Clinical and molecular spectrum of patients with 17β-hydroxysteroid dehydrogenase type 3 (17-β-HSD3) deficiency

Espectro clínico e molecular de pacientes com deficiência de 17β-hidroxiesteroide desidrogenase tipo 2 (17-β-HSD3)

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SUMMARY

The enzyme 17β-hydroxysteroid dehydrogenase type 3 (17-β-HSD3) catalyzes the conversion of androstenedione to testosterone in the testes, and its deficiency is a rare disorder of sex development in 46,XY individuals. It can lead to a wide range of phenotypic features, with variable hormonal profiles. We report four patients with the 46,XY karyotype and 17-β-HSD3 deficiency, showing different degrees of genital ambiguity, increased androstenedione and decreased testosterone levels, and testosterone to androstenedione ratio < 0.8. In three of the patients, diagnosis was only determined due to the presence of signs of virilization at puberty. All patients had been raised as females, and female gender identity was maintained in all of them. Compound heterozygosis for c.277+2T>G novel mutation, and c.277+4A>T mutation, both located within the intron 3 splice donor site of the *HSD17B3* gene, were identified in case 3. In addition, homozygosis for the missense p.Ala203Val, p.Gly289Ser, p.Arg80Gln mutations were found upon *HSD17B3* gene sequencing in cases 1, 2, and 4, respectively. Arq Bras Endocrinol Metab. 2012;56(8):533-9

SUMÁRIO

A enzima 17β-hidroxiesteroide desidrogenase tipo 3 (17-β-HSD3) catalisa a conversão de androstenediona a testosterona nos testículos, e sua deficiência é uma forma rara de distúrbio do desenvolvimento do sexo em indivíduos 46,XY. A desordem apresenta um amplo espectro de características fenotípicas e de resultados de dosagens laboratoriais. Neste trabalho, são relatados quatro casos de deficiência da 17-β-HSD3 com cariótipo 46,XY, ambiguidade genital em diversos graus, androstenediona aumentada, testosterona diminuída, e relação testosterona e androstenediona < 0,8. Em três das pacientes, o diagnóstico foi suspeitado devido à presença de sinais de virilização na puberdade. Todos os pacientes foram criados como mulheres, e a identidade de gênero feminino foi mantida em todas elas. A heterozigose composta da mutação nova c.277+2T>G e da mutação c.277+4A>T, ambas localizadas no sítio doador de *splicing* do íntron 3 do gene *HSD17B3*, foi identificada no caso 3. Além dessas, as mutações missense p.Ala203Val, p.Gly289Ser, p.Arg80Gln foram identificadas em homozigose pelo sequenciamento do gene *HSD17B3* dos casos 1, 2 e 4, respectivamente. Arq Bras Endocrinol Metab. 2012;56(8):533-9

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INTRODUCTION

The isoenzyme 17β-hydroxysteroid dehydrogenase type 3 (OMIM *605573, 17-β-HSD3), also known as 17-ketosteroid reductase, catalyses the conversion of the weak androgen substrate, androstenedione (Δ4), to the more biologically active testosterone

(T) in the testes. This conversion is essential for normal fetal development of male internal and external genitalia. The human gene, designated *HSD17B3*, contains 11 exons and is located on 9q22 (1-3).

17-β-HSD3 deficiency (OMIM #264300) is a rare autosomal recessive disorder form of male sex differentiation, characterized by hypoplastic-to-normal internal genitalia, absent or hypoplastic prostate, testes within the inguinal region, and female external genitalia at birth (4-9). Mutations in the *HSD17B3* gene are responsible for the disease, in which homozygous or compound heterozygous affected 46,XY individuals are usually born with female genitalia, and the disorder remains undetectable until puberty (10), when virilization of the external genitalia occurs. This is probably due to the conversion of the abundant Δ4 to T by other extragonadal 17-β-HSD isoenzymes, or due to residual 17-β-HSD3 activity (11). Many individuals raised as females develop a male gender identity, and decide to be reassigned as males after puberty (4-9,12,13).

