Distinguishing primary from secondary Δ4-3-oxosteroid 5β-reductase (SRD5B1, AKR1D1) deficiency by urinary steroid analysis

Short title: Urinary steroid analysis of SRD5B1 deficiency

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Abbreviations: 5β-reductase (SRD5B1), Δ4-3-oxosteroid 5β-reductase; GGT, γ-glutamyltransferase; TBA, total bile acids; NH, neonatal hemochromatosis; THF, tetrahydrocortisol; THE, tetrahydrocortisone; Cr, creatinine; GC-MS, gas chromatography-mass spectrometry; MRI, magnetic resonance imaging.
Abstract

Objective: Deficiency of Δ⁴-3-oxosteroid 5β-reductase (5β-reductase), a bile acid synthesis disorder, presents findings of neonatal cholestasis and hyper-3-oxo-Δ⁴ bile aciduria. The 5β-reductase enzyme participates in not only bile acid synthesis but also hepatic steroid metabolism. Deficiency of 5β-reductase includes 2 types: primary deficiency, with an SRD5B1 gene mutation; and secondary deficiency, lacking a mutation. Secondary deficiency is caused by fulminant liver failure from various etiologies including neonatal hemochromatosis (NH).

Distinguishing primary from secondary deficiency based on γ-glutamyltransferase (GGT), serum total bile acids (TBA), and urinary bile acid analysis using gas chromatography-mass spectroscopy (GC-MS) is very difficult. SRD5B1 gene analysis is the only reliable method. We examined urinary steroid analysis as a way to distinguish primary from secondary 5β-reductase deficiency.

Design, patients and measurements: We examined 12 patients with cholestatic jaundice, normal or slightly elevated GGT, and hyper-3-oxo-Δ⁴ bile aciduria using urinary steroid analysis by GC-MS of both cortisol and cortisone compounds, such as 5β-tetrahydrocortisol (5β-THF) and 5β-tetrahydrocortisone (5β-THE). Patients previously were diagnosed with primary 5β-reductase deficiency (n=3), deficiency secondary to NH (n=3), and deficiency secondary to other liver disorders (n=6).

Results: Urinary steroid analysis in 3 primary deficiency and 3 NH patients showed low 5β-THE and elevated 5α/5β-THE ratios, making distinction difficult without also considering the clinical course and abdominal magnetic resonance imaging (MRI) findings, such as a very
low signal intensity in liver and/or pancreas, especially in T₂-weighted images. In the 6 patients with other secondary deficiencies, urinary 5β-THF and 5α/5β-THF differed from those in primary deficiency (p<0.05).

Conclusions: Urinary steroid analysis can distinguish primary and NH-related deficiencies from other secondary deficiencies.
Introduction

Deficiency of 5β-reductase is a bile acid synthesis defect clinically evident from neonatal cholestasis and hyper-3-oxo-Δ^4 bile aciduria [1, 2]. Cholestasis can be alleviated by primary bile acids therapy, such as chenodeoxycholic acid and/or cholic acid [3, 4], when early diagnosis is possible [3]. The most common inborn error of bile acid synthesis in Europe is 3β-hydroxy-Δ^5-C_27-steroid dehydrogenase/isomerase deficiency, which has a clinical presentation similar to that of 5β-reductase deficiency: neonatal cholestatic jaundice, elevated serum transaminases but normal serum GGT and TBA, and evidence of fat-soluble vitamin malabsorption [5]. Deficiency of 5β-reductase exhibits autosomal recessive inheritance; the gene encoding 5β-reductase, SRD5B1, is located on chromosome 7q32-33 [6]. However, all Japanese patients with 5β-reductase deficiency represent sporadic cases [7, 8]. The 5β-reductase enzyme participates not only in bile acid synthesis but also in hepatic steroid hormone metabolism [8-10].

Deficiency of 5β-reductase includes 2 types: primary 5β-reductase deficiency with an SRD5B1 gene mutation [11,12], and the secondary 5β-reductase deficiency not caused by SRD5B1 gene mutations but rather by fulminant liver failure from any of several etiologies. Primary deficiency is very difficult to distinguish from secondary deficiency, which similarly exhibits normal serum GGT and/or TBA, together with excessive 3-oxo-Δ^4 bile acids in urine. Tyrosinemia type 1 [13] and NH are among the various causes of secondary deficiency [7, 14, 15]. Currently, SRD5B1 gene analysis is the only method for definitive diagnosis.

