Commentary

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Congenital Adrenal Hyperplasia: Time to Replace 17OHP with 21-Deoxycortisol

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Keywords

Adrenal steroid · Androgens · Congenital adrenal hyperplasia · Disorders of sexual development · Hormone assay · Newborn · Screening

Abstract

Congenital adrenal hyperplasia (CAH) due to steroid 21-hydroxylase deficiency (21OHD) has a worldwide incidence of 1 in 15–20,000. Affected individuals have adrenal insufficiency and androgen excess; the androgen excess begins during fetal life, typically resulting in 46,XX disordered sexual development. In 21OHD, 17-hydroxyprogesterone (17OHP), the steroid proximal to 21-hydroxylase, accumulates. Most industrialized countries have newborn screening programs that measure 17OHP; such screening has permitted rapid detection of newborns with 21OHD, saving lives previously lost to mineralocorticoid deficiency and salt wasting. However, newborn screening is plagued by false positives. 17OHP is above most "cutoff values" in the first 24 h of life, is high in otherwise normal premature infants, and in many term infants with physiologic stress from unrelated diseases. In addition, newborn 17OHP may be elevated in other forms of CAH, including 11-hydroxylase deficiency, 3β-hydroxysteroid dehydrogenase deficiency, and P450 oxidoreductase deficiency. In 21OHD, some of the accumulated intra-adrenal 17OHP is converted to 21-deoxycortisol (21-deoxy) by 11β-hydroxylase (CYP11B1); 21-deoxy is not elevated in premature infants or in other forms of CAH, and hence is a more specific marker for 21OHD. However, 21-deoxy assays have not been generally available until recently, hence experience is limited. We urge clinical investigators, commercial reference laboratories, and newborn screening programs to investigate replacing 17OHP with 21-deoxy as the analyte of choice for studies of 21OHD.

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Background on 21-Hydroxylase Deficiency

Congenital adrenal hyperplasia (CAH) includes multiple disorders of cortisol synthesis, but steroid 21-hydroxylase deficiency (21OHD) accounts for ~95% of the CAH cases worldwide [1, 2]. The incidence of 21OHD is about 1: 15,000 to 1:20,000 [1, 2]. Adrenal steroid 21-hydroxylation is catalyzed by microsomal P450c21, encoded by the *CYP21A2* gene [3]. P450c21 converts progesterone to deoxycorticosterone in the biosynthesis of aldosterone and converts 17-hydroxyprogesterone (17OHP) to 11-deoxycortisol in the biosynthesis of cortisol. Aldosterone deficiency, seen in up to 75% of the cases, can cause life-threatening salt loss with hyponatremia, hyperkalemia, and acidosis. Cortisol deficiency results in adrenal insufficiency with consequent increased secretion of ACTH, which drives adrenal synthesis of steroids proximal to the impaired 21-hy-

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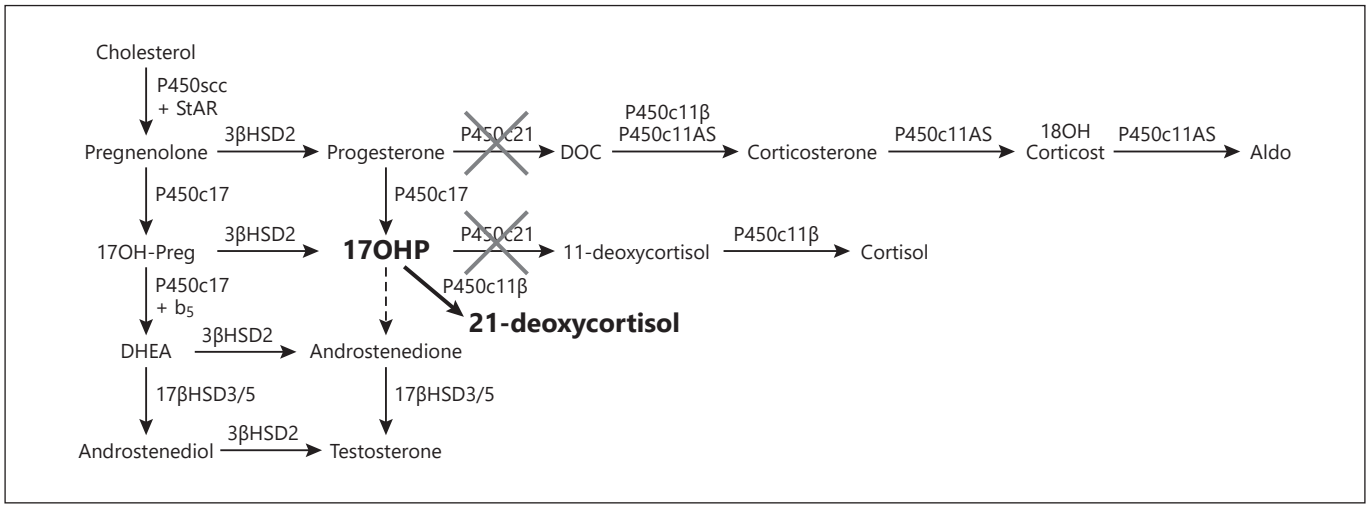