17-β-HSD3 deficiency in prepubertal patients is clinically indistinguishable from partial androgen insensitivity syndrome, 5α-reductase type 2 deficiency, and other disorders of T biosynthesis. Diagnosis can be established by elevated Δ4 and low T serum levels that result in a T/Δ4 ratio lower than 0.8. In addition, there is a poor response to hCG stimulation test. In the absence of suggestive signs during childhood, the disorder will only be diagnosed at puberty upon virilization of affected individuals (7,14).

To date, a total of 29 mutations in the *HSD17B3* gene have been identified (Figure 1), including 21 missenses, one nonsense mutation, two frameshifs leading to downstream premature stop codon, four located at splice junctions leading to aberrant transcripts, and one duplication of exons 3-10 (4,15,16).

Here, we report the clinical and molecular pictures of four cases of 17-β-HSD3 deficiency.

MATERIALS AND METHODS

Genomic DNA was obtained from peripheral blood by proteinase K/phenol extraction method (17). The 11 exons and exon/intron junction sequences of *HSD17B3* gene were amplified by polymerase chain reaction (PCR). Primers used for PCR were chosen with Prime 3 primer designing tool; primer sequences are available upon request. Purification of PCR products was carried out using the Wizard® SV Gel and PCR clean-up system (Promega, Madison, WI, USA). Direct PCR fragment sequencing with sense and antisense primers was performed using Big Dye® Terminator Cycle Sequencing Kit V3.1 Ready Reaction (ABI PRISM/PE Biosystems, Foster City, CA, USA). Sequences were obtained in an automatic sequencer ABI 3130 DNA Analyzer (ABI PRISM/PE Biosystems), and were compared with the *HSD17B3* normal sequence (ENSEMBL – ENSG00000130948) using Chromas (reduced version-free software) and CLC Sequence Viewer v.6.2 (free software).
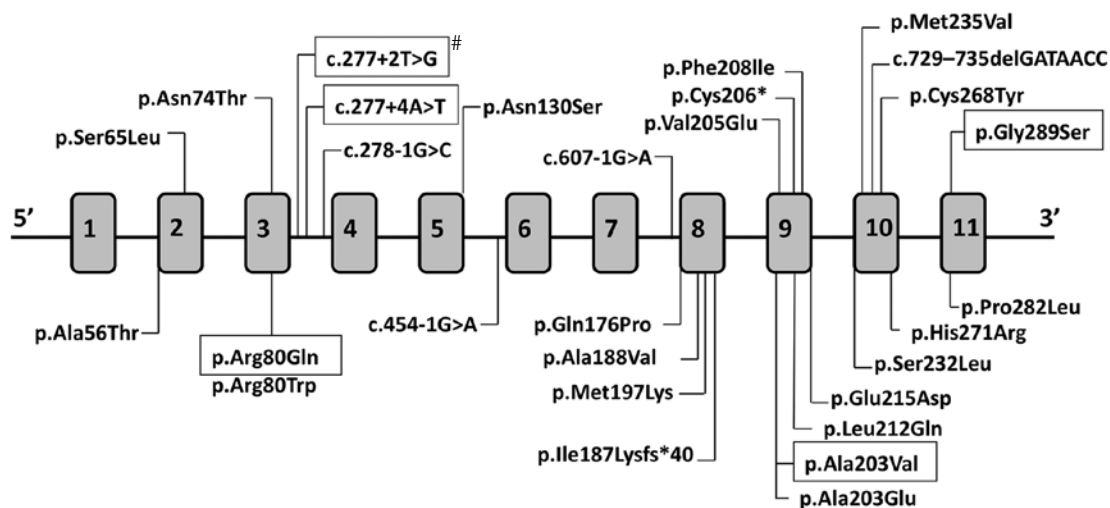
Figure 1. *HSD17B3* gene is represented with its 11 exons. Mutations found in 17-β-HSD3 deficiency are indicated. Black boxes highlight recurrent mutations also identified in this paper, and the # shows the mutation identified here for the first time.