We hypothesized that urinary steroid analysis could distinguish primary from secondary
deficiency because both cortisol and cortisone have, a 3-oxo-\(\Delta^4\) structure; accordingly, the first step in hepatic conversion to THF and THE is catalyzed by 5\(\beta\)-reductase [9, 10]. In this report, we show that urinary steroid analysis can provide evidence favoring diagnosis of either primary or NH-related deficiency as opposed to another secondary deficiency. We further examined how primary deficiency can be distinguished from secondary deficiency including NH.

Patients and Methods

Patients with 5\(\beta\)-reductase deficiency

In this study, we analyzed 12 patients with cholestatic jaundice, normal or slightly elevated GGT, and hyper-3-oxo-\(\Delta^4\) bile aciduria. Patients previously were diagnosed with primary deficiency (n=3), NH-associated deficiency (n=3), or secondary deficiency caused by liver disorders apart from NH (secondary non-NH, n=6), according to \textit{SRD5B1} gene analysis, clinical course, liver histopathology, and/or biochemical examination (Tables 1 and 2 accompanying the main text; also, supplementary Figure 1 and Table 1) [7, 8].

Diagnostic criteria for NH

We based diagnosis on the clinical course, especially hepatic failure occurring less than 1 or 2 months after birth, plus abdominal MRI findings such as a very low signal intensity in liver and/or pancreas, especially in T\(_2\)-weighted images. Moreover, liver pathologic findings at autopsy included the presence of ductular metaplasia, pericellular fibrosis, and hemosiderin granules.
Qualitative and quantitative bile acid analysis

Serum and urine samples were collected and stored at -25°C until analysis. Patients’ parents gave informed consent prior to analysis. Concentrations of individual bile acids in the urine were corrected for the creatinine (Cr) concentration and expressed as μmol/mmol Cr.

By comparison with control standard samples that we synthesized to represent specific unusual bile acids such as 3-oxo-Δ4 [16] and allo-bile acids [17], that occur in inborn errors of bile acid synthesis, we analyzed bile acids in the patients’ urine and serum using GC-MS with monitoring of selected ions. Specifically, we selectively monitored characteristic ionic fragments of methyl ester-dimethylethylsilyl ether-methoxime derivatives of bile acids after enzymatic hydrolysis (choloylglycine hydrolase, 30 units) and solvolysis (sulfatase, 150 units; Sigma, St Louis, MO), as described previously [18].

Qualitative and quantitative steroid analysis in urine

Each urine sample was collected and stored at -25°C until analysis after informed consent. We studied each infant for 4 urinary cortisol metabolites; 5α-THF, 5β-THF, 5α-THE, and 5β-THE (Figure 1) by GC-MS/selected-ion monitoring as previously reported [19] with minor modifications. In brief, urine samples (0.05 to 0.2 mL) were subjected to enzymatic hydrolysis (Ampullaria enzymes), organic solvent extraction (dichloromethane), and methyloxime-trimethylsilyl derivatization (o-methylhydroxyamine hydrochloride and 1-trimehylsilyl imidazole). The derivative (dissolved in
N-methyl-N-trimethyltrifluoroacetamide after purification) then was subjected to GC-MS/selected-ion monitoring analysis (performed on an HP6850N gas chromatograph with an HP-Ultra-1 fused silica column coupled to an HP5973 mass spectrometer). We quantified each steroid (mg/g Cr) using stigmasterol as an internal standard. A calibration curve was plotted using 5 standard solutions (4, 10, 20, 50, and 100 ng/tube [final amounts, 0.2, 0.5, 1.0, 2.5, and 5 ng/injection, respectively]). All values for inter-assay coefficient of variation (n=12) were under 7% and those for intra-assay coefficient of variation (n=5) were under 18%. The determination limit was 0.2 ng/injection or 0.001 mg/g creatinine.

Statistical analysis

The Kruskal-Wallis test was used to determine significance of differences between groups. A p value less than 0.05 was accepted as indicating statistical significance.

