CASE REPORT

A novel missense (R80W) mutation in 17-β-hydroxysteroid dehydrogenase type 3 gene associated with male pseudohermaphroditism

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Abstract

Objective: Deficit of the testosterone converting enzyme 17-β-hydroxysteroid dehydrogenase (17β-HSD) has been shown to be responsible for male pseudohermaphroditism (MPH). We analysed the gene encoding 17β-HSD type 3 (17β-HSD3) in a patient with MPH.

Methods: We studied a 46, XY new-born diagnosed as having MPH. The child also had other congenital disorders, including a giant omphalocele and Fallot’s tetralogy, and died of post-surgical complications at age 4.5 months. Basal hormonal levels, and after human chorionic gonadotrophin stimulation, suggested a deficiency in 17β-HSD as the biochemical defect underlying this MPH. PCR amplification and subsequent sequencing of all coding exons of the 17β-HSD3 gene were performed on genomic DNA from the patient and both parents. Messenger RNA was extracted from the patient’s testis and 17β-HSD3 cDNA was synthesized, PCR amplified and sequenced.

Results: Sequencing revealed the presence of a homozygous missense mutation (R80W) in exon 3 of the 17β-HSD3 gene, which was also present in single doses in both parents, in accordance with the recessive inheritance of the defect. No other mutation was found, and cDNA sequencing confirmed correct synthesis and processing of 17β-HSD3 mRNA.

Conclusions: Confirming the abnormal Δ4-androstenedione/testosterone ratios that suggested 17β-HSD deficiency, a homozygous missense mutation in the gene coding for this enzyme was identified in the patient with MPH. This study adds further genetic evidence to the role of 17β-HSD3 in male sexual development. There is no evidence supporting the association of this mutation in 17β-HSD3 with the congenital malformations other than MPH present in the child.

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Introduction

The developmental pathway that leads to the establishment of the male phenotype in human XY embryos can be divided into two sets of consecutive events that lead first to the formation of testes, directed by a cascade of genes, including the Y chromosome gene SRY (1–3), and secondly to the development of male genitalia through the action of the Mullerian inhibiting hormone and testosterone, both secreted by the foetal testes (4, 5). Male pseudohermaphroditism (MPH) consists of the presence of female or incompletely virilized external genitalia in 46, XY individuals, who nevertheless present testes and Wolffian structures, and is often due to a defect in testosterone action resulting from mutations in the androgen receptor gene (6) or in the steroid 5α-reductase 2 gene which encodes the enzyme responsible for conversion of testosterone into dihydrotestosterone in the urogenital tract (7). Deficiency of the enzymes involved in the conversion of cholesterol into testosterone has also been associated with MPH (8) and the gene encoding one of these enzymes, 17-β-hydroxysteroid dehydrogenase type 3 (17β-HSD3) has been cloned and mapped to chromosome 9q22, and is expressed only in testes (9, 10). To date, 17 recessive mutations in this gene have been associated with MPH (9, 11–13) and 46, XY patients with deficiency of this enzyme are usually individuals with testes and male Wolffian structures who are nevertheless classified as females at birth because of female or mildly virilized external genitalia. However, at the time of expected puberty, in those cases where testes have not been removed, these individuals virilize, probably as a response to elevated serum testosterone levels, which are thought to be a product of extra-glandular testosterone formation, or also to a reduction.
in the severity of the testosterone biosynthesis defect with age (14, 15).

In this report, we have studied the \(^{17}B\)-HSD3 gene in a case of MPH who also presented other congenital defects, and in his healthy parents. We report a homozygous mutation in exon 3 of \(^{17}B\)-HSD3 that may account for the MPH phenotype, since this gene is only expressed in testes.

**Subject and methods**

**Clinical and hormonal data**

The patient was a 46, XY new-born (39 weeks’ gestation, 3.5 kg at birth) showing an MPH phenotype with female external genitalia but with ovoid masses compatible with testes in the labia majora and absence of Mullerian structures. The child also presented other congenital malformations including omphalocele and Fallot’s tetralogy. Serial basal hormonal levels, as well as after human chorionic gonadotrophin (hCG) stimulation (1000 IU/24 h \(\times 3\)) (Table 1) demonstrated a reduction of testosterone synthesis, and identified \(\Delta_4\)-androstenedione as the most elevated precursor, suggesting a deficiency in \(^{17}B\)-HSD as the biochemical defect underlying this MPH. The karyotype did not show any major chromosomal defect. Although surgery corrected the omphalocele successfully, the child died because of complications resulting from surgery of his cardiac defect at age 4.5 months. Autopsy confirmed the absence of a uterus or other Mullerian structures, and revealed the presence of well developed Wolffian structures, including epididymis and vas deferens. Microscopical examination of the testes showed normal testicular histology (Fig. 1).

