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The urinary steroidome of treated children with classic 21-hydroxylase deficiency

Clemens Kamrath[1], Lisa Wettstaedt[1], Claudia Boettcher, Michaela F. Hartmann, Stefan A. Wudy, MD, PhD*

Steroid Research & Mass Spectrometry Unit, Laboratory for Translational Hormone Analytics, Division of Pediatric Endocrinology and Diabetology, Center of Child and Adolescent Medicine, Justus Liebig University, Giessen, Germany

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ABSTRACT

Monitoring treatment of children with classic congenital adrenal hyperplasia (CAH) is difficult and biochemical targets are not well defined.

We retrospectively analysed 576 daily urinary steroid hormone metabolite profiles determined by gas chromatography–mass spectrometry of 150 children aged 3.0–17.9 years with classic 21-hydroxylase deficiency (21-OHD) on hydrocortisone and fludrocortisone treatment.

Daily urinary excretion of glucocorticoid-, 17α-hydroxyprogesterone (17-OHP)-, and androgen metabolites as well as growth and weight gain are presented. Children with classic CAH exhibited increased height velocity during prepubertal age, which was then followed by diminished growth velocity during pubertal age until final height was reached. Final height was clearly below the population mean. 11β-Hydroxyandrosterone was the dominant urinary adrenal-derived androgen metabolite in CAH children. Adrenarche is blunted in children with CAH under hydrocortisone treatment and androgen metabolites except 11β-hydroxyandrosterone were suppressed. Cortisol metabolite excretion reflected supraphysiological hydrocortisone treatment dosage, which resulted in higher body-mass-indices in children with CAH.

Reference values of daily urinary steroid metabolite excretions of treated children with CAH allow the clinician to adequately classify the individual patient regarding the androgen-, 17-OHP-, and glucocorticoid status in the context of the underlying disorder. Additionally, urinary 21-OHD-specific reference ranges will be important for research studies in children with CAH.

1. Introduction

21-Hydroxylase deficiency (21-OHD) is the most common form of congenital adrenal hyperplasia (CAH). 21-OHD is caused by mutations in *CYP21A2*, the gene encoding the adrenal steroid 21-hydroxylase enzyme (CYP21A2). Inefficient cortisol synthesis in patients with CAH leads to adrenal stimulation, but rather than cortisol, the adrenals produce excess androgen precursors that do not require 21-hydroxylation for their synthesis [1] (Fig. 1).

The aim of treatment of children with classic CAH with glucocorticoids consists in replacing the lack of cortisol and suppressing excess adrenal androgen production. Clinical management of classic CAH is a difficult balance between androgen and cortisol excess [2]. However, monitoring of treatment is difficult in CAH [3]. Laboratory data should indicate the need for dose adjustment. Serum or plasma concentrations of 17α-hydroxyprogesterone (17-OHP), androstenedione and testosterone are currently the most widely used indicators to monitor glucocorticoid treatment [2,3]; however, biochemical targets of disease control are not well defined and random measurement of plasma concentrations of 17-OHP and androgens on a clinical visit is of only limited value in patients with CAH because it does not reflect a patient's circadian pattern of adrenal steroid secretion [4,5].

Analysis of urinary steroid hormone metabolites by gas chromatography–mass spectrometry (GC–MS) (urinary steroidomics) is a non-invasive diagnostic means and provides an overview of the whole spectrum of adrenal steroids in a CAH patient, including glucocorticoid, androgen and 17-OHP metabolites in parallel (Fig. 1). In contrast to the determination of single steroids

* Corresponding author at: Division of Pediatric Endocrinology and Diabetology, Steroid Research & Mass Spectrometry Unit, Laboratory of Translational Hormone Analytics, Center of Child and Adolescent Medicine, Justus Liebig University, Feulgenstr. 12, 35385 Giessen, Germany.
E-mail address: stefan.wudy@uni-giessen.de (S.A. Wudy).
[1] Both authors contributed equally to this work.

