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COMMENT

Substitution Mutation C268Y Causes 17β-Hydroxysteroid Dehydrogenase 3 Deficiency*

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ABSTRACT

The 17β-hydroxysteroid dehydrogenase (HSD) type 3 isozyme catalyzes the conversion of androstenedione to testosterone in the testis. Deleterious mutations in the *HSD17B3* gene cause undermasculinization in genetic males attributable to impaired testosterone biosynthesis. Hence, a hallmark of this autosomal recessive disorder is a decreased plasma testosterone-to-androstenedione ratio. Here, a novel C268Y substitution mutation in exon 10 of the *HSD17B3* gene, in a subject with 17β-HSD 3 deficiency, is reported. Reconstitution experiments with recombinant protein reveal that substitution of tyrosine for cysteine at position 268 of 17β-HSD type 3 abrogates the enzymatic activity. This finding brings to 20 the number of mutations in the *HSD17B3* gene that cause male undermasculinization. (*J Clin Endocrinol Metab* **86:** 921–923, 2001)

THE ROLE OF the 17β-hydroxysteroid dehydrogenase (HSD) type 3 isozyme is to convert androstenedione to testosterone in the testes. Its gene, designated *HSD17B3*, contains 11 exons and is located on human chromosome 9q22 (1). 17β-HSD 3 deficiency is an autosomal recessive disorder that manifests, in males, as undermasculinization characterized by hypoplastic-to-normal internal genitalia (epididymis, vas deferens, seminal vesicles, and ejaculatory ducts) but female external genitalia and the absence of a prostate. This phenotype is caused by inadequate testicular synthesis of testosterone, which, in turn, results in insufficient formation of dihydrotestosterone in the anlage of the external genitalia and prostate during fetal development. At the expected time of puberty, there is a marked increase in plasma LH and, consequently, in testicular secretion of androstenedione. Hence, a diagnostic hallmark of this disorder is a decreased plasma testosterone-to-androstenedione ratio. Significant amounts of the circulating androstenedione are, however, converted to testosterone, in peripheral tissues, by an unidentified member of the 17β-HSD isozyme family, thereby causing virilization (2, 3). Women who are homozygous or compound-heterozygous for mutations that, in men, cause 17β-HSD 3 deficiency are asymptomatic (4, 5).

To date, 19 mutations in the *HSD17B3* gene that impair testosterone biosynthesis and cause male undermasculinization have been found. Fifteen of these molecular lesions are missense mutations, 3 are splice junction abnormalities, and 1 is a frame shift mutation (1, 2, 6–8). Here, we report 1 additional missense mutation in the *HSD17B3* gene of a genetic male who was severely undermasculinized.

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Subjects and Methods

Subjects

Patient designated 17HSD3-Cambridge 3. The patient was born with apparently normal female genitalia, after an uneventful pregnancy. The parents were of Pakistani origin and were first cousins. The infant presented, at age 3 weeks, with a right inguinal hernia. Physical examination revealed palpable gonads in both inguinal regions and a rather prominent clitoris. Biopsies were taken of both gonads, and the histology confirmed testicular tissue composed of Sertoli cells and spermatogonia in seminiferous tubules, with normal interstitial stroma. The karyotype was 46,XY, and an ultrasound examination of the pelvis showed no evidence of a uterus. There were four female siblings, each of whom had a 46,XX karyotype. Endocrine studies included a human (h)CG-stimulation test (1500 IU daily for 3 days) performed at 7 and 18 months of age. Basal LH and FSH at 18 months of age were 3.3 and 5.6 IU/L, respectively. Bilateral gonadectomy was performed at age 2 yr-3 months. A normal vas deferens was seen bilaterally, and an epididymis was identified on the right. The vagina was 2.5 cm in length; the slightly prominent clitoris was recessed. A genital skin biopsy was obtained to establish a fibroblast line. Histology of the testes showed Sertoli cells and spermatogonia in the seminiferous tubules, with normal interstitial stroma. Androgen receptor protein binding studies in genital skin fibroblasts were performed (9), and they revealed a B_{max} (receptor concentration) of 0.4 fmol/μg DNA (normal, >0.3 fmol/μg DNA) and binding affinity of 0.25 nmol/L (normal, 0.08–0.17 nmol/L). All investigations were performed after approval from the Local Ethics Committee for the program of research on disorders of sex differentiation.

Mutation detection and expression analysis

Genomic DNA was extracted from white blood cells, and mutations in the *HSD17B3* gene were analyzed by DNA sequencing of amplified exons using a thermostable DNA polymerase (2). Oligonucleotide-directed mutagenesis of the 17β-HSD type 3 complementary DNA (cDNA) was performed using the QuikChange Site-Directed Mutagenesis Kit (Stratagene, La Jolla, CA) according to the manufacturer's in-

structions. Complementary DNA expression in human embryonic 293 cells were performed using the Fugene 6 transfection reagent (Roche Diagnostics, Indianapolis, IN). Enzyme activity assays in intact cells were performed and analyzed in duplicates of 3 independent experiments, as described previously (6). Immunoblotting was performed using the monoclonal antibody MAb-C3–10 as described (6).

