21-HYDROXYLASE DEFICIENT NON-CLASSIC ADRENAL HYPERPLASIA: SCREENING, DIAGNOSIS AND TREATMENT
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Prevalence of NCAH
Twenty-one hydroxylase (21-OH) deficient non-classic adrenal hyperplasias (NCAH) is one of the most common genetic disorders known to man. This autosomal recessive disorder results in a defect in adrenocortical 21-OH enzyme function. NCAH affects one of every 1000 to 2000 non-Jewish White individuals 1, and between 1% and 10% of hyperandrogenic women, depending on the ethnicity of the population studied 2-12. In the United States others and we have observed an NCAH prevalence of 1% to 2% among populations of White and Hispanic hyperandrogenic patients 2-5. In contrast, studies in France, Italy and Canada yield frequencies of 4% to 6% 6-8, and other studies originating in Israel, India and Jordan demonstrate prevalences of 6% to 10% 6-12. In contrast, NCAH due to defects of 11β-hydroxylase and 3β-hydroxysteroid dehydrogenase are extremely strange, if existent at all, particularly in patients presenting with postpubertal hyperandrogenism 2,13-15.

Genetics of NCAH
NCAH is a homozygous recessive disorder due to the mutation of CYP21, the gene encoding for the enzyme P450c21. In turn, P450c21 is located exclusively in the zona fasciculata of the adrenal cortex, and demonstrates 21-OH activity, converting 17-HP to 11-deoxycortisol and P4 to deoxycorticosterone 16. The gene encoding for P450c21 (i.e. CYP21) exists in tandem with a pseudogene (i.e. CYP21P). Each gene has 10 exons and 9 introns, although CYP21P has many mutations that render it non-transcribable. These genes are located within the HLA locus on chromosome 6 next to the gene for the fourth component of complement (C4) and a gene for the extracellular matrix protein tenacin-x 17.

There are multiple mutations reported to result in NCAH, some defined as "mild" mutations (preserving about 20% of native 21-OH activity) and others as "severe" (preserving 0% to 5% of native 21-OH activity). Most of these mutations are similar to the sequences found in a proximate pseudogene (CYP21P), suggesting that the mechanism for acquiring the mutations is gene conversion. Clinically evident NCAH may result from mild/mild (e.g. V281L/V281L) or from mild/severe allele combinations, the so-called "compound heterozygotes" (e.g. V281L/I172N). We should note that there is a loose association between the severity of the clinical presentation and the severity of the CYP21 mutations carried 18.

Because of the autosomal recessive nature of NCAH, the incidence of this disorder among family members is actually lower than that observed among other hyperandrogenic patients, e.g. PCOS patients. For example, full sisters will have a 25% chance of being affected, while few mothers are actually homozygous and clinically affected. This contrasts to our finding that in PCOS approximately 35% of mothers and 40% of sisters of PCOS patients are affected by the disorder 19.

Diagnosis of NCAH
NCAH results in hyperandrogenic symptoms some time after birth, in contrast to the symptoms of classic adrenal hyperplasia (CAH) that are apparent at or near birth. The endocrine diagnosis of 21-OH deficient NCAH is performed by a basal or acute
ACTH-stimulated 17-hydroxyprogesterone (17-HP) level >10 ng/mL (30.3 nmol/L)\textsuperscript{20,21}. The diagnosis can be confirmed by genotyping, although it should be noted that not all mutations can be readily identified, either because they are new and unknown, or because they are extremely rare and not generally included in screening assays\textsuperscript{18,22}.

Clinical presentation: Comparison to PCOS

Overall, it is generally difficult to distinguish NCAH from PCOS solely on clinical grounds, as both disorders demonstrate varying degrees of hyperandrogenism and ovulatory dysfunction\textsuperscript{23}. We recently studied 905 consecutive untreated hyperandrogenic patients diagnosed between 1987 and 2001 at the University of Alabama at Birmingham, including 654 patients with endocrinologically diagnosed PCOS and 17 with 21-OH deficient NCAH\textsuperscript{24}. Thirteen of the NCAH were non-related probands, while four of the patients were sisters of four separate probands. Although the significant discordance in number of subjects reduces the validity of a direct statistical comparison between the two patient populations, we should note that PCOS and NCAH patients had similar mean ages, body mass indices, waist hip ratio, hirsutism score, and prevalence of acne (Table 1).

