994). Probably, DHT-deficient local formation more greatly affected seminal plasma formation, which requires development and function of prostate and seminal vesicles, than spermatogenesis. Paternity by intrauterine insemination with sperm from one such patient was achieved (Katz et al, 1997), and fertility in 2 brothers from a Swedish family harboring 2 mutations in exon 4 has also been demonstrated (Nordenskjold and Ivarsson, 1998). Whether abnormal spermatogenesis in these patients is the consequence of *SRD5A2* gene mutations or is secondary to the position of the cryptorchid testis that was uncorrected during early infancy has not been clarified. Testis morphology has been described in few of these patients at pubertal and postpubertal ages, and most reports have described abnormal Sertoli cell maturation and absent or incomplete spermatogenesis (Imperato-McGinley et al, 1980; Okon et al, 1980; Johnson et al, 1986; Steger et al, 1999). A recent report described an adult Japanese patient who was homozygous for the Q6X mutation in whom an inguinal testis presented a giant seminoma (Sasaki et al, 2003a). In the patient of our study, the testicular morphology was almost completely normal at 20 months of age, presenting a normal tubular diameter, spermatogonial index, and Sertoli cell number, as did the testis morphology at 4 months of age in another compound heterozygous patient for whom the *SRD5A2* mutations were present (personal data). This would mean that early testis descent is advisable when the male gender is officially assigned and that gonadectomy is to be performed as soon as possible when the female gender is chosen in order to avoid both virilization at puberty and testicular malignancy at postpubertal ages.

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