Hormonal and genetic analysis of a patient with congenital adrenal hyperplasia

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We describe a patient with signs and symptoms of virilization caused by 21-hydroxylase deficiency. The patient, a Hispanic woman, first sought medical attention at age 24, when she presented to a medical clinic with an uncomplicated urinary tract infection. At that time several signs of virilization were noted and she was referred to the endocrinology clinic. Evaluation revealed temporal balding, hyperpigmentation, acne, absent breast development, a muscular habitus, and clitoromegaly. Radiological studies revealed bilaterally enlarged adrenal glands and ovaries. Laboratory evaluation revealed markedly increased concentrations of 17-hydroxyprogesterone, androstenedione, and testosterone. The patient was diagnosed with congenital adrenal hyperplasia (CAH) and received hormone therapy. In her sister, encouraged to undergo testing for this autosomal recessive disorder, HLA testing demonstrated that certain haplotypes in this family were associated with CAH. The case highlights key steps in the laboratory diagnosis and genetics of CAH.

INDEXING TERMS: inherited disorders • 21-hydroxylase deficiency • endocrinology

Congenital adrenal hyperplasia (CAH) is a relatively common genetic disease that presents several challenges to the primary care physician and the clinical chemist. Although the disease typically presents in the perinatal period or in early childhood, it may present in adulthood and can be an important cause of infertility and morbidity in certain populations. In addition to the variability of age at presentation, the clinical signs and symptoms range from asymptomatic to life-threatening (see below). The disease results from a deficiency in one of several adrenal enzymes that have wide-ranging effects on the hormone and chemical balance throughout the body. These imbalances require an accurate laboratory assessment. Given the frequency of the disease and the importance of the clinical laboratory in the diagnosis, every laboratory should be well rehearsed in the pathophysiology of CAH.

This specific case serves as an excellent example of a patient who presented with the late-onset form of CAH. Her age at presentation, 24 years, serves as a reminder of the variability of age at presentation. Her body habitus displayed many of the classic features of virilization, e.g., short stature, hirsutism, frontal balding, acne, and clitoromegaly. Her increased concentration of 17-hydroxyprogesterone was typical of one form of CAH, 21-hydroxylase deficiency. HLA typing of the patient and her sister, who was also affected, illustrates the autosomal recessive nature of the disease and provides useful information for evaluating other members of her family for genetic counseling. In this paper we emphasize the features that will be most useful in the laboratory evaluation of this important disorder.

Case Report

The patient is a 24-year-old Hispanic woman who presented to a community clinic with symptoms of an uncomplicated urinary tract infection. Examination revealed clitoromegaly and, after treatment for the urinary tract infection, she was referred to the endocrinology clinic for further evaluation.

The patient was unaware of any medical problems. She had never before seen a physician, never been hospitalized, never had any surgeries, did not take any medications, and did not use any street drugs or alcohol. She felt well throughout childhood and noted the onset of axillary and pubic hair at age 12 or 13. She did not undergo thelarche or menarche. She was sexually active, but had never become pregnant despite the absence of birth control. She described herself as muscular and short, even compared with members of her own family, but she was never worried about her appearance until the nurse mentioned it to her at the medical clinic. She had a normal energy level and did not note any salt craving.

Physical examination revealed a well-nourished muscular young Hispanic woman of short stature (144 cm). Her weight was 57.2 kg, pulse 68/min, and blood pressure 115/51 mmHg.
She had hyperpigmentation of the gums and creases on her palms. Acne was present on her face, chest, and back. Mild temporal balding and sideburns were present, in addition to hair growth on the upper lip. No breast tissue was palpable. There was a male-pattern escutcheon and the clitoris measured 5 cm by 2 cm. Structure of the urethra, vagina, and rectum was normal; there was no labioscrotal fusion.