Results

Urinary 3-oxo-Δ4 bile acids in 12 patients

We detected large amounts of 3-oxo-Δ4 bile acids, such as 7α,12α-dihydroxy-3-oxo-4-cholenoic acid and 7α-hydroxy-3-oxo-4-cholenoic acid bile acid, by GC-MS/selected-ion monitoring in urine from the 12 patients. Proportions of 3-oxo-Δ4 bile acids among total bile acids in urine exceeded 70% in 9 patients. Patients 4, 10, and 11 showed smaller proportions (Tables 1 and 2).
Urinary steroid analysis in 12 patients

In patients 1 to 3, excretion of 5\(\beta\) metabolites, such as 5\(\beta\)-THE and 5\(\beta\)-THF, was very low. Similarly, in patients 4 to 6, excretion of 5\(\beta\) metabolites such as 5\(\beta\)-THE, was scant. Overall alteration in cortisol and cortisone metabolism in patients 1 to 6 was evident from high 5\(\alpha\)/5\(\beta\)-THE ratios.

In patients 7 to 12, however, excretion of 5\(\beta\) metabolites, such as 5\(\beta\)-THF and/or 5\(\beta\)-THE, varied from within the normal range to slightly decreased. Further, alteration of cortisol and cortisone metabolism was evident as low 5\(\alpha\)/5\(\beta\) ratios even though 5\(\alpha\)/5\(\beta\)-THF ratios were less depressed in patients 7 to 10 and patient 12 (Table 3).

We could distinguish primary deficiency from non-NH secondary deficiency based upon these data (Tables 3 and 4, and Figure 2). As statistical data in Figure 2, a significance difference 5\(\beta\)-THE was found between controls (n=996) and patients with primary 5\(\beta\)-reductase deficiency (n=3, \(p=0.0113\)), NH (n=3, \(p=0.0113\)), or secondary 5\(\beta\)-reductase deficiency caused by other liver disorders (n=6, \(p=0.0001\)), respectively. In 5\(\alpha\)-THE, a significant difference was found between controls and patients with secondary 5\(\beta\)-reductase deficiency caused by liver disorders apart from NH (\(p=0.002\)). In 5\(\alpha\)/5\(\beta\)-THE, a significant difference was found between controls and primary 5\(\beta\)-reductase deficiency (\(p=0.0113\)) or NH (\(p=0.0113\)), respectively.

As shown in the Figure 2, a significant difference concerning 5\(\beta\)-THF was found between controls (n=166) and patients with primary 5\(\beta\)-reductase deficiency (\(p=0.00705\)) or NH (\(p=0.00163\)), respectively. Also, a significant difference was found between primary 5\(\beta\)-reductase deficiency and secondary 5\(\beta\)-reductase deficiency caused by liver disorders apart
from NH ($p=0.04332$). For $5\alpha$-THF, a significant difference was found between controls and patients with primary $5\beta$-reductase deficiency ($p=0.04467$) or NH ($p=0.00023$), respectively. In $5\alpha/5\beta$-THF, a significant difference was found between patients with primary $5\beta$-reductase deficiency and control ($p=0.00724$) or patients with secondary $5\beta$-reductase deficiency caused by liver disorders apart from NH ($p=0.04454$).

**Discussion**

At present, primary $5\beta$-reductase deficiency is difficult to distinguish from secondary deficiency based solely on a normal serum GGT concentration in the presence of elevated urinary $3$-oxo-$\Delta^4$ bile acids concentrations more than $70\%$ of TBA [7, 13-15, 20]. Examination of serum TBA by an enzymatic technique using $3\alpha$-hydroxysteroid dehydrogenase simultaneously with examination of serum GGT and urinary $3$-oxo-$\Delta^4$ bile acids can facilitate diagnosis of $5\beta$-reductase deficiency, but many institutions in Japan and elsewhere do not analyze serum TBA by such methods. However, we have found urinary steroid analysis using GC-MS to be capable of distinguishing primary from secondary deficiency except in secondary deficiency from NH, which requires additional examinations (Tables 3 and 4; Figure 1).