CASE REPORTS

Case 1

A 16-year-old girl from Pariconha (Alagoas – Northeastern region of Brazil) was referred to us due to genital ambiguity and virilization at puberty. She was born at term by cesarean section to a 48-year-old mother, and weighted over 4.5 kg. Her parents were consanguineous (second cousins), and family history showed a sister with primary amenorrhea and absence of uterus, who had both gonadectomy and genitoplasty done during childhood. On physical examination, her weight and height were 68 kg and 168.4 cm, respectively; genitalia presented a 5.7-cm phallus and palpable gonads in labioscrotal folds with volumes of 15 cm³ and 10 cm³ for the right and the left gonad, respectively. A single perineal urethral opening with a short vagina were also observed. In addition, she presented facial hair and pubertal development Tanner 3 for breasts, and Tanner 5 for pubic hair (Figures 2A and 2B). The karyotype was 46,XY and laboratory data are shown in table 1. *HSD17B3* gene sequencing revealed the homozygous p.Ala203Val missense mutation within exon 9 (Figure 3A). After psychological evaluation of the patient and the family, they decided to maintain the female gender. Bilateral gonadectomy and feminizing genitoplasty were performed, and hormonal replacement with estrogens was administrated. The analysis of both gonads indicated pubertal testes with discrete peritubular fibrosis. As she maintained the female gender, vaginal dilation will be performed as soon as she decides to initiate sexual activity.

Case 2

A 4-year-old girl, from Oliveira dos Brejinhos (Bahia – Northeastern region of Brazil), was referred to us to investigate genital ambiguity with palpable gonads. She was the second child of non-consanguineous parents, and was born at term after an uneventful pregnancy. At physical examination, her weight was 16.2 kg and height 102 cm. A 3.7-cm phallus, single perineal opening, labioscrotal fusion, and bilateral palpable gonads within inguinal region were observed. Cytogenetic analysis indicated a 46,XY karyotype. Laboratory data are shown in table 1. *HSD17B3* gene sequencing showed homozygosis for the p.Gly289Ser substitution in exon 11 (Figure 3B). Her family decided to maintain the female

gender. Therefore, gonadectomy, clitoroplasty, and introitoplasty were performed. Histological analysis of the gonads showed normal pre-pubertal testes.

Case 3

A 13-year-old girl from São Luís (Maranhão –Northeastern region of Brazil) was referred to us due to signs of virilization such as acne, hirsutism, voice deepening, and phallus enlargement since the age of 11. She was born at term after an uneventful pregnancy. Her parents were healthy, non-consanguineous, and from small cities Maranhão State (Fortuna and Buriti Bravo). She had two sisters, both with telarche, and without signs of virilization, and one brother without sex ambiguity. On physical examination, the patient was 164 cm tall and weighed 59.8 kg. Her breasts were Tanner stage 1. She had increased hair on the abdomen and face, phallus enlargement (3.7 cm), and the skin of labioscrotal folds were pigmented and rugged. Pubic hair was Tanner stage 4. Her vagina ended in a 3-cm pouch, and no gonads were palpable (Figures 2C and 2D). Karyotype was 46,XY. Results of hormonal evaluation are shown in table 1. MRI of the abdomen and pelvis did not show an uterus or a prostate. However, seminal vesicles were identified, and both gonads were found at the inguinal region. *HSD17B3* gene sequencing showed heterozygosis for two nucleotide changes within intron 3 splice donnor consensus sequence, c.277+4A>T, and the novel c.277+2T>G (Figure 3C). The analysis of the family indicated that the first variant was inherited from her father, and the second from her mother (data not shown). After female gender identity was confirmed by long-term psychological evaluation, video laparoscopy was performed to remove the gonads, and estrogen replacement therapy was initiated, followed by vaginal dilation. Histologic examination of both gonads showed bilateral testes with slight peritubular fibrosis, germ-cell aplasia, and Leydig cell hyperplasia, without evidence of malignancy.