Fig. 1. Adrenal steroidogenic pathways in 21OHD. The enzymes and factors indicated are: P450scc, cholesterol side-chain cleavage enzyme (CYP11A1); StAR, steroidogenic acute regulatory protein; 3βHSD2, 3β-hydroxysteroid dehydrogenase type 2 (HSD3B2), P450c21, steroid 21-hydroxylase (CYP21A2); P450c11β, 11β-hydroxylase (CYP11B1); P450c11AS, aldosterone synthase (CYP11B2); P450c17, 17α-hydroxylase/17,20-lyase (CYP17A1); b_5, cytochrome b_5 (CYB5); 17βHSD3/5, 17β-hydroxysteroid dehydrogenase, type 3 or type 5 (HSD17B3 or AKR1C3). DOC, deoxycorticosterone; Aldo, aldosterone; DHEA, dehydroepiandrosterone.

droxylation. Some of these proximal steroids are diverted to the production of androgens; the hyperandrogenemia in affected 46,XX females typically leads to genital virilization. However, 21OHD in 46,XY males and milder forms in 46,XX females, often goes unrecognized, resulting in rapid growth, disproportionate advancement in skeletal maturation, sexual precocity, and severely compromised adult stature. Newborn screening programs, instituted in most industrialized countries, facilitate early diagnosis and treatment, mitigating these outcomes [1, 2, 4, 5].

17OHP in 21OHD

As 17OHP is the major substrate for P450c21, it accumulates in 21OHD and is the analyte most often measured in studies of 21OHD. Very little 17OHP is converted to androstenedione, as human P450c17 (17-hydroxylase/17,20 lyase) catalyzes this reaction very poorly [6, 7] (Fig. 1). However, 17OHP may be converted to 21-deoxycortisol (21-deoxy) [8], catalyzed by 11β-hydroxylase (P450c11β, encoded by *CYP11B1*). The pathways shown in Figure 1 were not known to the early pioneers of CAH, hence both Wilkins [9] and Bartter [10] assessed responses of CAH to treatment with cortisone by measuring urinary 17-ketosteroids (metabolites of androgen precursors), but they did not know they were dealing with 21OHD. Butler and Marrian [11] showed that pregnanetriol was elevated in the urine of patients with CAH, and Bongiovanni et al. [12] later showed that 17OHP was metabolized to pregnanetriol. But it was not until 1955–1960 that it was demonstrated that most CAH was caused by an error in 21-hydroxylation [8, 13, 14]. Jailer reported that both 17OHP and 21-deoxy were elevated in CAH [15] (Fig. 2), and Jailer et al. [8] studied the roles of both 17OHP and 21-deoxy in CAH but focused on 17OHP because they thought it was an androgenic steroid. Thus, the lack of apparent hormonal activity for 21-deoxy, and the close correlations among urinary 17-ketosteroids, urinary pregnanetriol, and serum 17OHP, resulted in 17OHP becoming the analyte of choice in 21OHD. Over the past 60 years, massive amounts of data have been reported concerning 17OHP and its metabolites in normal and CAH-affected fetuses, newborns, and adults, and the responses of 17OHP to ACTH and other stimuli in 21OHD and other forms of CAH. Thus, endocrinologists have become familiar (and comfortable) with 17OHP.

