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# Human $\Delta^4$-3-oxosteroid 5β-reductase (AKR1D1) deficiency and steroid metabolism☆

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ABSTRACT

Conclusive *in vivo* evidence regarding the enzyme responsible for steroid hormone 5β-reduction has not been obtained, although studies have suggested it may be the same enzyme as that utilized for cholic acid and chenodeoxycholic bile-acid synthesis. We have recorded the steroid metabolome of a patient with a defect in the "bile-acid" 5β-reductase (AKR1D1) and from this confirm that this enzyme is additionally responsible for steroid hormone metabolism. The 13-year old patient has been investigated since infancy because of a cholestasis phenotype caused by bile-acid insufficiency. Several years ago it was shown that she had a 662C>T missense mutation in AKR1D1 causing a Pro198Leu substitution. It was found that the patient had an almost total absence of 5β-reduced metabolites of corticosteroids and severely reduced production of 5β-reduced metabolites of other steroids. The patient is healthy in spite of her earlier hepatic failure and is on no treatment. All her vital signs were normal, as were results of many biochemical analyses. She had normal pubertal changes and experiences regular menstrual cycles. There was no evidence for any clinical condition that could be attributed to attenuated ability to metabolize steroids in normal fashion. Both parents were heterozygous for the mutation but the steroid excretion was entirely normal, although an older female sibling showed definitive evidence for attenuated 5β-reduction of cortisol. A younger brother had a normal steroid metabolome. The sibling genotypes were not available.



☆ Steroid metabolites without specified 5-hydrogen orientation are 3α-hydroxy-5β-steroids.

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*Abbreviations:* And, androsterone; Etio, etiocholanolone; 11OxoAnd, 11-oxoandrosterone; 11βOHAnd, 11β-hydroxyandrosterone; 11βOHEtio, 11β-hydroxyetiocholanolone; THE, tetrahydrocortisone; 5αTHE, 5α-tetrahydrocortisone; THF, tetrahydrocortisol; 5αTHF, 5α-tetrahydrocortisol; αC′one, α-cortolone; αC′one5α, (5α)-α-cortolone (equivalent for β-cortolones); αC′ol, α-cortol; αC′ol5α, (5α)-α-cortol (equivalent for β-cortols); THA, tetrahydro-11-dehydrocorticosterone; 5αTHA, 5α-tetrahydro-11-dehydrocorticosterone; THB, tetrahydrocorticosterone; 5αTHB, 5α-tetrahydrocorticosterone; P′diol, pregnanediol; 5αP′diol, 5α-pregnanediol; P′triol, pregnanetriol; 5αP′triol, 5α-pregnanetriol; 17HP, 17-hydroxypregnanolone; 17HP5α, (5α)-17-hydroxypregnanolone; 6βOHF, 6β-hydroxycortisol; 20αDHE, 20α-dihydrocortisone; 20βDHE, 20β-dihydrocortisone; 20α-DHF, 20α-dihydrocortisol; 20βDHF, 20β-dihydrocortisol.

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## 1. Introduction

Reduction of sterols and steroids with a 3-oxo-4-ene structure is a common transformation. This is a two-step procedure, 5α- or 5β-reduction occurring first followed by the action of 3α-hydroxysteroid dehydrogenase. Reduction at 5α- has been most extensively studied following the finding that this reaction was involved in generation of the hormone 5α-dihydrotestosterone (5α-DHT), while comparatively little attention has been paid to 5β-reduction. However, this reaction has vital synthetic roles as an integral part of bile-acid synthesis, and a catabolic role in the degradative metabolism of active steroid hormones, about 2/3 of the mass of which are deactivated by this mechanism prior to excretion.

The human bile-acid biosynthetic enzyme has been cloned and is now known as AKR1D1. It has been expressed in COS cells and shows high 5β-reductase activity to bile-acid intermediates but low or absent activity to steroid hormones [1]. This suggests that may be more than one enzyme is involved in human 5β-reduction, a question that could be resolved by studying the urinary steroid metabolome of a human "knock-out" of the enzyme, and over a period of 20 years there have been several reports of patients with putative 5β-reductase deficiency [2–6]. This disorder (congenital bile-acid synthesis defect type 2 (CBAS2), OMIM:235555) is known for a cholestasis and liver failure phenotype, which can be fatal in infancy. Lemonde et al. found inactivating AKR1D1 mutations in three patients with the disorder [7]. Typically the treatment for these patients is administration of exogenous bile-acids or liver transplantation. However, 11 years after publication of a case report on one of these individuals [3,7], the female patient is thriving and has apparently no current need for bile-acid supplementation, possibly as a result of active formation of "replacement" 5α- (or "allo") bile-acids. Daugherty et al. [8] have also studied patients with the disorder and have noted near-normalization of liver function with age and/or oral bile-acid administration.