We use GC-MS to measure urinary $5\beta$-metabolites, such as tetrahydrometabolites including $5\beta$-THF and $5\beta$-THE produced by hepatic $5\beta$-reductase [10]. In primary deficiency, urinary $5\beta$-metabolites should be decreased. Our results (Table 3) indeed showed urinary $5\beta$-metabolites to be decreased in primary deficiency, including 2 patients heterozygous for a mutation in the $SRD5B1$ gene (patients 1 and 3 in Table 1). Patients 7 to 12 in Tables 3 had
secondary deficiency but not NH; their urinary 5β-metabolites were not sharply decreased. Therefore, we believe that urinary steroid analysis can distinguish primary deficiency from those secondary deficiencies not related to NH.

One needs to distinguish primary deficiency from NH-related deficiency by analyzing urinary 5β-metabolites such as tetrahydrometabolites including 5β-THF and 5β-THE, and 5α/5β ratios by urinary steroid analysis using GC-MS. All NH patients showed very low 5β-THE and sharply elevated 5α/5β-THE ratios (patients 4 to 6 in Table 3). Interestingly, however, urinary 5β-THF in 3 patients with NH were not low; remaining in the normal range (Table 3). In passing, one must note difficulty in interpreting results for 5α-THF and 5β-THF in patients less than 2 months old when considering results of urinary steroid analyses using GC-MS. Specifically, many ion peaks representing similar substances may occupy the same position and show the same retention time as 5α-THF and 5β-THF resulting in a falsely elevated value. Therefore, when 5α-THF and 5β-THF are elevated in NH, much care in interpretation is needed. Unfortunately, we lack data oconcerning 5α-THF and 5β-THF in primary deficiency at less than 2 months of age. We concluded that distinguishing primary deficiency from NH-related deficiency would be difficult based on urinary steroid analysis alone.

Assuming that 5α-THF and 5β-THF elevations in NH are not false findings, urinary steroid analysis data in primary deficiency or NH may resemble findings in other secondary deficiencies. The cause could be impairment of type 1 or type 2 11β-hydroxysteroid dehydrogenase activity, as well as reduced 5β-reductase activity (Figure 1). Moreover, in NH,
activity of 5β-reductase and/or a coenzyme required by 5β-reductase may be compromised by the abnormality of iron metabolism.

Despite difficulties distinguishing primary from NH-related deficiency is very important. Distinction may be made using additional data such as details of the clinical course and abnormalities detected by abdominal MRI findings such as a very low signal in liver and/or pancreas, especially in T₂-weighted images. About 40% of acute liver failure in the neonatal period (less than 28 days after the birth) results from NH [21]. Not only do symptoms develop in less than 1 month, but also outcome becomes irreversible within 1 month – a particularly rapid course. NH presents with jaundice, coagulopathy, and impairment of consciousness. Although liver function values such as alanine aminotransferase may be moderately elevated, the process of cirrhosis has begun and normal values are common. Importantly, biopsy specimens show no histopathologically demonstrable iron deposition but only severe fibrosis or cirrhosis [22]. Detection of glyceroluria by organic acid analysis using GC-MS may be useful for diagnosis of NH [23]. Although primary 5β-reductase deficiency progresses more rapidly than many other inborn errors of bile acid synthesis, hepatic failure less than 1 month after birth is rare.

Since 5β-reductase exists only in the liver [10], one wonders why 5β-metabolites of urinary steroids do not decrease sharply in secondary deficiency not caused by NH (Table 4). We believe that 5β-reductase is very important for bile acid synthesis in liver, so diminished enzyme activity results in large amounts of ketonic bile acids such as 3-oxo-Δ⁴ bile acids. In hepatic steroid metabolism, however, 5α-reductase is much more important than 5β-reductase.
Steroid metabolism in liver therefore is little affected by diminished 5β-reductase activity. In hepatic steroid metabolism, 5β-metabolites do not reflect onset of cholestasis or acute liver failure in secondary deficiency unrelated to NH. In NH, however, activity of 5β-reductase is as low as in primary deficiency. Activity of 5β-reductase may be reduced significantly by massive, rapidly progressive hepatic necrosis caused by iron overload.

In conclusion, primary and secondary 5β-reductase deficiency are difficult to differentiate using laboratory data such as GGT, TBA in serum, and even bile acid analysis. However, we could distinguish primary from non-NH secondary deficiency by urinary steroid analysis (Figure 2). Nonetheless, NH may not be distinguished from primary deficiency by urinary steroid analysis. Additional factors such as clinical course and abdominal MRI findings, -- such as a very low signal intensity in liver and/or pancreas, especially in T2-weighted images together with laboratory data including results of urinary steroid and bile acid analysis -- can aid in making this important distinction.