Newborn Screening for CAH via 17OHP

Newborn screening programs throughout the industrialized world now incorporate measurements of 17OHP to detect 21OHD. By reducing the time to diagnosis,

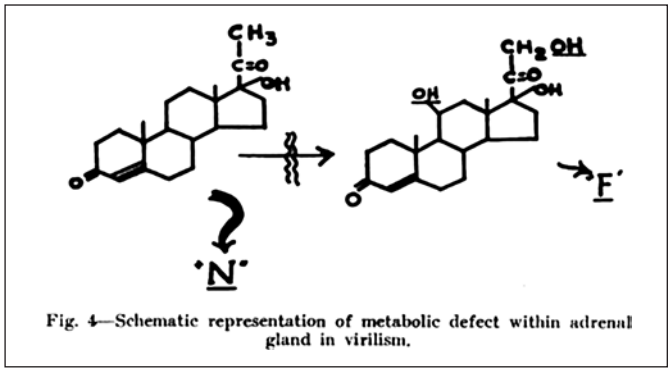

Fig. 4—Schematic representation of metabolic defect within adrenal gland in virilism.

Fig. 2. Illustration from Joseph Jailer's 1953 review 'Virilism' [15] showing defects in both the 11-hydroxylation and 21-hydroxylation of 17OHP to cortisol. The original caption designated 'Fig. 4' is retained for historical context. Reprinted with permission.

screening dramatically reduces mortality, morbidity, length of hospitalization, cost, learning disability, and errors of sex assignment [2, 4, 5, 16]. However, screening programs in different jurisdictions use different 17OHP assays, thresholds, and follow-up procedures; hence the physician must become familiar with local procedures. Current guidelines recommend a standardized 17OHP immunoassay from the dried blood samples used for other newborn screens [2]. The automated time-resolved dissociation-enhanced lanthanide fluoroimmunoassay permits high throughput with small samples extracted from newborn heel-sticks ("Guthrie cards") and has almost completely supplanted classical radioimmunoassays (RIAs) and enzyme-linked immunosorbent assays for newborn screening of 17OHP. Because 17OHP is elevated in healthy babies in the first 1–2 days of life and can be grossly elevated in premature and sick infants, secondary screening by liquid chromatography followed by tandem mass spectrometry (LC-MS/MS) is recommended for all elevated results [2]. Initial screening results are improved when small babies are stratified for gestational age rather than birth weight [17]. Because immunoassays can yield false-positive results, follow-up testing should be done with an intravenous ACTH test if LC-MS/MS is not available.

Shortcomings of Newborn Screening by 17OHP

Widespread use of newborn screening for 21OHD via measurements of 17OHP has revealed numerous shortcomings to this approach. About 40% of the samples that are initially reported as positive will have normal 17OHP levels when subsequently measured by LC-MS/MS [2]. Several factors cause increased 17OHP in babies who do not have 21OHD. First, 17OHP is high in cord blood and falls slowly during the first 1–2 days of life [18], hence obtaining the sample too soon after birth is a common cause of false-positive results [19, 20]. Second, stress from other illnesses may result in the 17OHP remaining high in unaffected babies [21]. Third, because the fetal adrenal produces large amounts of 17OHP [22], premature infants very frequently test as false positives [17, 23]. Fourth, other forms of CAH, including 11-hydroxylase deficiency [4, 24–26], 3β-hydroxysteroid dehydrogenase deficiency [4, 27, 28], and P450 oxidoreductase deficiency [29, 30] may also result in elevated 17OHP values detected on newborn screening. Measuring additional steroids and their ratios may improve the specificity of screening by LC-MS/MS: one study found that the ratio of sum of 17OHP plus 21-deoxy divided by the cortisol level identified all 16 affected children among 1,609 preliminary positive tests among 242,500 newborns, with no false positives [31].