We have revisited this case to determine if steroid (as distinct from sterol) 5β-reduction was affected, and evaluate the impact of the disorder on steroid hormone homeostasis, particularly related to clinical problems of an endocrine nature.

## 2. Materials and methods

### 2.1. Patient

The patient is from the Italian province of Sardinia, the second child of parents not known to be consanguineous. Sardinia is a Mediterranean island with much of the population relatively isolated from mainland European communities for centuries. The patient's ancestors are autochthonous and the parents are both from the north-west region of the island. The patient presented at 3 weeks of age with cholestasis associated with steatorrhoea, failure to thrive and rickets. A liver biopsy at 3 months showed lobular disarray, and hepatocytes contained fat and bile pigments. Failure to thrive, malabsorbtion of fat soluble vitamins continued despite ursodeoxycholic acid treatment. At 8 months the liver still showed giant cell hepatitis with steatosis. Treatment with cholic acid and chenodeoxycholic acid led to normalization of liver function within 3 months. She remained well on this treatment for several years. Lemonde et al. [7] published biochemical and clinical data at the age of 9 years. Her bile-acid synthesis had been evaluated in detail when she was 8 months old using liquid ion mass spectrometry (LSIMS) of a urine extract, which showed a complete absence of chenodeoxycholic acid and cholic acid, strong evidence for 5β-reductase deficiency. The AKR1D1 gene was sequenced and it was determined that she had a 662C>T missense mutation causing a Pro198Leu substitution. Currently she is 13 years old and even after termination of treatment (by personal decision, not medical advice) at the age of 10 years she has apparently remained healthy. She has shown normal growth (height 156, weight 53, 50th centile for Sardinian population); regular menses started at age of 11, and secondary sexual characteristics are normal. Neither acne nor hirsutism was evident. Blood pressure was recently determined to be normal at 110/70 mmHg and clinical signs of cholestasis or jaundice were no longer evident. Liver analytes (transaminase, bilirubin, gGT, cholinesterase) were normal. Blood glucose, uric acid, cholesterol, electrophoresis of proteins, plasma electrolytes were all in the normal range. Plasma creatinine was 0.45 mg/dl (normal 0.50–1.4), urea 19.5 mg/dl (normal 15–50) and urine creatinine 75.8 mg/dl. Serum levels of the lipophilic vitamins A, D, E and K were all normal, thus showing no indication of malabsorbtion. Liver sonography showed no abnormalities. Serum cortisol, 17-hydroxyprogesterone and ACTH were also all in the normal range. The patient appears well and takes no medication. We suggest that she is compensating for her conventional bile-acid deficiency through increased synthesis of 5α- (or "allo") bile-acids which could be hepatoprotective through their ability, shared with conventional bile-acids, to activate bile flow. Serum bile-acids were measured at the age of 11 years [3] 1 year after cessation of supplementary bile-acid therapy, results are given in Table 1. It was found that 62% of her circulating acids were of 5α-configuration.

### 2.2. Family members

The mother and father were heterozygous for the 662C>T mutation. Genotypes of the brother and sister (7 and 16 years, respectively) were not determined. They are asymptomatic. The patient's grandfathers died of bowel cancer and stroke, respectively, but the grandmothers are alive and healthy.

### 2.3. Methods

Informed consent for these studies was obtained from all family members and the study was approved by the institutional review board of ASL, Sassari. Urine samples were collected from the patient and immediate family members for measurement of urinary steroids. In view of the family being rural and not under current formal investigation only random samples were collected during a single visit by one of us (MCP) to their home. This was not considered to be a disadvantage since the interrelationships between individual steroid metabolite excretions would be sufficient to establish the activity of 5β-reductase to hormonal steroids.