A Case of 3 β -Hydroxysteroid Dehydrogenase Type II (HSD3B2) Deficiency Picked up by Neonatal Screening for 21-Hydroxylase Deficiency: Difficulties and Delay in Etiologic Diagnosis

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Established Facts

- 3 β -HSD due to HSD3B2 deficiency results in cortisol and aldosterone deficiency with varying degrees of salt loss.
- Males present with incomplete masculinization, while females may present with mild virilization of the external genitalia.
- Patients with 3 β -HSD may have elevated 17 α -hydroxyprogesterone levels due to normal peripheral HSD3B1 activity.

Novel Insights

- A patient with HSD3B2 deficiency was detected in the neonatal screening for CYP21 deficiency.
- The correct diagnosis was delayed in part because of cross-reactivity of the antibodies included in the commonly used assays.
- Discrimination between CYP21 and HSD3B2 deficiency was facilitated by the use of more specific antibodies.
- This case emphasizes the importance of confirming the specific enzyme deficiency with molecular genetic methods, particularly in neonatally detected congenital adrenal hyperplasia.

Key Words

3 β -Hydroxysteroid dehydrogenase type II • *HSD3B2* gene • Neonatal screening, congenital adrenal hyperplasia • Assay cross-reactivity

Abstract

Background: 3 β -Hydroxysteroid dehydrogenase type II deficiency, a rare form of congenital adrenal hyperplasia, is characterized by varying degrees of salt loss and incomplete masculinization in males and mild virilization or normal external genitalia in females. The clinical signs may be difficult

to recognize, increasing the risk of a neonatal adrenal crisis. In addition, elevated 17 α -hydroxyprogesterone and androstenedione levels due to peripheral HSD3B1 activity may lead to a delay of the correct diagnosis and even to misdiagnosis as CYP21 deficiency. **Method:** We report a patient who was detected on neonatal screening for 21-hydroxylase deficiency, in part because of cross-reactivity in the commonly used assay. **Results:** The diagnostic difficulties in this case were overcome by the use of more specific antibodies. **Conclusion:** This case emphasizes the importance of confirming the etiological diagnosis with molecular genetic analyses.

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Introduction

Congenital adrenal hyperplasia (CAH) consists of a group of disorders caused by enzyme deficiencies in the steroid hormone synthesis in the adrenal cortex. Classical 3 β -hydroxysteroid dehydrogenase (3 β -HSD) deficiency is a rare cause of CAH, resulting in cortisol and aldosterone deficiency and in varying degrees of salt loss. In males, the inability to synthesize testosterone in the testis results in incomplete masculinization of the external genitalia of varying degrees. Females may present with mild virilization of the external genitalia neonatally or with normal sexual differentiation [1–3]. The most severe classical form of 3 β -HSD deficiency results in salt loss during the first few months of life and is fatal, if not diagnosed and treated. Milder forms of classical 3 β -HSD deficiency may be diagnosed anytime before puberty and can lead to salt-wasting crises that are precipitated by, e.g., infections.

There are two isoenzymes of 3 β -HSD in humans, converting the Δ^5 -steroids to the Δ^4 -keto metabolites, i.e. 17 α -hydroxypregnenolone (Δ^5 -17-OHP) to 17 α -hydroxyprogesterone (17-OHP) and dehydroepiandrosterone to androstenedione. Type II 3 β -HSD (*HSD3B2*) is expressed in the adrenals and gonads, and type I 3 β -HSD (*HSD3B1*) is active in numerous peripheral tissues. Both genes are located on chromosome 1 [4]. Deficiency of *HSD3B2* causes classical 3 β -HSD deficiency. There are (at least) 39 mutations described in patients with *HSD3B2* deficiency [1] associated with clinical symptoms, varying from neonatal onset of salt loss to adrenarche later in childhood or even infertility and menstrual irregularities in adult life [2]. A less severe form of 3 β -HSD deficiency defined by an excess of Δ^5 -steroids has not been shown to be caused by mutations in the *HSD3B2* gene nor in the *HSD3B1* gene [5–7]. It is presently of unknown cause.