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Figure legends

Figure 1. Metabolic pathways of cortisol (compound F) and cortison (compound E)
11β-HSD, 11β-hydroxysteroid dehydrogenase; 3α-HSD, 3α-hydroxysteroid dehydrogenase.

Figure 2. Plots of urinary steroid data in primary and secondary 5β-reductase deficiency
We plotted values for 5α- and 5β-tetrahydrocortisone (THE), 5α- and 5β- tetrahydrocortisol (THF), and 5α/5β ratios, in primary 5β-reductase deficiency and neonatal hemochromatosis (NH) for comparison with secondary deficiency caused by liver disorders apart from NH.
However, we need to exclude data for NH from panel presented THF, since in subjects younger than 2 months old, many ion peaks representing similar substances may occupy the same position and show the same retention time as 5α-THF and 5β-THF. This would result a falsely elevated value. Accordingly, when 5α-THF and 5β-THF are elevated in NH, much care in interpretation is needed. In 5β-THE and 5α/5β-THE, we could not see a statistically significant difference between primary 5β-reductase deficiency and secondary deficiency caused by liver disorders other than NH. However, the plotted data show no overlap, we consider this sufficiently finding important to be considered together with the results for 5β-THF and 5α/5β-THF. We provide statistical data in the Results for the main text.

THE, tetrahydrocortisone; THF, tetrahydrocortisol; •, control; ◆, primary 5β-reductase deficiency; △, neonatal hemochromatosis; ○, acute liver failure; ●, chronic liver failure.
Cortisol (F) \[\xrightarrow{11\beta\text{-HSD2}}\] Cortisone (E) \[\xleftarrow{11\beta\text{-HSD1}}\]

- 5α-reductase
- 5β-reductase
- 5α-reductase
- 5β-reductase

- 5α-dihydrocortisol \[\xrightarrow{3\alpha\text{-HSD}}\] 5α-tetrahydrocortisol
- 5β-dihydrocortisol \[\xrightarrow{3\alpha\text{-HSD}}\] 5β-tetrahydrocortisol
- 5α-dihydrocortisone
- 5β-dihydrocortisone
Table 1. Laboratory data from patients with primary Δ<sup>4</sup>-3-oxosteroid 5β-reductase deficiency

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age</th>
<th>Gender</th>
<th>T.Bil (mg/dl)</th>
<th>ALT (U/L)</th>
<th>GGT (U/L)</th>
<th>TBA (µmol/L)</th>
<th>Urinary 3-oxo-Δ&lt;sup&gt;4&lt;/sup&gt; bile acids</th>
<th>Mutation of SRD5B1</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6 mo</td>
<td>M</td>
<td>4.4</td>
<td>441</td>
<td>46</td>
<td>n.d.</td>
<td>98.4</td>
<td>R266G (866 G&gt;A)</td>
</tr>
<tr>
<td>2</td>
<td>8 mo</td>
<td>F</td>
<td>6.9</td>
<td>333</td>
<td>45</td>
<td>2.2</td>
<td>99.6</td>
<td>G223E (737 G&gt;A), R261C (850 C&gt;T)</td>
</tr>
<tr>
<td>3</td>
<td>1 yr, 2 mo</td>
<td>F</td>
<td>0.5</td>
<td>16</td>
<td>9</td>
<td>3.0</td>
<td>85.4</td>
<td>G223E (737 G&gt;A)</td>
</tr>
</tbody>
</table>

Normal range 0.3-1.5 8-42 10-47 <10 <5

T.Bil, total bilirubin; ALT, alanine aminotransferase; GGT, γ-glutamyltransferase; TBA, total bile acids; M, male; F, female; n.d., not determined.
Table 2. Laboratory data from patients with secondary Δ⁴-3-oxosteroïd 5β-reductase deficiency.