All patients with PCOS, by definition had ovulatory dysfunction. Likewise the majority of NCAH patients in this study had ovulatory dysfunction. This is consistent with the observation by other investigators that not all patients with NCAH have oligo-ovulation\textsuperscript{25}. In the present study, all patients with NCAH were White while 13% of our PCOS population was non-White, primarily Black, consistent with our referral population (Table 1). The paucity of NCAH among Black patients is not surprising, as this racial trend was also observed in a large international multicenter study\textsuperscript{21}, and is in agreement with the low prevalence CAH among Black subjects\textsuperscript{1}.

Polycystic ovaries are observed by ultrasonography in about 75% of patients with PCOS\textsuperscript{26}. Likewise about 40% of patients with NCAH demonstrate this clinical feature\textsuperscript{23,25}, consistent with the fact that “polycystic ovaries” can be observed in a variety of patients with ovulatory disorders.

In conclusion, both PCOS and 21-OH deficient

<table>
<thead>
<tr>
<th>Features</th>
<th>PCOS (n=654)</th>
<th>NCAH (n=17)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>27.1±7.1</td>
<td>28.5±10.9</td>
<td>NS</td>
</tr>
<tr>
<td>Race (% non-White)</td>
<td>13.4</td>
<td>0</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>Ovulatory dysfunction</td>
<td>654/654</td>
<td>15/16</td>
<td>NS</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>33.2±9.1</td>
<td>28.9±6.5</td>
<td>NS</td>
</tr>
<tr>
<td>WHR</td>
<td>0.84±0.10</td>
<td>0.83±0.07</td>
<td>NS</td>
</tr>
<tr>
<td>Acne (%)</td>
<td>92/654 (14.1%)</td>
<td>3/17 (17.6%)</td>
<td>NS</td>
</tr>
<tr>
<td>F-G score</td>
<td>8.1±4.8</td>
<td>8.1±3.8</td>
<td>NS</td>
</tr>
<tr>
<td>Total T (nmol/L)</td>
<td>3.14±2.2</td>
<td>4.26±2.28</td>
<td>NS</td>
</tr>
<tr>
<td>Free T (nmol/L)</td>
<td>0.03±0.03</td>
<td>0.04±0.02</td>
<td>NS</td>
</tr>
<tr>
<td>DHEAS (mmol/L)</td>
<td>6.42±3.55</td>
<td>9.50±4.75</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>SHBG (nmol/L)</td>
<td>182.2±71.6</td>
<td>239.4±162.7</td>
<td>p&lt;0.05</td>
</tr>
</tbody>
</table>

Abbreviations: BMI is body mass index, F-G score is modified Ferriman-Gallwey hirsutism score, T is testosterone, DHEAS is dehydroepiandrosterone sulfate, SHBG is sex hormone binding globulin.

*All PCOS patients had ovulatory dysfunction by definition; one eumenorrheic NCAH patient was excluded.*
NCAH present with similar phenotypes, making it virtually impossible to distinguish between the two disorders solely on clinical grounds. We should note that a relatively unique clinical feature of NCAH is the presence of minimal clitoromegaly, which is in sharp contradistinction to the otherwise relatively mild androgenic symptoms, observable in some children with the disorder 21.

Clinical presentation: Biochemical profile

An exaggerated luteinizing hormone (LH) to follicle-stimulating hormone (FSH) ratio has been a recognized finding in PCOS. However, women with NCAH may have also mildly elevated levels of LH, either basally or in response to GnRH stimulation, although generally to a lesser degree than patients with PCOS 25,27. Mean serum measurements of total and free testosterone (T) also do not differ significantly between patients with PCOS and those with NCAH, either in our study (Table 1) or in the experience of others 6,25.