The most common form of CAH, comprising more than 95% of all cases, is 21-hydroxylase (*CYP21*) deficiency [8]. A number of countries, including Sweden, have neonatal screening programs for *CYP21* deficiency, where 17-OHP, the metabolite prior to the enzyme block, is used as an indicator of the disease [9, 10]. It is known that patients with severe forms of 11 β -hydroxylase deficiency, another form of CAH, where the enzyme deficiency is downstream from *CYP21*, may be detected in the screening due to increased measurements of 17-OHP. It is also known that newborns with 3 β -HSD deficiency have elevated levels of 17-OHP [11]. One patient has been identified through neonatal screening [12], and it has retrospectively been shown that a patient presenting with

HSD3B2 deficiency could have been detected in a neonatal screening program, had there been one [13]. However, it is not known to what extent these patients are actually found in the screening. The commercially available antibody against 17-OHP was changed in 2004, and this new reagent is in general use in screening programs around the world since 2005. The profile of the new antibody has prompted many screening programs to increase their recall levels.

We report a girl presenting with *HSD3B2* deficiency with no prenatal virilization who was detected in the neonatal screening program for *CYP21* deficiency and thereby escaped clinical symptoms of severe salt loss and adrenal crisis. However, the diagnosis was delayed by the contradictory laboratory results, i.e., elevated 17-OHP and aldosterone levels, which were, at least in part, caused by methodological issues. She was detected in the screening using the previous antibody, but retrospective analysis of the neonatal filter paper blood sample using the new antibody that is in general use since 2005 gave an even higher 17-OHP value. Our results indicate that the possibility of detecting patients with *HSD3B2* deficiency is increased as a result of this change in methodology. The diagnostic difficulties this may impose underline the importance of a confirmation of the enzyme deficiency by molecular genetic methods.

Case Report

The full-term girl was born after an uneventful pregnancy to unrelated parents of Russian origin. The birth weight was 3,690 g, the length was 51.5 cm, and the Apgar scores were 9, 10, and 10 at 1, 5, and 10 min, respectively. The girl developed hypoglycemia (blood glucose 0.1 mmol/l) on the 2nd day of life which required intravenous glucose infusion. She was noted to have bilateral four-finger creases, slight hypotonia, and a cardiac murmur that prompted chromosome analysis. She was discharged from the hospital with a scheduled appointment for follow-up. The neonatal screening results showed an elevated plasma 17-OHP level of 125 nmol/l (recall level 75 nmol/l plasma for full-term infants). When the girl was investigated on the return visit, she had no signs of prenatal virilization and no cardiac murmur. She had a normal female karyotype, 46,XX. Laboratory investigations showed a low sodium level of 127 (normal range 133–146) mmol/l and an elevated potassium level of 6.0 (normal range 3.4–4.9) mmol/l, compatible with salt loss due to mineralocorticoid deficiency. Treatment with hydrocortisone 5 mg $\times$ 3, fludrocortisone 0.05 mg $\times$ 2, and sodium chloride 0.5 g $\times$ 3 was initiated.

A second filter paper sample taken on day 14 showed a grossly elevated 17-OHP value of >900 nmol/l. Because of the discrepancy between the clinical presentation and the salt loss, more detailed hormonal studies were performed.

Table 1. Hormone levels analyzed in the routine laboratory on day 14 and the results of serum hormone analysis using more specific antibodies at 8 weeks

	Serum levels on day 14	Serum levels at 8 weeks	Reference levels
17 α -OH progesterone, nmol/l	760	52.5	3.5–5
Dehydroepiandrosterone, nmol/l	5.8	136.7	4–10
Androstenedione, nmol/l	53	7.51	0.5–1.7
Testosterone, nmol/l	>55	1.48	0.1–0.4
Cortisol, nmol/l	400		180–350 (08.00–09.00 h)
ACTH, pmol/l	180		4–12
Aldosterone, pmol/l	>3,200		age dependent, <830 in adults
Renin, ng/l	600		<50
Progesterone, nmol/l		1.71	0.1–1
17 α OH pregnenolone, nmol/l		640.6	84–16