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age</th>
<th>Gender</th>
<th>T.Bil (mg/dl)</th>
<th>ALT (U/L)</th>
<th>GGT (U/L)</th>
<th>TBA (µmol/L)</th>
<th>Urinary 3-oxo-Δ⁴ bile acids</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>14 d</td>
<td>F</td>
<td>11.9</td>
<td>16</td>
<td>19</td>
<td>n.d.</td>
<td>63.3</td>
<td>Neonatal hemochromatosis</td>
</tr>
<tr>
<td>5</td>
<td>36 d</td>
<td>M</td>
<td>13.0</td>
<td>10</td>
<td>29</td>
<td>n.d.</td>
<td>74.8</td>
<td>Neonatal hemochromatosis</td>
</tr>
<tr>
<td>6</td>
<td>40 d</td>
<td>M</td>
<td>26.6</td>
<td>21</td>
<td>36</td>
<td>92.9</td>
<td>91.4</td>
<td>Neonatal hemochromatosis</td>
</tr>
<tr>
<td>7</td>
<td>3 mo</td>
<td>M</td>
<td>25.6</td>
<td>72</td>
<td>31</td>
<td>90.2</td>
<td>71.2</td>
<td>Acute liver failure of unknown origin</td>
</tr>
<tr>
<td>8</td>
<td>5 mo</td>
<td>F</td>
<td>32.3</td>
<td>386</td>
<td>68</td>
<td>77.4</td>
<td>85.4</td>
<td>PFIC-2 ?</td>
</tr>
<tr>
<td>9</td>
<td>7 mo</td>
<td>F</td>
<td>10.5</td>
<td>52</td>
<td>55</td>
<td>15.2</td>
<td>76.8</td>
<td>Acute liver failure of unknown origin</td>
</tr>
<tr>
<td>10</td>
<td>7 mo</td>
<td>M</td>
<td>9.6</td>
<td>155</td>
<td>44</td>
<td>n.d.</td>
<td>68.7</td>
<td>Acute liver failure of unknown origin</td>
</tr>
<tr>
<td>11</td>
<td>10 mo</td>
<td>M</td>
<td>12.6</td>
<td>864</td>
<td>20</td>
<td>12.0</td>
<td>55.3</td>
<td>Acute liver failure of unknown origin</td>
</tr>
<tr>
<td>12</td>
<td>16 mo</td>
<td>M</td>
<td>15.2</td>
<td>82</td>
<td>41</td>
<td>84.0</td>
<td>85.3</td>
<td>Chronic liver failure due to biliary atresia</td>
</tr>
</tbody>
</table>

Normal range: T.Bil, total bilirubin; ALT, alanine aminotransferase; GGT, γ-glutamyltransferase; TBA, total bile acids; F, female; M, male; n.d., not determined.
determined; PFIC-2 ?, suspected progressive familial intrahepatic cholestasis type 2 (PFIC-2) or other inborn error of bile acid synthesis such as an amidation defect, considering giant cell transformation and weak immunofluorescent staining for bile salt export pump (BSEP) observed in the liver. In neonatal hemochromatosis cases, we did not detect a mutation in the SRD5B1 gene (patients 4 to 6).
<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age</th>
<th>Gender</th>
<th>5α-THF</th>
<th>5β-THF</th>
<th>5α-THE</th>
<th>5β-THE</th>
<th>5α/5β-THF</th>
<th>5α/5β-THE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6 mo</td>
<td>M</td>
<td>1.579</td>
<td>&lt;0.001</td>
<td>1.685</td>
<td>0.003</td>
<td>&gt;1500</td>
<td>561.7</td>
</tr>
<tr>
<td>2</td>
<td>8 mo</td>
<td>F</td>
<td>0.080</td>
<td>0.001</td>
<td>0.101</td>
<td>0.030</td>
<td>80</td>
<td>3.367</td>
</tr>
<tr>
<td>3</td>
<td>1 yr, 2 mo</td>
<td>F</td>
<td>2.034</td>
<td>0.001</td>
<td>0.291</td>
<td>0.008</td>
<td>2034</td>
<td>36.375</td>
</tr>
<tr>
<td>4</td>
<td>14 d</td>
<td>F</td>
<td>0.170*</td>
<td>0.018*</td>
<td>0.748</td>
<td>0.034</td>
<td>9.444*</td>
<td>21.744</td>
</tr>
<tr>
<td>5</td>
<td>36 d</td>
<td>M</td>
<td>0.161*</td>
<td>0.021*</td>
<td>0.749</td>
<td>0.016</td>
<td>7.667*</td>
<td>46.813</td>
</tr>
<tr>
<td>6</td>
<td>40 d</td>
<td>M</td>
<td>0.938*</td>
<td>0.165*</td>
<td>1.754</td>
<td>0.261</td>
<td>5.685*</td>
<td>6.720</td>
</tr>
<tr>
<td>7</td>
<td>3 mo</td>
<td>F</td>
<td>0.135</td>
<td>0.049</td>
<td>0.174</td>
<td>0.564</td>
<td>2.747</td>
<td>0.306</td>
</tr>
<tr>
<td>8</td>
<td>5 mo</td>
<td>F</td>
<td>0.319</td>
<td>0.585</td>
<td>0.146</td>
<td>3.182</td>
<td>0.545</td>
<td>0.046</td>
</tr>
<tr>
<td>9</td>
<td>7 mo</td>
<td>F</td>
<td>0.430</td>
<td>0.656</td>
<td>0.269</td>
<td>4.276</td>
<td>0.655</td>
<td>0.063</td>
</tr>
<tr>
<td>10</td>
<td>7 mo</td>
<td>M</td>
<td>0.601</td>
<td>0.186</td>
<td>0.041</td>
<td>0.439</td>
<td>3.231</td>
<td>0.093</td>
</tr>
<tr>
<td>11</td>
<td>10 mo</td>
<td>M</td>
<td>0.593</td>
<td>0.090</td>
<td>0.131</td>
<td>0.237</td>
<td>6.589</td>
<td>0.553</td>
</tr>
<tr>
<td>12</td>
<td>16 mo</td>
<td>M</td>
<td>0.232</td>
<td>0.154</td>
<td>0.069</td>
<td>0.060</td>
<td>1.510</td>
<td>1.152</td>
</tr>
</tbody>
</table>
(Table 3, continued)