Results and Discussion

Initial endocrine analyses of a 46,XY prepubertal undermasculinized male, designated 17HSD3-Cambridge 3, revealed a plasma testosterone-to-androstenedione ratio of 0.25 and 0.29, after hCG stimulation performed at 7 and 18 months of age, respectively. These findings were indicative of the patient having 17β-HSD 3 deficiency, given that the plasma testosterone-to-androstenedione ratio normally is more than 0.8 after hCG stimulation, based on studies in 84 prepubertal undermasculinized patients without this genetic disorder (10). The patient demonstrated a slight decrease in androgen receptor binding affinity, as studied in genital skin fibroblasts. We have previously reported similar subtle abnormalities in androgen receptor binding characteristics in prepubertal subjects with 17β-HSD 3 deficiency (11), perhaps the result of deficient androgen production in fetal and early postnatal life. The changes, however, are not as conclusive as those observed in patients with androgen receptor-mutant syndromes of androgen insensitivity (9). DNA sequence analysis was performed on DNA fragments amplified from genomic DNA of 17HSD3-Cambridge 3, who was found to be homozygous for a novel C268Y substitution mutation attributable to a G → A transition in the second base of codon 268 in exon 10 of the *HSD17B3* gene (Fig. 1).

To determine the functional consequence of the C268Y mutation in the 17β-HSD type 3 protein, cDNA constructs encoding the normal enzyme and the C268Y substitution were individually transfected into cultured mammalian human embryonic kidney 293 cells. Figure 2A shows the results of a representative time-course experiment in intact cells

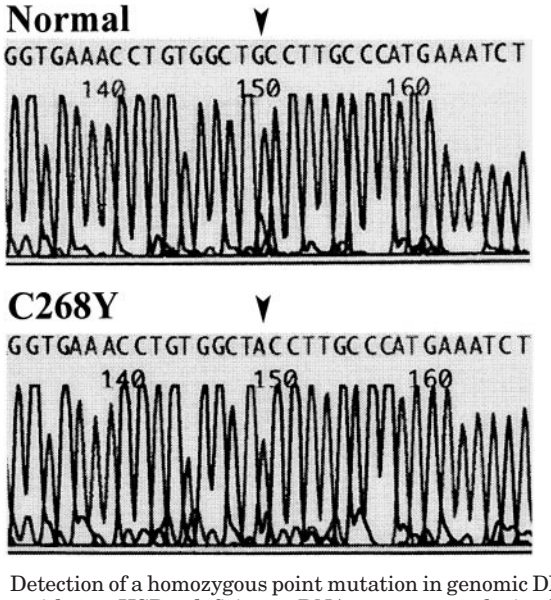

FIG. 1. Detection of a homozygous point mutation in genomic DNA of a subject with 17β-HSD 3 deficiency. DNA sequence analysis of exon 10 of the *HSD17B3* gene revealed a G → A transition mutation (*arrowheads*), resulting in a C268Y substitution at the protein level.

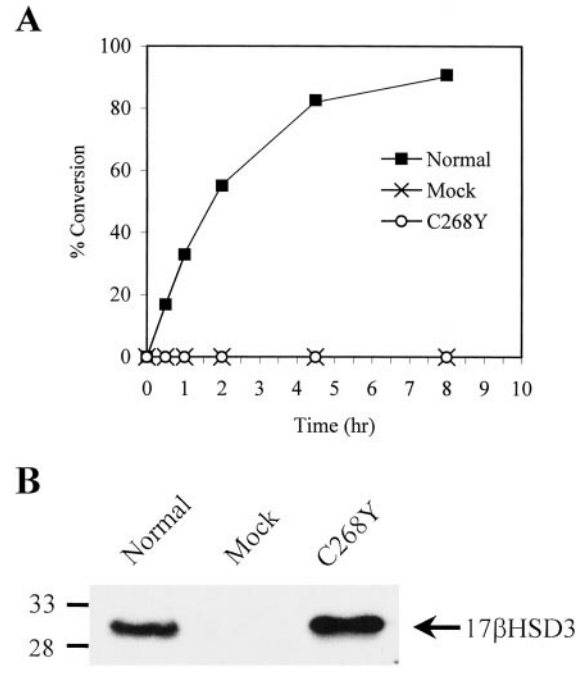

FIG. 2. Expression analysis of normal and mutant 17βHSD type 3 enzymes. Expression vectors containing the indicated cDNAs were transfected into human embryonic kidney 293 cells and assayed for activity by adding [^{3}H]androstenedione (0.1 μmol/L) to the medium. Aliquots of the medium were collected at the indicated times, and the conversion of [^{3}H]androstenedione to [^{3}H]testosterone was monitored by thin-layer chromatography and radioactivity scanning (A). The amount of 17β-HSD type 3 protein was detected by immunoblotting of total cell lysate (5 μg protein) using a monoclonal antibody directed against the 17βHSD type 3 protein (B). The positions of prestained molecular size markers are shown *on the left*.

using 0.1 μmol/L [^{3}H]androstenedione as substrate. The mutated protein was compared with the normal enzyme and vector transfected cells (mock), and the data show that the C268Y mutation abolished the 17β-HSD type 3 enzyme activity. To ensure equal expression of the different recombinant proteins in the transfected cells, we performed immunoblotting using a monoclonal antibody against the 17β-HSD type 3 protein (Fig. 2B). To investigate whether the mutation altered the enzyme's affinity for the substrate, a time-course experiment using 20 μmol/L [^{3}H]androstenedione was performed. However, no 17β-HSD activity over background (mock transfected cells) was observed with the Y268 protein (data not shown).

In the present study, we have characterized one novel missense mutation in the *HSD17B3* gene of a severely undermasculinized male. The C268Y mutation abrogated the enzyme activity, as revealed by expression studies of recombinant protein in transfected 293 cells. It is conceivable that substitution of tyrosine for cysteine at position 268 of 17β-HSD type 3 may result in a misfolded protein. Alternatively, the bulky hydroxyphenyl side-chain of tyrosine may interfere with substrate or cofactor binding. These issues remain to be investigated.

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