Materials and Methods

Neonatal screening measurements of 17-OHP on filter paper blood spots collected on day 3, 4, or 5 after birth were used. 17-OHP was determined using the Delfia assay (PerkinElmer, Waltham, Mass., USA) with gestational-age-related recall levels as described earlier [10]. Cross-reactivity reported for the antibodies in the previous kit: progesterone 3.6%, 4-pregnene-11 β -17 α -diol-3,20-diol 1.75%, 11-deoxy-17-hydroxycorticosterone 0.33%, 16 α -hydroxyprogesterone 0.30%, Δ^5 -17-OHP 0.13%, 11-deoxycortisol 0.04%, deoxycorticosterone 0.03%, and tetrahydrocortisone 0.02%. Cross-reactivity reported for the antibody in the currently used kit: 11-deoxycortisol 1.82%, Δ^5 -17-OHP 1.20%, progesterone 0.47%, 21-deoxycortisol 0.46%, deoxycorticosterone 0.09%, and spironolactone 0.09%.

Retrospective analysis of the filter paper blood spot from day 3 was performed using the new currently used antibody in the Delfia assay kit.

Serum 17-OHP and plasma renin levels were determined by a radioimmunoassay (RIA) obtained from CIS Bio International (Gif-sur-Yvette, France); serum testosterone was determined by means of a Coat-a-Count RIA (Diagnostic Products, Los Angeles, Calif., USA); serum androstenedione was analyzed by RIA after ether extraction [14, 15]; adrenocorticotrophic hormone was measured using a RIA (Nichols Institute Diagnostics, San Juan Capistrano, Calif., USA), and plasma aldosterone was also measured by RIA (Diagnostic Products). The urinary steroid metabolites were determined by gas chromatography and gas chromatography-mass spectrometry [16].

Genomic DNA was prepared from peripheral leukocytes. All translated exons, including exon/intron junctions of the *HSD3B2* gene, were PCR amplified using a set of primers that were designed to flank each exon. Sequencing was performed using the BigDye Terminator version 1.1 sequencing kit and the model 3130 sequencer (Applied Biosystems, Foster City, Calif., USA).

Results

The serum hormone levels analyzed on day 14 are shown in table 1. There was a discrepancy between the

salt loss, the grossly elevated 17-OHP levels, and the lack of virilization pointing against 21-hydroxylase deficiency, and *CYP21* mutation analysis did not detect any of the common mutations.

Urinary steroid analyses showed that the dominating peaks were P5-3,11,21-triol-20-one, P5-3,21-diol-20-one, and P5-3,17,20-triol, i.e., 3 β -hydroxy- Δ^5 -steroids pointing to a severe *HSD3B2* defect, but malignancy could not be ruled out. Dexamethasone decreased the adrenal steroid production efficiently, hence ruling out malignancy as a cause.

During the investigation and workup procedures, the parents stopped the treatment completely on their own initiative. After 2 days, they saw that the girl was unwell and started sodium chloride again. We were informed 2 weeks later, when the child developed pubic hair. The girl showed poor weight gain and growth. Laboratory investigation showed signs of salt loss and increased adrenal metabolites.

At 8 weeks of age, samples taken when the girl was off medication were sent to Lyon for analysis by specific RIAs after purification by celite column chromatography [17]. The results are shown in table 1. The elevated levels of Δ^5 -17-OHP and progesterone strongly suggested that the girl had *HSD3B2* deficiency.

Sequence analysis of the *HSD3B2* gene showed that the patient was a compound heterozygote for two different previously not described mutations. A GCC-to-GAC transversion in exon 3, causing alanine at residue 82 to be replaced by aspartic acid (A82D), was inherited from the father, whereas a nonsense mutation in exon 4 (W230X) caused by a TGG-to-TGA substitution was inherited from the mother.

A retrospective analysis of the blood spot filter paper taken during the neonatal period was performed, with the new antibody that is currently used in the Delfia assay. It showed a 17-OHP level of 405 nmol/l, which is well above the cutoff level used in Sweden of 100 nmol/l for full-term infants.