<p>| | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>normal range</td>
<td></td>
<td>M, F</td>
<td>0.01-0.96*</td>
<td>0.02-0.28*</td>
</tr>
<tr>
<td></td>
<td>(2.5-97.5%ile)</td>
<td></td>
<td>0.23-6.5</td>
<td>5.8-21</td>
<td>0.30-9.1*</td>
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<td>0.03-0.53</td>
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<tr>
<td></td>
<td>7 d-2 mo</td>
<td></td>
<td>0.55-3.7</td>
<td>0.44-2.8</td>
<td>3.3-14</td>
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<td>1.7-7.9</td>
</tr>
<tr>
<td></td>
<td>3 mo-3 yr</td>
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<td></td>
<td></td>
<td>0.07-0.39</td>
</tr>
<tr>
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<td></td>
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<td></td>
</tr>
<tr>
<td>THF, tetrahydrocortisol; THE, tetrahydrocortisone; Patient numbers are the same as in Tables 1 and 2. *, Values of 5α-THF, 5β-THF, and 5α/5β-THF may be falsely increased between 7 days and 2 months of age (see Discussion).</td>
<td></td>
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Table 4. Differential diagnosis in patients with cholestasis by urinary steroid analysis

<table>
<thead>
<tr>
<th>Condition</th>
<th>5β-THF</th>
<th>5β-THE</th>
<th>5α/5β-THF</th>
<th>5α/5β-THE</th>
</tr>
</thead>
<tbody>
<tr>
<td>5β-Reductase deficiency</td>
<td>↓↓↓</td>
<td>↓↓↓</td>
<td>↑↑↑</td>
<td>↑↑↑</td>
</tr>
<tr>
<td>Neonatal hemochromatosis</td>
<td>normal range to ↓†</td>
<td>↓</td>
<td>normal range†</td>
<td>↑↑↑</td>
</tr>
<tr>
<td>Liver failure*</td>
<td>normal range to ↓</td>
<td>normal range to ↓</td>
<td>normal range to ↓</td>
<td>↑ to ↓</td>
</tr>
</tbody>
</table>

5β-reductase deficiency, Δ⁴-3-oxosteroid 5β-reductase deficiency; THF, tetrahydrocortisol; THE, tetrahydrocortisone; *, acute liver failure of unknown origin, suspected PFIC-2, and chronic liver failure of cirrhosis due to biliary atresia; †, Values of 5β-THF and 5α/5β-THF may be falsely increased between 7 days and 2 months of age (see Discussion).