THE JOURNAL OF
SEXUAL MEDICINE

ORIGINAL RESEARCH

TRANSGENDER HEALTH

Biochemical Analysis of Four Missense Mutations in the *HSD17B3* Gene Associated With 46,XY Disorders of Sex Development in Egyptian Patients

Roger T. Engeli, MSc,[1] Maria Tsachaki, PhD,[1] Heba A. Hassan, PhD,[2] Christoph P. Sager, MSc,[3] Mona L. Essawi, MD,[2] Yehia Z. Gad, MD,[2] Alaa K. Kamel, MD,[4] Inas Mazen, MD,[5] and Alex Odermatt, PhD[1]

ABSTRACT

Background: Mutations in the *HSD17B3* gene are associated with a 46,XY disorder of sexual development (46,XY DSD) as a result of low testosterone production during embryogenesis.

Aim: To elucidate the molecular basis of the disorder by chemically analyzing four missense mutations in *HSD17B3* (T54A, M164T, L194P, G289S) from Egyptian patients with 46,XY DSD.

Methods: Expression plasmids for wild-type 17β-hydroxysteroid hydrogenase type 3 (17β-HSD3) and mutant enzymes generated by site-directed mutagenesis were transiently transfected into human HEK-293 cells. Protein expression was verified by western blotting and activity was determined by measuring the conversion of radiolabeled Δ^4-androstene-3,17-dione to testosterone. Application of a homology model provided an explanation for the observed effects of the mutations.

Outcomes: Testosterone formation by wild-type and mutant 17β-HSD3 enzymes was compared.

Results: Mutations T54A and L194P, despite normal protein expression, completely abolished 17β-HSD3 activity, explaining their severe 46,XY DSD phenotype. Mutant M164T could still produce testosterone, albeit with significantly lower activity compared with wild-type 17β-HSD3, resulting in ambiguous genitalia or a microphallus at birth. The substitution G289S represented a polymorphism exhibiting comparable activity to wild-type 17β-HSD3. Sequencing of the *SRD5A2* gene in three siblings bearing the *HSD17B3* G289S polymorphism disclosed the homozygous Y91H mutation in the former gene, thus explaining the 46,XY DSD presentations. Molecular modeling analyses supported the biochemical observations and predicted a disruption of cofactor binding by mutations T54A and M164T and of substrate binding by L196P, resulting in the loss of enzyme activity. In contrast, the G289S substitution was predicted to disturb neither the three-dimensional structure nor enzyme activity.

Clinical Translation: Biochemical analysis of mutant 17β-HSD3 enzymes is necessary to understand genotype-phenotype relationships.

Strengths and Limitations: Biochemical analysis combined with molecular modeling provides insight into disease mechanism. However, the stability of mutant proteins in vivo cannot be predicted by this approach.

Conclusion: The 17β-HSD3 G289S substitution, previously reported in other patients with 46,XY DSD, is a polymorphism that does not cause the disorder; thus, further sequence analysis was required and disclosed a mutation in *SRD5A2,* explaining the cause of 46,XY DSD in these patients. **Engeli RT, Tsachaki M, Hassan HA, et al. Biochemical Analysis of Four Missense Mutations in the *HSD17B3* Gene Associated With 46,XY Disorders of Sex Development in Egyptian Patients. J Sex Med 2017;14:1165–1174.**

Key Words: 46,XY Disorder of Sexual Development; *HSD17B3*; Hydroxysteroid Dehydrogenase; Mutation; Androgen; Testosterone

Received April 18, 2017. Accepted July 11, 2017.

[1]Division of Molecular and Systems Toxicology, Department of Pharmaceutical Sciences, University of Basel, Basel, Switzerland;

[2]Department of Medical Molecular Genetics, Division of Human Genetics and Genome Research, Center of Excellence for Human Genetics, National Research Center, Cairo, Egypt;

[3]Molecular Modeling, Department of Pharmaceutical Sciences, University of Basel, Basel, Switzerland;

[4]Department of Human Cytogenetics, Division of Human Genetics and Genome Research, Center of Excellence for Human Genetics, National Research Center, Cairo, Egypt;

[5]Department of Clinical Genetics, Division of Human Genetics and Genome Research, Center of Excellence for Human Genetics, National Research Center, Cairo, Egypt

http://dx.doi.org/10.1016/j.jsxm.2017.07.006

INTRODUCTION

17β-Hydroxysteroid dehydrogenase type 3 (17β-HSD3) converts the weak androgen Δ^4-androstene-3,17-dione (AD) into the potent androgen testosterone (T) using nicotinamide adenine dinucleotide phosphate (NADPH) as a cofactor.[1,2] This enzyme is predominantly expressed in testicular Leydig cells.[3,4] T can be further converted into 5α-dihydrotestosterone (DHT) in fetal genital tissues by 5α-reductase type 2 (SRD5A2).[5] T and DHT syntheses are crucial for normal male genital development during embryogenesis. T mediates the masculinization of the Wolffian ducts, seminal vesicles, and vas deferens, whereas DHT is responsible for the growth of the prostate and external genital development.[6,7]