Case 4

A 14-year-old girl from Alto do Araguaia (Mato Grosso – Central-Western region of Brazil) was brought to us due to genital ambiguity and virilization at puberty. She was the second child of a consanguineous marriage (first cousins). Family history

showed a 2-year-old female maternal cousin who presented clitoromegaly, and a 12-year-old female paternal cousin with hirsutism. Upon physical examination, the patient was 160 cm tall and weighed 41.4 kg. She had a 4.5-cm phallus, a single perineal opening, partial fusion of labioscrotal folds with pigmentation, and bilateral palpable gonads in the lower third of inguinal canal. Facial, axillary, abdominal, and pubic hair (Tanner 3) were observed, as well as Tanner 3 breast development. Karyotype was 46,XY. Hormonal evaluation data are shown in table 1. Pelvic ultrasonography did not show a uterus or a prostate. Genitography showed a urogenital sinus with a short vagina. *HSD17B3* gene sequencing showed homozygosis for p.Arg80Gln mutation in exon 3 (Figure 3D). The patient decided to maintain the female gender; therefore, gonadectomy and feminizing genitoplasty were performed. Histological gonadal analysis indicated normal pubertal testes. Estrogen replacement therapy was initiated, and the patient decided to postpone vaginal dilation.
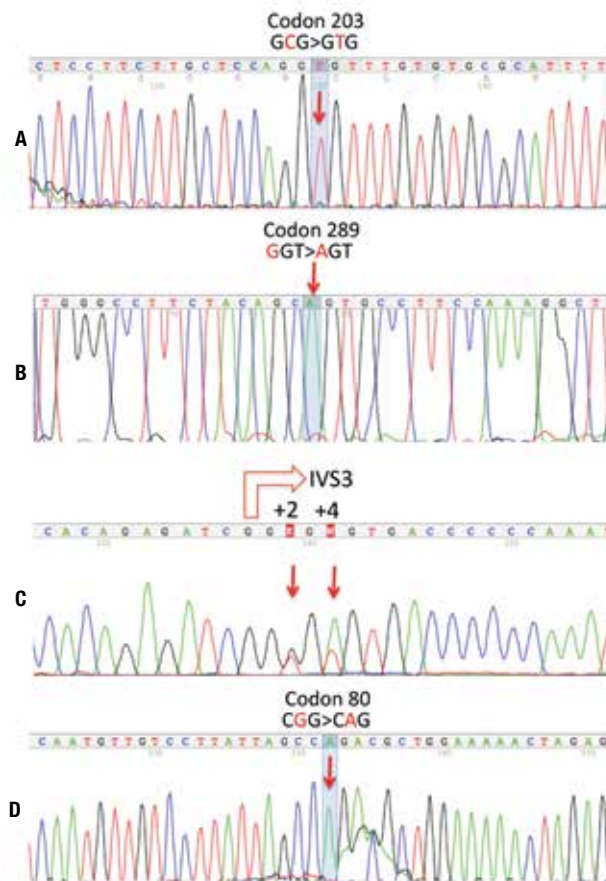

Figure 3. Parts of the eletropherograms obtained for *HSD17B3* gene sequencing: **A)** Homozygous GCG>GTG nucleotide change within exon 9 identified in case 1; this substitution causes the replacement in residue 203, usually alanine, by valine (p.Ala203Val). **B)** Homozygous GGT>AGT nucleotide change within exon 11 identified in case 2; this substitution causes the replacement in residue 289, usually glycine by serine p.Gly289Ser. **C)** Compound heterozygosis for c.277+2T>G and c.277+4A>T nucleotide changes in case 3, which were inherited from the mother and the father, respectively (data not shown); the two mutations are within the donor splice site consensus sequence of intron 3; the empty red arrow indicates the beginning of IVS3 at exon 3/intron 3 boundary. **D)** Homozygous CGG>CAG nucleotide change within exon 3 identified in case 4; this substitution causes the replacement in residue 80, usually arginine by glutamine (p.Arg80Gln).

Figure 2. External genitalia of patients with HSD17B3 deficiency. Case 1 – figures **A** and **B**, and case 3 – figures **C** and **D**.

DISCUSSION

We report here four cases of 17-β-HSD3 deficiency, a rare disorder that was described for the first time in 1971 (18,19). It is a rare form of 46,XY DSD that affects testosterone biosynthesis (4). Its prevalence is higher among the Arab population, ranging from 1:100 to 1:300, due to a high frequency of consanguineous marriages (20). Two out of the four cases described here were born to consanguineous parents (cases 1 and 4), and showed homozygosis