While some investigators have noted that the mean dehydroepiandrosterone sulfate (DHEAS) levels of NCAH patients is similar to that of other hyperandrogenic women 25, we actually observed a lower mean DHEAS level among patients with NCAH compared to women with well-defined PCOS (Table 1). Others have also observed that DHEAS level in NCAH patients are similar to that of controls, and lower than that of patients with PCOS 6.

That a commonly used marker of adrenal androgen excess should be similar or even lower than that of PCOS women is not entirely unexpected. Firstly, about 25-50% of PCOS women have frankly supranormal DHEAS levels 26,28. Secondly, adrenocortical enzyme kinetics primarily favors the production of cortisol, such that little of excess 17-HP produced in NCAH is actually back-converted to 17-hydroxypregnenolone and then to dehydroepiandrosterone and its metabolite DHEAS. We observed a similar finding when studying other markers of adrenocortical androgen secretion, including 11β-hydroxyandrostenedione 29.

Finally, although Δ4-androstenedione (A4) levels are frequently supranormal in patients with NCAH 30, the overlap between women with NCAH and other hyperandrogenic patients is significant 6. This not unexpected, considering the relatively little Δ17,20-lyase activity that human P450c17α demonstrates 31 which effectively preventing the direct conversion of the excess 17-HP to A4.

Screening for NCAH

An important biochemical marker of NCAH is the presence of supranormal levels of the immediate 17-hydroxylated precursor to P450c17α, 17-HP. In a prospective study in 266 hyperandrogenic women, we observed that 15.4% had one time 17-HP levels > 6.0 nmol/L (2 ng/mL) 20. Of these about 60% had a repeat 17-HP that was below this value, while in another 30% the acute ACTH stimulation test was normal 20. The remaining patients had NCAH diagnosed upon the performance of their ACTH stimulation test. Hence, 10-15% of patients with a basal level of 17-HP above 6.0 nmol/L (2 ng/mL) will eventually prove to have NCAH 20, while practically 100% of hyperandrogenic women with a basal 17-HP below this cutoff will not have the disorder 5.

It should be noted that about 10% of patients with NCAH will not have a basal 17-HP level above 6.0 nmol/L; and that the level of this steroid will be decreased in the evening, as it follows the adrenocortical circadian rhythm, and will be spuriously increased in the follicular phase to levels greater than 6.0 nmol/L in about 50% of ovulatory controls 20. Hence, to use a basal 17-HP to screen for NCAH, it is necessary to preferable to obtain this measurement in the morning, and shortly after the completion of a spontaneous or induced-vaginal bleed.

In conclusion, a higher mean basal follicular phase 17-HP level serves as a useful screening marker for NCAH. About 90% of NCAH patients have a basal 17-HP > 6.0 nmol/L, which is observable in only about 15% of PCOS.

Mechanisms of Adrenocortical Excess in NCAH

An understanding of the mechanisms underlying the excessive secretion of androgens and progestogens by the adrenal cortex of patients with NCAH is important to appropriately understand and design therapeutic regimens. Firstly, over-activation of the hypothalamic-pituitary-adrenal (HPA) axis and an increased ACTH secretion appears to be an important mechanism resulting in steroid excess in untreated patients, at least in the classic forms of the disorder. However, most NCAH patients do not demonstrate over-activity of the HPA axis. Nonetheless, a few of these patients may demonstrate a mild degree of ACTH hyper-responsiveness to CRH stimulation, and up to 40% have radiologic evidence of adrenocortical hyperplasia and/or isolated adenomas, suggesting
that some degree of chronic ACTH excess is present.

Another mechanism resulting in adrenocortical excess in adrenal hyperplasia, and primarily NCAH, results from altered enzyme kinetics in the abnormal 21-OH. The mutated enzyme product is less efficient than the wild type, resulting in an increased precursor to product ratio, independent of ACTH levels. Hence progesterone (P4) and 17-HP levels in these patients may remain above normal even in the presence of excess glucocorticoid administration 31.