21-Deoxycortisol in CAH

Measuring 21-deoxy in patients with 21OHD is not a new idea. Early studies relied on tedious chromatography followed by RIAs based on the cross-reactivity of 21-deoxy with antisera directed against cortisol, permitting the measurement of 21-deoxy in samples stripped of cortisol [32, 33]. Because of these crude technologies, the absolute values of 21-deoxy in these two early studies differed substantially, but nevertheless, both studies showed that 21-deoxy was very high in untreated infants, in children with salt-wasting 21OHD, and in untreated adults with simple virilizing 21OHD, but very low in controls. Fukushima et al. [34] raised an antiserum to 21-deoxy and reported its first specific RIA, again reporting substantially elevated values in 21OHD and values below the limit of detection in controls. Using Fukushima's antiserum, Cassorla et al. [35] reported that obligate heterozygous parents of patients with 21OHD had similar basal 21-deoxy values compared to controls, but that their 21-deoxy values were hyper-responsive to stimulation with ACTH. Milewicz et al. [36] raised a high-affinity antiserum (used at 1:180,000) that had a low cross-reactivity with other steroids and compared results measuring 21-deoxy and 17OHP. In 22 normal children, 21-deoxy was 7.5 ± 5 ng/dL and 17OHP was 136 ± 53 ng/dL; in 9 untreated chil-

dren with 21OHD (ages 5 months–12 years), 21-deoxy was 2,225 ± 876 ng/dL, and 17OHP was 7,201 ± 2,529 ng/dL [36]. Gueux et al. [37] also raised a specific antiserum and set up a RIA, which they used to show that 21-deoxy and 17OHP tracked similarly in the amniotic fluid of pregnancies carrying fetuses with and without 21OHD [38] and that 21-deoxy was a reliable diagnostic marker for "late-onset" (now termed "non-classical") 21OHD [39]. Together, these studies show that 21-deoxy is abundant in 21OHD, tracks well with 17OHP, and is a clinically reliable diagnostic analyte.

However, in the last 30 years, few groups have studied the dynamics of 21-deoxy in 21OHD and other forms of CAH, and 21-deoxy has been relegated to use as a secondary analyte measured by LC-MS/MS in a few CAH screening programs. The reason for this diminished interest in 21-deoxy has been the lack of generally available antisera to 21-deoxy that might be used in newborn screening programs, the lack of commercial assays for 21-deoxy for routine clinical use, the overwhelmingly prevalent use of 17OHP in the diagnosis of 21OHD at all ages, and endocrinologists' lack of familiarity with 21-deoxy. Thus, detailed information about 21-deoxy as a function of hours or days after birth in term and premature infants without and with 21OHD is absent; however, it appears that 21-deoxy is not grossly elevated in the premature as is 17OHP [31], and hence newborn screening assays based on 21-deoxy should not have the high false-positive rate that plagues current screening programs based on 17OHP.

Next Steps

Newborn screening programs require rapid analysis of huge numbers of samples (~500,000/year in California); contemporary LC-MS/MS technologies are poorly suited to this task; hence immunoassays remain most widely used. Thus, a key question is whether specific, high-affinity antisera to 21-deoxy can be generated in high quantity and made generally available. Commercial laboratories should aggressively pursue the generation of such reagents. Alternatively, new technologies might be used to measure 21-deoxy. A single order of magnitude increase in the throughput of LC-MS/MS technologies would make these practical for initial newborn screening. The data summarized above suggest that converting newborn screening programs from 17OHP to 21-deoxy should dramatically reduce the rate of false-positives, thus reducing the need to recall infants for additional, expensive testing, and eliminating much parental stress (and expense) associated with recall visits. Nevertheless, while the scientific logic behind screening for CAH with 21-deoxy is compelling, such a revision of current screening procedures will require careful validation. Screening programs will need to continue measuring 17OHP for several years while screening with 21-deoxy is implemented, so as to compare the results with both analytes objectively.

Pediatric endocrinologists should encourage their local, state, or national screening programs to explore the possibility of measuring 21-deoxy as the primary screen for CAH, initially in combination with established 17OHP measurements. Pediatric endocrinologists should also begin to measure 21-deoxy in their patients with CAH or being evaluated for possible CAH, especially in the newborn, again, in combination with conventional 17OHP measurements. Both the newborn screening community and the practicing endocrinologists must become familiar with this superior mode of diagnosing and monitoring CAH.

Disclosure Statement

The author is a consultant for Spruce Biosciences and Adrenas Therapeutics.

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