Materials and Methods

Testosterone concentrations were performed by both RIA and mass spectrometry. Mass spectrometry was performed at the VA Medical Center, San Diego, CA, with a trideuterated internal standard and gas chromatography/negative chemical ionization mass spectrometry as described by Fitzgerald and Herold [1]. The concentrations of hormones were determined by immunological techniques at Corning Laboratory (San Diego, CA), with the Coat-A-Count (Diagnostic Products Corp., Los Angeles, CA) solid-phase RIA based on testosterone-specific antibody immobilized to the wall of a polypropylene tube. 125I-labeled testosterone competes for a fixed time with testosterone in the patient's sample for antibody sites. The tube is then decanted, to separate bound from free radioactivity, and the former is counted in a gamma counter. Concentrations of other hormones were determined at Corning Laboratory with in-house assays based on standard RIA techniques [2, 3], as follows:

- The concentration of 17-hydroxyprogesterone was determined by extracting the serum with methylene chloride, evaporating the solvent under nitrogen, and quantifying the hormone in the reconstituted dried extract by RIA with radiolabeled 17-hydroxyprogesterone and anti-17-hydroxyprogesterone.

- The concentration of androstenedione was determined by extracting the serum sample with ethyl acetate in hexane (20:80 by vol), followed by Celite chromatography and RIA.

- The concentration of 11-deoxycortisol was determined by extracting the serum sample with methylene chloride, followed by Celite chromatography. RIA of the effluent was then performed with 125I-labeled 11-deoxycortisol and rabbit anti-11-deoxycortisol. Bound/free separation was achieved by using second-antibody/polyethylene glycol precipitation.

- Cortisol concentration was determined by using an RIA with 125I (without an extraction step).

- The concentration of dihydroepiandrosterone was determined by RIA after extraction with ethyl acetate in hexane (20:80 by vol) and Celite chromatography.

Electrolyte analysis was performed by well-accepted techniques on the Hitachi 911 automated analyzer (Boehringer Mannheim, Indianapolis, IN).

HLA class I typing was performed at the University of California–San Diego immunology laboratory, which used the microdroplet assay of human serum cytotoxins described by Terasaki and McClelland [4]. HLA DR typing was performed by PCR amplification with sequence-specific primers (PCR-SSP), according to the method published by Olerup and Setterquist [5]. HLA DQ typing was performed by PCR-SSP as described by Salazar et al. [6].

<table>
<thead>
<tr>
<th>17-Hydroxyprogesterone</th>
<th>Baseline</th>
<th>Corticosterone</th>
<th>After treatmenta</th>
</tr>
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<tbody>
<tr>
<td>0.2–5</td>
<td>9.2</td>
<td>232</td>
<td>1.42</td>
</tr>
<tr>
<td>DHEA</td>
<td>450–3800</td>
<td>5770</td>
<td>30690</td>
</tr>
<tr>
<td>11-Deoxycortisol</td>
<td>&lt;1.2</td>
<td>—</td>
<td>3.4</td>
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<tr>
<td>Cortisol</td>
<td>55–200</td>
<td>40</td>
<td>81</td>
</tr>
<tr>
<td>Androstenedione</td>
<td>0.5–2.5</td>
<td>4.07</td>
<td>22.3</td>
</tr>
<tr>
<td>Testosterone</td>
<td>0.1–0.7</td>
<td>1.2</td>
<td>1.9</td>
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DHEA, dihydriopandosterone.

* With dexamethasone, 0.25 mg/day.
anterior pituitary. Cholesterol is converted to pregnenolone, and the adrenal gland hypertrophies. The concentration of cortisol remains low because of the faulty 21-hydroxylase enzyme. Some studies have found that the activity of the deficient enzyme is only 10–20% of normal [11]. The precursor hormones such as 17-hydroxyprogesterone are shunted into the sex steroid pathway, which has fully functional enzymes. Testosterone itself is not formed in the adrenal gland; instead, the adrenal gland produces androstenedione, which is converted to testosterone in peripheral tissues and results in virilization [12].

In the case we report, the patient presented with signs of increased androgen production, which was apparent from the history and physical examination. The first step in the analysis was to locate the point of the biochemical lesion. Our results indicated an increased testosterone concentration in conjunction with low cortisol, consistent with a lesion in the adrenal glands itself or primary adrenal dysfunction.