Mutations in the *HSD17B3* gene (9q22) can lead to a 46,XY disorder of sex development (46,XY DSD) because of the lack of T and DHT production.[3] The prevalence of 17β-HSD3 deficiency in European countries is rather low, representing approximately 4% of all 46,XY DSD cases.[8] A study in the Netherlands reported an incidence of 1:147,000 in newborns.[9] However, in some Arabic populations, the prevalence is much higher because of the high rate of consanguineous marriages.[10–12] To date, more than 40 different mutations in introns and exons of the *HSD17B3* gene are known.[13–15] Patients with this disorder have the XY combination that characteristically produces undermasculinization. The external genitalia often appear to be female with or without clitoromegaly or labial fusion and a blind-ended vagina.[16,17] The spectrum of phenotypes can vary from ambiguous female genitalia to a male microphallus, depending on T and DHT levels during development.[18] Unfortunately, the diagnosis of 46,XY DSD is sometimes missed until puberty; moreover, the clinical phenotype overlaps with that of SRD5A2 deficiency to a great extent.[19] Patients frequently can be raised as girls and undergo sex change surgery after puberty because of ongoing masculinization owing to partial activity of the mutant 17β-HSD3 enzyme or extra-testicular T production by 17β-hydroxysteroid dehydrogenase type 5 (AKR1C3).[17,20]

In this study, we biochemically investigated four missense mutations in the *HSD17B3* gene that were previously proposed to cause 46,XY DSD in Egyptian patients.[13] The mutant enzymes were biochemically analyzed to check for their expression and remaining activity. In addition, a 17β-HSD3 homology model was applied to clarify the effect of the mutated amino acid residues on enzyme activity.

METHODS

Patient Details and Sequencing

Blood samples were collected from seven patients with 46,XY DSD, representing five unrelated families. The patients were recruited from the outpatient Clinical Genetics and Endocrinology Clinics at the Egyptian National Research Center (Cairo, Egypt). Written informed consent was obtained from all patients or their guardians according to the medical ethics committee at the Egyptian National Research Center. Clinical examinations, hormonal profile, and karyotyping provisionally suggested the diagnosis of 46,XY DSD with 17β-HSD3 enzyme deficiency. The age at referral ranged from very young (2 months) to postpubertal stages (16–20 years; Table 1). Postpubertal patients presented with primary amenorrhea or virilization, and prepubertal patients presented with ambiguous genitalia. Patients 5 to 7 were ascertained from one family; the older siblings (patients 6 and 7) were dizygotic twins who were reared as girls and seeking sex reversal later in development. Human chorionic gonadotropin (hCG) stimulation was performed only in prepubertal patients. Only the short hCG stimulation protocol was performed (hCG 2,500 IU administered by intramuscular injection daily for 3 consecutive days). Steroid hormone levels were measured before and after stimulation.

Genomic DNA extraction was performed according to a standard protocol.[21] For all patients, the coding regions and flanking intron and exon boundaries of the *HSD17B3* gene were sequenced. The *SRD5A2* gene was sequenced in selected cases bearing the *HSD17B3* G289S substitution. Polymerase chain reaction conditions and oligonucleotide primers were described previously.[13,22]

Plasmids and Molecular Cloning

The pcDNA3 plasmid containing the coding sequence of 17β-HSD3 followed by a C-terminal FLAG epitope tag (17β-HSD3-FLAG) was described previously.[23] This plasmid was used as a template for site-directed mutagenesis using the Pfu Polymerase (Promega, Madison, WI, USA) to introduce the corresponding mutation into the coding sequence of 17β-HSD3. Expression of these constructs led to mutant 17β-HSD3 proteins T54A, M164T, L194P, and G289S. All constructs were verified by sequence. The oligonucleotide primer sequences used for polymerase chain reaction are available on request.

Cell Culture and Western Blotting

For enzymatic activity determination, the wild-type and mutant 17β-HSD3-FLAG constructs were transiently expressed in the HEK-293 cell line (ATCC, Manassas, VA, USA). HEK-293 cells were cultured in Dulbecco's Modified Eagle's Medium (Sigma-Aldrich, St Louis, MO, USA) supplemented with 10% fetal bovine serum (Connectorate, Dietikon, Switzerland), penicillin 100 U/mL, streptomycin 100 μg/mL (Sigma-Aldrich), HEPES buffer 10 mmol/L, pH 7.4 (Life Technologies, Grand Island, NY, USA), and non-essential amino acid solution (Sigma-Aldrich). Cells were cultivated under standard conditions (37°C, 5% CO_2). Western blotting for confirmation of proper protein expression was performed as described previously.[23] Briefly, cells were transfected with the corresponding cDNA constructs using the calcium phosphate precipitation method and lysed 48 hours later with RIPA buffer (Sigma-Aldrich) containing protease inhibitor cocktail (Roche, Basel, Switzerland). For detection of the FLAG epitope,