**Molecular studies**

DNA was extracted from peripheral blood samples using standard methods. Independent amplification of the 11 exons of the \(^{17}B\)-HSD3 gene was done in the patient using primers flanking each of the exons (11). Amplification products were purified using ‘Wizard-PCRpreps’ resin (Promega, Madison, WI, USA) and direct sequencing was carried out with each of the amplification primers using either Sequenase (USB, Cleveland, OH, USA) and radiolabelling by \(^{35}\)S-dATP incorporation or a Cy5-Autocycle sequencing kit (Pharmacia-Biotech, Uppsala, Sweden) with Cy5-labelled primers. Reactions were run on a 6% sequencing gel and data were collected either manually (radioactive sequencing) or automatically with an ‘Alf express’ automated sequencer (Pharmacia-Biotech).

Restriction enzyme digestions of exon 3 of the patient, both parents and 42 unrelated healthy individuals were done using 20% of purified PCR products with HpaII (Boehringer Mannheim, Mannheim, Germany) following the manufacturer’s instructions, and resulting products were separated by gel electrophoresis in 3% agarose.

Messenger RNA was purified from 100 mg of the patient’s gonadal tissue removed after autopsy using a ‘Quick-Prep Micro’ mRNA purification kit (Pharmacia-Biotech). First strand cDNA was produced using M-MuLV reverse transcriptase and oligo(dT) priming, and was subsequently amplified with 25 pmol each of the flanking primers \(17B1\) and \(17B22\) (11) with 30 cycles of 1 min at 95 \(^\circ\)C, 30 s at 65 \(^\circ\)C and 1 min 30 s at 72 \(^\circ\)C. The amplified cDNA was sequenced using cycle sequencing and Cy5-labelled primers \(17B1\), \(17B22\) and an internal primer \(17BI\) (5'0-CTAGCAAAACGTGGACTCAATGTTGT-3') to confirm correct processing of the message.

**Results and discussion**

Early diagnosis of \(^{17}B\)-HSD deficiency is difficult because many cases show no significant clinical problems and are only characterized at puberty because of primary amenorrhoea and different degrees of virilization. Even

<table>
<thead>
<tr>
<th>Days of postnatal life</th>
<th>3</th>
<th>13</th>
<th>15</th>
<th>22</th>
<th>42</th>
<th>51</th>
<th>64</th>
<th>72</th>
<th>75 hCG*</th>
<th>126</th>
</tr>
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<tbody>
<tr>
<td>T (nmol/l)</td>
<td>3.46</td>
<td>0.66</td>
<td>2.25</td>
<td>1.31</td>
<td>&lt;0.35</td>
<td>&lt;0.35</td>
<td>&lt;0.35</td>
<td>0.38</td>
<td>1.69</td>
<td>&lt;0.35</td>
</tr>
<tr>
<td>(\Delta_4) (nmol/l)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>22.68</td>
<td>6.45</td>
<td>1.74</td>
<td>0.70</td>
<td>4.19</td>
<td>16.75</td>
<td>1.26</td>
</tr>
<tr>
<td>(\Delta_4/T)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>17</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>11</td>
<td>9.8</td>
<td>–</td>
</tr>
<tr>
<td>DHEA (nmol/l)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>7.95</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>11</td>
<td>9.8</td>
<td>–</td>
</tr>
<tr>
<td>17-OH-P (nmol/l)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>26.97</td>
<td>15.15</td>
<td>–</td>
<td>8.18</td>
<td>6.97</td>
<td>–</td>
<td>–</td>
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<tr>
<td>E(_2) (pmol/l)</td>
<td>462.5</td>
<td>187.2</td>
<td>187.2</td>
<td>106.4</td>
<td>(&lt;36.7)</td>
<td>44.0</td>
<td>(&lt;36.7)</td>
<td>36.7</td>
<td>58.7</td>
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<tr>
<td>LH (mIU/ml)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1.6</td>
<td>(&lt;1.5)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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</tr>
<tr>
<td>FSH (mIU/ml)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>(&lt;1.5)</td>
<td>(&lt;1.5)</td>
<td>–</td>
<td>–</td>
<td>–</td>
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</tr>
</tbody>
</table>