Table 1. Hormonal profile of four cases with HSD17B3 deficiency

	Case 1	Case 2	Case 3	Case 4	Male reference range (12 – 19 yr)
Age (years)	16	4	13	14	
Puberty	+	-	+	+	
LH (IU/L)	12.1	0.4	16.0	10.5	0.5 - 5.3 [#]0.1 - 1.2
FSH (IU/L)	7.4	2.0	6.7	8.7	1.4 - 18.1 [#]0.8 - 2.3
T (ng/mL)	4.2	0.8*	3.4	3.1	0.1 - 5.0
Δ4 (ng/mL)	6.8	1.3*	6.5	4.8	0.7 - 1.9
DHT (ng/mL)	0.49	0.21*	0.42	0.58	0.25 - 1.20
E1 (pg/mL)	32.1	NP	41.0	42.7	17.0 - 44.0
E2 (pg/mL)	20.9	NP	25.8	22.2	< 50.0
DHEA sulfate (µg/mL)	3.8	1.9	2.5	3.9	1.0 - 4.2
T/DHT ratio	8.6	3.8*	8.1	5.3	< 10.0
T/Δ4 ratio	0.62	0.61	0.50	0.65	> 0.8
E2/E1 ratio	0.65	NP	0.63	0.52	-

* After 3 consecutive days of 1,500 IU of intramuscular of human chorionic gonadotropin;

[#] pre-pubertal male reference range; Δ4: androstenedione; E1: estrone; E2: estradiol;

NP: not performed; T: testosterone.

for *HSD17B3* mutations. On the other hand, case 2, who has non-consanguineous parents, also showed a homozygous mutation in the *HSD17B3* gene. In this case, the patient and her parents were born at a small city of the countryside of the State of Bahia.

Different *HSD17B3* gene mutations confer a wide range of phenotypic characteristics to 46,XY affected individuals. These may vary from predominantly female genitalia, as in case 3; to mild virilized female genitalia, as in case 2; to evident genital ambiguity with palpable gonads, as in cases 1 and 4; to predominantly male genitalia with microphallus and hypospadias (4-8). This variability in phenotypes may correlate with partial activity of mutated 17-β-HSD3 in the testes, or to an extragonadal conversion of Δ4 to T by other 17-β-HSD isoenzymes (11).

As the most common clinical presentations of 17-β-HSD3 deficiency are female or mild virilized female genitalia, most patients do not have the diagnosis of 46,XY DSD at birth, and are registered and raised as females (4,5,8). Therefore, diagnosis is only established at puberty for most patients (4-8). Conversely, some cases may be diagnosed early, because they seek medical care during childhood due to some degree of virilization with palpable gonads (4,5,7).

If 17-β-HSD3 deficiency is not diagnosed in childhood, and gonadectomy is not performed, patients may present virilization at puberty (4,5,7).

Main signs of virilization are increased hair growth all over the body and face, deepening of the voice, and android fat distribution, in addition to phallus elongation reaching 5-8 cm in length, which may also be observed in response to peripheral T conversion. However, the phallus will always be shorter than a normal-sized penis (4,5,7).

The diagnosis of 17-β-HSD3 deficiency may be suspected upon laboratory investigation, and may be confirmed with molecular analysis. As verified in all cases reported here, patients have, respectively, high and low to normal Δ4 and T serum concentrations (4,5,7). When T/Δ4 ratio is less than 0.8, 17-β-HSD3 deficiency is suggested (7,14). The literature also refers to increased serum concentrations of DHEA and DHEA sulfate (4,5,7). However, these elevated values are not always observed.

The mutation p.Arg80Gln identified in case 4 is the most common one in Mediterranean (21) and Brazilian patients (6). McKeever and cols. (22) demonstrated that p.Arg80Gln causes a significant decrease in the rate of enzymatic reaction leading to approximately 5% of residual enzyme activity, and a 1/60 reduction in the binding affinity of the mutant protein to NADPH cofactor. Therefore, it is suggested that the R in residue 80 of the protein structure should be critical in maintaining appropriate levels of enzymatic activity, and to promote normal human male devel-

opment. This mutation was first described in a Palestinian family from the Gaza Strip (20). Further, it was also identified in Brazilian families with no Palestinian ancestry, and of probable Portuguese origin (6). However, its recurrence, not only in patients of Arab origin, but also in Dutch patients, was reported for individuals who were homo- or heterozygous for this mutation (5). Therefore, p.Arg80Gln may represent a founder effect, common among Arabs from different regions of Israel, Lebanon, and Syria (23). This fact led to the hypothesis that it has been introduced in Portugal and Spain by Phoenicians who migrated from Syria, Lebanon, and Israel around 750 B.C. (24,25). Its introduction in the Netherlands could have occurred during the Spanish domination in the sixteenth and seventeenth centuries and, in Brazil, by Portuguese colonizers and during the Dutch invasion of the Northeastern region of the country.