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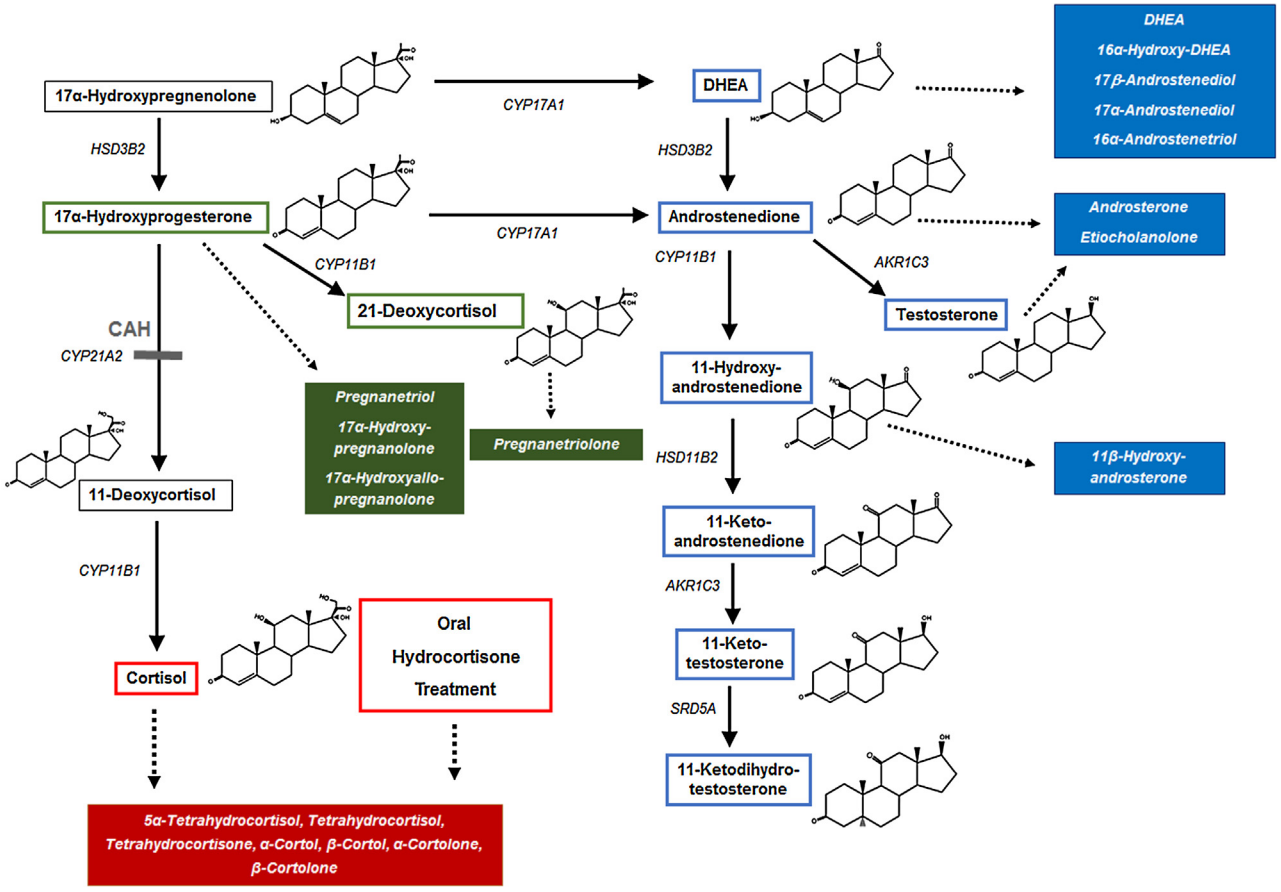

Fig. 1. Schematic overview of steroidogenesis and steroid metabolism in CAH. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
The enzymatic block in CAH is shown in gray. Metabolism in extra-adrenal tissues leading to urinary metabolites is shown in dotted arrows. Urinary metabolites are shown in color-filled boxes. 17α-hydroxyprogesterone and its urinary metabolites are highlighted green, androgens and their urinary metabolites blue, and cortisol and their urinary metabolites red.
Urinary metabolites of 17α-hydroxyprogesterone include pregnanetriol, 17α-hydroxypregnanolone and 17α-hydroxyallopregnanolone. Additionally, accumulating 17α-hydroxyprogesterone is further metabolized to 21-deoxycortisol by the 11β-hydroxylase activity of CYP11B1, which is metabolized to urinary pregnanetriolone. In CAH, accumulation 17α-hydroxyprogesterone is converted into androgens. Dehydroepiandrosterone (DHEA) is metabolized to urinary DHEA, 16α-hydroxy-DHEA, 17β-androstenediol, 17α-androstenediol and 16α-androstenetriol, and additionally to urinary androsterone and etiocholanolone. The latter two also represent metabolites of androstenedione and testosterone [14]. Additionally adrenal androstenedione is converted by 11β-hydroxylase activity to 11β-hydroxyandrostenedione, which is further metabolized to urinary 11β-hydroxyandrosterone [16]. 11β-Hydroxyandrostenedione can be further metabolized to the active androgens 11-ketotestosterone and 11-ketodihydrotestosterone that activate the androgen receptor comparable to testosterone and dihydrotestosterone, respectively [16,17].
Urinary metabolites of endogenous cortisol as well as exogenous hydrocortisone replacement comprise tetrahydrocortisol, 5α-tetrahydrocortisol, tetrahydrocortisone, α- and β-cortols, as well as α- and β-cortolones [13].

in a single plasma sample 24-h urinary steroid profile analysis provides an assessment of daily steroid excretion rates [6–8].

The aim of our study was to analyse retrospectively 24-h urinary steroid metabolite excretion in a large cohort of children with classic CAH due to 21-OHD treated with hydrocortisone and fludrocortisone to characterize their daily excretion pattern of androgen-, 17-OHP-, and cortisol metabolites in the context of their growth and weight gain, and to provide 21-OHD specific reference values. 21-OHD specific reference values could help to classify the individual CAH patient's urinary steroid metabolome on the basis of a large reference cohort and could be important for research studies in children with CAH.

2. Patients and methods

2.1. Patients

Inclusion criteria for our retrospective analysis were: 1.) classic CAH due to 21-OHD. The diagnosis of classic 21-OHD was made on the basis of the characteristic urinary steroid metabolite profile determined by GC–MS analysis with highly elevated concentrations of 17-OHP and 21-deoxycortisol metabolites [8–10] and of requirement of mineralocorticoid (fludrocortisone) replacement therapy; 2.) oral hydrocortisone given in three divided doses, as the only glucocorticoid replacement treatment; 3.) children aged 3–18 years; and 4.) data of height (in 0.1 cm intervals) and weight (in 0.1 kg intervals).

The data were collected in a local database and analysed anonymously. All collected data were part of the routine care of patients with CAH due to 21-OHD [2]. The retrospective analysis was approved by the local ethics committee (AZ: 84/13).

We retrieved 576 daily urinary steroid metabolite excretion profiles (317 males, 259 females) of 150 patients aged 3.0–17.9 years with classic CAH who matched the above criteria. Urinary steroid metabolite excretion was divided into classes of one-year intervals. Data of 24-h urinary steroid hormone metabolite analysis were included only once a year for every patient to avoid overrepresentation of single patients. Standard-deviation-scores (SDS) of height and body-mass-index (BMI) were calculated according to actual German references (KIGGS (German Health