* hCG stimulation test. T: testosterone; \(\Delta_4\): androstenedione; DHEA: dehydroepiandrosterone; 17-OH-P: 17-hydroxy-progesterone; E\(_2\): oestradiol; LH: luteinizing hormone; FSH: follicle-stimulating hormone.
If MPH is suspected during the neonatal period, and testes are identified, basal testosterone levels can be near the normal range and be misleading, and 17β-HSD deficiency is not identified. Unequivocal diagnosis of 17β-HSD deficiency may require an hCG stimulation test as well as observation of the Δ4-androstenedione/testosterone ratio, rather than the sole measurement of testosterone levels (Table 1). In our patient, such elevated ratios (Table 1) suggest 17β-HSD deficiency as the cause of MPH, and make the presence of androgen resistance unlikely (13). Testicular histology may help to rule out other abnormalities, such as complete testicular dysgenesis or Leydig cell hypoplasia/aplasia, which could also account for decreased testosterone levels. Our patient presented normal testicular structures (Fig. 1), further supporting a defect in testosterone biosynthesis as responsible for the MPH phenotype.

Amplification of the 11 exons of the 17β-HSD3 gene in our MPH patient resulted in individual bands of the expected size, and sequence analysis revealed the presence of a homozygous C to T transition in position 37 of exon 3 (i.e. an Arg (CGG) to Trp (TGG) change in codon 80 of the enzyme) (Fig. 2). The mutation destroys the only HpaII restriction site in exon 3, providing a useful screening tool to determine if this amino acid change is also present, as a normal polymorphism, in the general population. No other substitution was found in the whole open reading frame, and sequencing of the 1300 bp reverse transcriptase PCR product confirmed the presence of the transcript in testes and correct processing, as well as the R80W mutation. This mutation was also present in single doses in both parents, as confirmed by sequencing and restriction enzyme analysis (Fig. 3), and agrees with the accepted recessive inheritance of the trait. Restriction enzyme

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Figure 1 Light photomicrograph of the patient’s testis showing normal testicular structure. (400 ×, haematoxylin/eosin staining).

Figure 2 Partial sequence data of exon 3 of 17β-HSD3 in a patient with MPH, showing a homozygous missense point mutation (C to T) in codon 80 of the enzyme which results in an amino acid substitution (Arg to Trp).
coincidental event. The association of the mutation in the parents, although it could well be a manifestation of accumulation of unidentified genetic alterations, could suggest the presence of a common ancestor harbouring the mutation in this position, together with their common geographical origin (a small rural village in Central Spain), could suggest the presence of an identical point mutation in both parents. Analysis of exon 3 from 42 unrelated individuals did not detect the mutation in any of the 84 independent chromosomes, reducing the probability of the amino acid change being present in a normal variant of the enzyme. This residue has been previously implicated in optimal binding of NADPH cofactor, and a different missense mutation in the same position (R80Q) has been previously associated with several cases of MPH (9, 12, 16) and is the most frequent mutation in 17β-HSD3 associated with MPH. Although in vitro studies demonstrate a residual activity of the enzyme with the R80Q mutation, the number of cases of MPH harbouring a mutation in this position, together with the present report, confirms the importance of arginine 80 in enzyme function (9, 11).

Finally, although the parents deny consanguinity, the presence of an identical point mutation in both, together with their common geographical origin (a small rural village in Central Spain), could suggest the presence of a common ancestor harbouring the mutation as a possible explanation. Moreover, the absence of chromosomal defects which could account for the severe congenital malformations in the child could indicate accumulation of unidentified genetic alterations in the parents, although it could well be a coincidental event. The association of the mutation in 17β-HSD3 with the child’s MPH phenotype adds genetic evidence to the role of this enzyme in the normal development of male reproductive structures, and in the implication of codon 80 in normal enzyme activity.

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