The second step was to localize which specific enzyme within the adrenal gland was faulty. This patient had markedly increased 17-hydroxyprogesterone concentrations and decreased cortisol concentrations consistent with a deficiency of either 21-hydroxylase or 11β-hydroxylase. Administration of Cortrosyn (α1–24 corticotropin, a synthetic subunit of ACTH) to stimulate the adrenal glands resulted in markedly increased concentrations of 17-hydroxyprogesterone and a relatively small increase in the concentration of 11-deoxycortisol. In addition, hypertension, frequently found in patients with 11β-hydroxylase deficiency, was not present in this patient. These results are consistent with deficiency of the 21-hydroxylase enzyme (see Fig. 1).

Of special note to the clinical chemist is the special environment created when concentrations of several hormones are increased to such a substantial degree. Several methods of determining the concentrations of steroid hormones, most notably, 17-hydroxyprogesterone and testosterone, rely on immunoassays that are acceptable in the normal hormonal environment but that can give misleading results in this setting because of the potential presence of cross-reacting hormones at high concentrations. Many of the cross-reacting steroids exist as conjugates that can be removed in the aqueous phase when 17-hydroxyprogesterone, which exists as a free steroid, is extracted with an organic solvent before analysis. Problems with immunological cross-reactivity can be avoided by analyzing urine or serum steroids by gas chromatography–mass spectrometry [11–13]. Thus, if the diagnosis of 21-hydroxylase deficiency is suspected, the laboratory results must be interpreted carefully and with an understanding of the type of laboratory methodology used.

Fig. 1. Synthetic pathways for adrenal steroid hormones.

The structures of cholesterol, aldosterone, cortisol, and androstenedione are shown. CMO, corticosterone methyl oxidase. Adapted from White et al. [14].
An understanding of the different types of 21-hydroxylase deficiency is also useful when evaluating the laboratory data. The four clinical categories of 21-hydroxylase deficiency are salt-wasting, simple virilizing, late-onset (or nonclassical), and cryptic [14–16]. The signs and symptoms of each of these categories are related to the degree of activity of the 21-hydroxylase enzyme. If the enzyme is totally absent, life cannot be sustained and the affected infant, if untreated, will die shortly after birth as a result of electrolyte imbalances and shock. In addition to the chemical imbalances, morphological abnormalities are also present because of the in utero exposure to high concentrations of androgens produced by the fetal adrenal glands. If the fetus is female, the high concentrations of androgens may cause labioscrotal fusion, enlargement of the clitoris, and a phallic urethra. The internal organs of the female reproductive system, however, are still present; their development is inhibited not by androgens but by Mullerian-inhibiting factor, which is produced by the testis. Therefore, the internal reproductive organs will develop in the female fetus even if exposed to high androgen concentrations. A male infant with this disorder will have a normal male appearance at birth and may not be as readily diagnosed with CAH as the female will be because he will lack pathognomonic external morphologic features [17].

The infants who present at birth have the "congenital" form of 21-hydroxylase deficiency and may have either simple virilization, if only cortisol production is affected, or the salt-wasting form, if both cortisol and aldosterone production are affected [18]. The 21-hydroxylase enzyme is necessary for the production of both cortisol and aldosterone, but not all cases of 21-hydroxylase deficiency result in noticeable decreases in aldosterone production. Aldosterone acts on the proximal renal tubule, enhancing sodium reabsorption and potassium excretion. In the absence of aldosterone, large amounts of sodium will be excreted in urine, and potassium will be conserved. This will result in a high serum potassium, low serum sodium, diuresis, and salt craving; thus, patients whose enzyme deficiency results in reduced cortisol and aldosterone production are termed salt-wasters. Those who display only cortisol deficiency have "simple" virilization, i.e., not associated with salt-wasting.