Discussion

It is well recognized that 17-OHP, androstenedione, and testosterone levels may be elevated in patients with *HSD3B2* deficiency [11]. The rationale for this finding is that the gene coding 3 β -HSD type I is intact and converts the Δ^5 -precursors to the corresponding delta 4-3-ketosteroids in peripheral tissues [3]. One patient with *HSD3B2* deficiency has been reported to be detected by neonatal screening for 21-hydroxylase deficiency [12], as well as a previous one at the very beginning of neonatal screening [Limal, personal commun. to M.G. Forest]. In addition, by retrospective analysis of a neonatal filter paper blood sample, it has been shown that a patient with late-diagnosed 3 β -HSD deficiency would have been found by neonatal screening, if such a program had been present [13]. However, the actual detection rate of these patients by neonatal analysis of 17-OHP is unknown. It is conceivable that a proportion of the CAH cases assumed to have 21-hydroxylase deficiency based on neonatal detection are in fact due to *HSD3B2* deficiency. Our findings confirm that patients with 3 β -HSD deficiency are detectable in the neonatal programs screening for *CYP21* deficiency.

This girl with *HSD3B2* deficiency was detected on neonatal screening for 21-hydroxylase deficiency due to an elevated 17-OHP level (125 nmol/l). At the time of detection, she had laboratory signs of salt loss, but had not yet developed clinical symptoms. She had a hypoglycemic episode on the 2nd day of life, but no prenatal virilization. However, when the parents stopped treatment at 2–3 months of age, the girl developed pubic hair within less than 2 weeks.

This postnatal virilization, despite the normal prenatal sexual differentiation, could be explained by the withdrawal of placental *HSD3B1*, no longer converting the Δ^5 -17-OHP to Δ^4 -steroids, i.e., more pronounced buildup of Δ^4 -precursors converted thereafter by *HSD3B1* on the periphery, resulting in excess of androgens and pubic hair development. Precocious pubarche has been previously reported as the first symptom of *HSD3B* deficiency [2, 13, 18, 19].

Diagnostic criteria for *HSD3B2* deficiency based on genetically confirmed patient data have recently been

published [20, 21]. The ratio of Δ^5 -17-OHP/cortisol has been reported to be significantly elevated in all genetically confirmed cases of *HSD3B2* deficiency as compared with controls. The hormonal levels were particularly difficult to compare for *HSD3B2* infants and controls during the neonatal period. The Δ^5 -17-OHP/17-OHP ratio was not significantly elevated in *HSD3B2* as compared with control neonatal infants. This is consistent with the finding that preterm infants have elevated levels of 17-OHP in the neonatal screening for *CYP21* deficiency [22]. This is likely due to the immaturity of enzyme activities (i.e., functional 11 β -hydroxylase deficiency) together with the high levels of sulfated metabolites of Δ^5 - and Δ^4 -steroids cross-reacting in the assay, thus leading to false elevated hormone assessments in the Delfia assay [23]. Immature kidney function with low excretion rate can also play a role. It is possible that all these mechanisms as well as peripheral *HSD3B1* activity contributed to the markedly elevated 17-OHP levels in our patient with *HSD3B2*. In fact, the best hormonal criterion of 3 β -HSD deficiency is an elevated Δ^5 -17-OHP level of ≥ 100 nmol/l, whether under basal or adrenocorticotrophic hormone stimulated conditions [3], and whatever the age.

Serum aldosterone measured during the neonatal period was surprisingly $>3,200$ pmol/l. The most plausible explanation is cross-reactivity in the immunoassay used. The aldosterone concentrations are normally given in picomoles per liter, while many of the steroid precursors were present at more than 500 nmol/l. Even a low degree of cross-reactivity will have obvious effects on the measurements, when the concentration of the cross-reacting steroid is more than 1,000 times higher. All steroid precursors have structural similarities. The most likely metabolites to be responsible for the cross-reactivity are of course the metabolites before the block, the Δ^5 -steroids (pregnenolone and Δ^5 -17-OHP). In rare disorders, where the levels of unusual compounds are grossly elevated, a nominally low level of cross-reactivity that normally does not have any significance becomes important.