The p.Ala203Val mutation identified in case 1 was first described by Geissler and cols. (26), in a patient from São Paulo – Brazil. This mutation was assayed for the ability to convert Δ4 into T, and it completely inactivated the enzyme 17-β-HSD3, indicating a good correlation with the phenotype of case 1.

Moghrabi and cols. (27) reported the missense substitution p.Gly289Ser (SNP – CM023631). This mutation has been considered a polymorphism since it is frequent in all populations reported in the screening of 1,000 genomes (28). It also did not alter the *in vitro* enzymatic activity. However, it was identified in a compound heterozygous patient who also carried the well-known deleterious p.Asn130Ser mutation. It was supposed that this patient carried alterations in regulatory regions of the *HSD17B3* gene that may explain the phenotype (5). In addition, p.Gly289Ser amino acid substitution has been associated with increased risk of developing prostate cancer, and to hypospadias in individuals carrying the S289 allele in homozygosis, once mRNA expression levels were significantly lower for the mutant S289 than for the wild-type G289, indicating that it may not be as neutral as it had been initially considered (29,30). Since we did not evaluate either 5' or 3' regulatory regions, we can speculate that the homozygous p.Gly289Ser in case 2 may be associated with the phenotype, and some other nucleotide change in regulatory regions could act synergistically with the mutation.

Case 3 was found to be compound heterozygous for two nucleotide changes affecting intron 3

splice donor region. The c.277+4A>T mutation was reported in 1996 by Andersson and cols. (9), who identified it in homozygosis in three families, and in heterozygosis in two other families. Those results indicated it as one of the most prevalent mutations found in subjects with 17-β-HSD3 deficiency. It is located within the intron 3 canonical splice donor site, and was shown to disrupt normal splicing by Boehmer and cols. (5), who analyzed the *HSD17B3* cDNA prepared from testis mRNA of a homozygous patient. The result of cDNA sequencing showed a transcript where exon 3 had been skipped and, in minor amounts, a transcript with deletion of both exons 3 and 4 (5,31).

The mutation c.277+4A>T is found in populations worldwide, including Dutch, Germans, white Australians and white Americans, who share the same marker genotype, and are likely to be identical by descent (5). The c.277+2T>G mutation was also identified in case 3. It changes the almost invariant GT at the splice donor site to GG in intron 3. This mutation was identified here for the first time, and it probably suppresses the normal splicing so that it can either activate a cryptic splice site, or lead to exon 4 skipping. The *in silico* search for splicing sites using the online Splice Site Prediction NNSPLICE 0.9 version indicated that the mutation eliminated the normal donor splice at that position (data not shown). As testicular samples are not accessible for *in vitro* studies, the construction of mini-genes will be an alternative to test an aberrant splicing process in this case.

Patients with 17-β-HSD3 deficiency undergo virilization if gonadectomy is not performed before puberty, and some may adopt the male gender (4-8,12,13). Such a decision usually occurs in late adolescence or early adulthood, and the frequency varies between 39% and 64% (13). The explanation for the male option to occur is still unclear. Conversely, most patients who undergo prepubertal gonadectomy remain in the female gender (4-8). According to the literature, patients who choose to remain as females must undergo gonadectomy not only to avoid virilization, but also because of the 28% risk of malignant germ cell tumors (32). These patients should receive estrogen replacement therapy for the development of secondary sexual characteristics (4). All patients reported here, even those diagnosed at puberty, decided to maintain female identity after psychological evaluation of the patient and family members.

In conclusion, we report mutations in *HSD17B3* gene, including the novel c.277+2T>G mutation, in four cases of 17-β-HSD3 deficiency with different clinical laboratorial and presentations.

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