In some instances the 21-hydroxylase enzyme will be partially deficient but will retain enough activity to produce sufficient cortisol that the patient remains asymptomatic for the first several years of life. In more-severe cases, patients begin to develop precocious secondary sexual characteristics because of increased androgen concentrations. In both males and females, this will result in virilization at an early age and a rapid growth spurt, followed by early fusion of the epiphyseal growth plate and, ultimately, short stature. The condition can cause infertility in both males and females. High concentrations of progesterone cause irregular or absent menses, and high concentrations of androgens cause reduced concentrations of gonadotropins, resulting in poor spermatogenesis. This form of the disease is termed late-onset or nonclassical 21-hydroxylase deficiency, to distinguish it from the "classic" form of the disease, which manifests in the neonatal period [18].

A subset of patients with 21-hydroxylase deficiency remain asymptomatic. These patients can be diagnosed by testing hormone concentrations, but the concentrations are not sufficiently abnormal to cause symptoms. These patients have the "cryptic" form of the disease [16, 19].

The patient in this study is a good example of a patient with the late-onset variety of CAH. Although she did not seek medical care until well after puberty, it is apparent from her physical examination that she had been symptomatic for some years. After treatment with dexamethasone (0.25 mg per day), the concentration of ACTH was decreased and the concentrations of adrenal hormones were reduced (see Table 1). Along with reduced concentrations of adrenal hormones, the patient experienced breast development and menstruation.

An understanding of the genetics of the disease is also useful for counseling the patient and evaluating other members of the family. The gene for 21-hydroxylase is located on the short arm of chromosome 6 in the class III region of the major histocompatibility complex. This segment also contains the DNA that codes for complement 2 and 4 (C2, C4) and factor B. Two 21-hydroxylase genes alternate with two repeated sequences for C4. One of the 21-hydroxylase genes, 21A, is a pseudogene and does not code for any protein product because of three point mutations in this gene. The 21-hydroxylase B gene encodes the functional enzyme [8].

Because the gene for 21-hydroxylase is in such proximity to the class I and class II regions of the major histocompatibility complex, there is linkage disequilibrium, and several haplotypes have been associated with 21-hydroxylase deficiency. The salt-wasting form is associated with A3, B47, and DR7; the simple virilizing form is associated with Bw51; and the nonclassical form is associated with B14 and DR1 [12, 20].

The arrangement of the DNA segments that encode the 21-hydroxylase enzyme may be important in the pathogenesis and frequency of the disease. The fourth component of complement and the 21-hydroxylase gene are randomly repeated, an arrangement that might lead to misalignment during meiotic metaphase. This would create two types of errors. First, because the 21A gene is a pseudogene, the point mutations it contains can be transferred to the active 21B gene. Second, unequal crossover may occur, such that the functional 21B gene is deleted on one chromatin. Gene deletion occurs in ~21% of patients; the rest have point mutations. Both of these mechanisms have been demonstrated [8, 21, 22].

The patient in this case study, and her sister, have an HLA type not previously known to be associated with 21-hydroxylase deficiency. This case may represent a new mutation not previously studied. The fact that the two affected siblings are homozygous for the same HLA types suggests the possibility of inbreeding in this family—which is consistent with the autosomal recessive nature of the disorder. Further family testing would be useful to provide genetic counseling for other members of the family. For example, a sibling who has one copy of the HLA type found in this patient would be a carrier of the disease.

Although 21-hydroxylase deficiency is associated with serious and even life-threatening consequences, it is a treatable disorder. Once glucocorticoid and, when necessary, mineralocorticoid replacement is accomplished, the concentration of ACTH nor-
nalizes, thereby reducing excess androgen production. If treatment is initiated early, the symptoms of virilization—including genital abnormalities, short stature, acne, and hirsutism—can be ameliorated [23]. Many reports have been published of healthy births to females who were appropriately treated for this disorder [24]. Therefore, in view of the high frequency of the disorder and the readily available and effective treatment options, the diagnosis of 21-hydroxylase deficiency should be considered in the patient with the appropriate clinical history and physical findings. We hope this paper will serve as a guideline to ordering and interpreting appropriate laboratory tests.

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References