The commercially available antibody in general use for detection of 17-OHP in neonatal screening programs was changed in 2004. The antibody introduced to replace the previous one in the Delfia assay has a slightly different pattern of cross-reactivity with considerably higher cross-reactivity for Δ^5 -17-OHP and progesterone: 1.20 and 0.47%, respectively.

Our patient had a 17-OHP value of 405 nmol/l in a retrospective analysis with the new Delfia kit which is considerably higher than with the previous kit (125 nmol/l). This indicates that the likelihood of a child with *HSD3B2*

deficiency to be detected by screening has 'technically' increased. This opens a possibility to prevent salt loss and adrenal crisis also in patients with *HSD3B2* deficiency. However, there is a risk that the correct diagnosis is delayed, as in this case, by the contradictory laboratory information caused by cross-reactivity in the RIAs used for hormone analysis. This emphasizes the importance of absolute confirmation of the diagnosis by means of molecular genetic analysis.

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References

- Simard J, Ricketts ML, Gingras S, Soucy P, Feltus FA, Melner MH: Molecular biology of the 3 β -hydroxysteroid dehydrogenase/ Δ^5 - Δ^4 isomerase gene family. *Endocr Rev* 2005; 26:525–582.
- Marui S, Castro M, Latronico AC, Elias LL, Arnhold JJ, Moreira AC, Mendonca BB: Mutations in the type II 3 β -hydroxysteroid dehydrogenase (*HSD3B2*) gene can cause premature pubarche in girls. *Clin Endocrinol (Oxf)* 2000;52:67–75. Erratum in: *Clin Endocrinol (Oxf)* 2004;60:527.
- Moisan AM, Ricketts ML, Tardy V, Desrochers M, Mebarki F, Chaussain JL, Cabrol S, Raux-Demay MC, Forest MG, Sippell WG, Peter M, Morel Y, Simard J: New insight into the molecular basis of 3 β -hydroxysteroid dehydrogenase deficiency: identification of eight mutations in the *HSD3B2* gene in eleven patients from seven new families and comparison of the functional properties of twenty-five mutant enzymes. *J Clin Endocrinol Metab* 1999;84:4410–4425.
- Simard J, Ricketts ML, Moisan AM, Tardy V, Peter M, Van Vliet G, Morel Y: A new insight into the molecular basis of 3 β -hydroxysteroid dehydrogenase deficiency. *Endocr Res* 2000;26:761–770.
- Carbunaru G, Prasad P, Scoccia B, Shea P, Hopwood N, Ziai F, Chang YT, Myers SE, Mason JI, Pang S: The hormonal phenotype of nonclassic 3 β -hydroxysteroid dehydrogenase (*HSD3B*) deficiency in hyperandrogenic females is associated with insulin-resistant polycystic ovary syndrome and is not a variant of inherited *HSD3B2* deficiency. *J Clin Endocrinol Metab* 2004;89:783–794.
- Marui S, Russell AJ, Paula FJ, Dick-de-Paula I, Marcondes JA, Mendonca BB: Genotyping of the type II 3 β -hydroxysteroid dehydrogenase gene (*HSD3B2*) in women with hirsutism and elevated ACTH-stimulated Δ^5 -steroids. *Fertil Steril* 2000;74:553–557.
- Pang S, Carbunaru G, Haider A, Copeland KC, Chang YT, Lutfallah C, Mason JI: Carriers for type II 3 β -hydroxysteroid dehydrogenase (*HSD3B2*) deficiency can only be identified by *HSD3B2* genotype study and not by hormone test. *Clin Endocrinol (Oxf)* 2003; 58:323–331.
- Clayton PE, Miller WL, Oberfield SE, Ritzen EM, Sippell WG, Speiser PW; ESPE/LWPES CAH Working Group: Consensus statement on 21-hydroxylase deficiency from the European Society for Paediatric Endocrinology and the Lawson Wilkins Pediatric Endocrine Society. *Horm Res* 2002;58:188–195.
- Pang S, Wallace MA, Hofman L, Thuline HC, Dorche C, Lyon IC, Dobbins RH, Kling S, Fujieda K, Suwa S: Worldwide experience in newborn screening for classical congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *Pediatrics* 1988;81:866–874.
- Nordenström A, Thilén A, Hagenfeldt L, Larsson A, Wedell A: Genotyping is a valuable diagnostic complement to neonatal screening for congenital adrenal hyperplasia due to steroid 21-hydroxylase deficiency. *J Clin Endocrinol Metab* 1999;84:1505–1509.
- Cara JF, Moshang T Jr, Bongiovanni AM, Marx BS: Elevated 17-hydroxyprogesterone and testosterone in a newborn with 3 β -hydroxysteroid dehydrogenase deficiency. *N Engl J Med* 1985;313:618–621.
- Tardy V, Toublanc J, Nivelon JL, Dorche C, Morel Y: Genotyping of 150 patients detected by French neonatal screening of 21-hydroxylase deficiency (1985–1997): more than 97% have a classic form (abstract). *Horm Res* 2000;53(suppl 2):77.
- Johannsen TH, Mallet D, Dige-Petersen H, Muller J, Main KM, Morel Y, Forest MG: Delayed diagnosis of congenital adrenal hyperplasia with salt wasting due to type II 3 β -hydroxysteroid dehydrogenase deficiency. *J Clin Endocrinol Metab* 2005;90:2076–2080.
- Brody S, Carlström K, Lagrelius A, Lunell NO, Rosenborg L: Serum levels of 4-androstene-3,17-dione in menstruating and postmenopausal women: evaluation of a radioimmunoassay and correlation with bone mineral content and endometrial pathology. *Acta Obstet Gynecol Scand* 1983;62:531–534.
- Stege R, Eriksson A, Henriksson P, Carlström K: Orchidectomy or oestrogen treatment in prostatic cancer: effects on serum levels of adrenal androgens and related steroids. *Int J Androl* 1987;10:581–587.
- Axelsson M, Sahlberg BL, Sjövall J: Analysis of profiles of conjugated steroids in urine by ion-exchange separation and gas chromatography-mass spectrometry. *J Chromatogr Biomed Appl* 1981;224:355–370.
- Forest MG, de Peretti E, Lecoq A, Cadillon E, Zabet MT, Thoulon JM: Concentration of 14 steroid hormones in human amniotic fluid of midpregnancy. *J Clin Endocrinol Metab* 1980;51:816–822.
- McCartin SR, Fisher RA, Wallace AM, Arnhold JJ, Mason JI, Varley J, Mendonca BB, Sutcliffe RG: Phenotypic variability and origins of mutations in the gene encoding 3 β -hydroxysteroid dehydrogenase type II. *J Mol Endocrinol* 2000;24:75–82.
- Reiter JC, Leveau P, Tardy V, Forest MG, Morel Y: Diagnostic criteria for the diagnosis of 3 β -hydroxysteroid dehydrogenase (3 β -HSD) deficiency: lessons from hormonal and molecular studies in 2 girls presenting with premature pubarche (abstract). *Horm Res* 2000;53(suppl 2):76.
- Lutfallah C, Wang W, Mason JI, Chang YT, Haider A, Rich B, Castro-Magana M, Copeland K, David R, Pang S: Newly proposed hormonal criteria via genotypic proof for type II 3 β -hydroxysteroid dehydrogenase deficiency. *J Clin Endocrinol Metab* 2002;87: 2611–2622.
- Mermejo LM, Elias LL, Moreira AC, Mendonca BB, Castro M: Refining hormonal diagnosis of type II 3 β -hydroxysteroid dehydrogenase deficiency in patients with premature pubarche and hirsutism based on *HSD3B2* genotyping. *J Clin Endocrinol Metab* 2005;90:1287–1293.
- Nordenström A, Wedell A, Hagenfeldt L, Marcus C, Larsson A: Neonatal screening for congenital adrenal hyperplasia: 17-hydroxyprogesterone levels and CYP21 genotypes in preterm infants. *Pediatrics* 2001;108:E68.
- Doerr HG, Sippell WG, Versmold HT, Bidlingmaier F, Knorr D: Plasma mineralocorticoids, glucocorticoids, and progestins in premature infants: longitudinal study during the first week of life. *Pediatr Res* 1988;23: 525–529.