Finally, alterations in ovarian and gonadotrophic function, with the appearance of a polycystic ovary syndrome-like picture, also contribute to the androgen excess of these patients. Functional ovarian abnormalities in patients with the classic and non-classic forms of adrenal hyperplasia may be secondary to a number of differences mechanisms, including prenatal masculinization of the hypothalamic-pituitary-ovarian (HPO) axis by adrenal androgens; continued disruption of the HPO axis by persistently elevated progesterone or androgen levels; and/or to a direct glucocorticoid effect. Finally, these data would suggest that the measurement of P4 or 17-HP may not be the most accurate marker of therapeutic efficacy, and suppression of both ovaries and adrenals may be necessary for optimum steroidogenic control.

Treatment of NCAH

Little is known regarding the long-term outcome and optimum treatment of patients with NCAH. As in other androgen excess disorders, therapy should be tailored to patient goals and presenting findings. Contrary to CAH, glucocorticoid administration is only one of the available therapies for the treatment of NCAH that should be considered. Our experience indicates that younger patients (usually < 20 years old) clinically respond well to lower doses of glucocorticoids, while older patients newly diagnosed with the disorder generally fail to respond fully to this therapy.

When using glucocorticoids we prefer to use oral dexamethasone (DEX), to improve compliance, although the use of prednisone is certainly an acceptable option. We begin at a dose of DEX 0.5 mg/day for the first 30-60 days, and then decrease the dose to 0.25 mg/day or 0.5 mg qod, according to patient preference. In the long-term we titrate the dose according to the improvement in clinical features and hormonal profile. Rarely, we encounter patients who require doses of DEX of 0.5 to 0.75 mg/day, although frequently these patients develop Cushingoid features. Adrenocortical suppression generally improves hirsutism, acne, alopecia, and oligo-ovulation, and the final height of affected children.

It is a matter of debate whether patients with NCAH are at risk for adrenal insufficiency. Although we have never observed either acute or chronic hypocortisolism in our patients, and many of them have undergone surgery or pregnancy deliveries without having required glucocorticoid supplementation, this possibility cannot be discarded. Therefore, we inform patients of this theoretical risk, and counsel them and their families regarding the presenting symptoms of adrenal insufficiency, particularly in the event of trauma, accidents or surgery.

To monitor the adequacy of treatment, we measure the circulating androstenedione (A4) and free testosterone levels at three and six months of therapy, and yearly thereafter, aiming to maintain these levels are or slightly above the upper normal limit. Alternatively, physicians should not aim to normalize the morning 17-HP circulating levels, since adrenal androgens are more sensitive to the suppressive effects of glucocorticoid administration than are C-21 steroids. As noted above, the 17-HP level may remain elevated in spite of adequate androgen and ACTH suppression 32. Alternatively, DHEAS is very sensitive to glucocorticoid administration, and is rapidly and dramatically suppressed, even when the level of other androgens (e.g. A4) remains elevated. While we have found that the A4 levels represent an adequate marker for monitoring therapy, it extremely important to keep in mind that these measures simply serve as a guideline and that clinical response is of greater value in determining the adequacy of therapy. In fact, androgen measures are primarily useful when clinical improvement is not evident, suggesting the possibility of persistent hyperandrogenemia (see below).

The principal marker for monitoring the adequacy of therapy is the improvement in clinical features. Acne improves relatively quickly in these patients. Alternatively, glucocorticoid suppression alone has a limited impact on established hirsutism in NCAH, similar to the response seen to oral contraceptives (OCPs) in patients with PCOS. In a randomized study, Spritzer and colleagues observed that treatment with the anti-androgen cyproterone acetate and percutaneous estradiol elicited a more rapid and sustained
The principal cause of the reduced fertility in patients with NCAH is probably ovulatory dysfunction, secondary to hyperandrogenism. However, chronic elevations in circulating P4 and 17-HP levels may also result in an inadequate cervical mucus, and/or a persistently atrophic endometrium. Nonetheless, while NCAH patients may be subfertile, it should be stressed that many of these women become pregnant without requiring treatment, and often prior to diagnosis. Assuming that other infertility factors have been ruled out, we first initiate glucocorticoid therapy to improve ovulatory function. If the patient fails to demonstrate adequate or satisfactory ovulation after four months of adrenocortical suppression, we then proceed to ovulation induction with clomiphene citrate, and hMG if necessary. As noted previously, it is possible that long-term chronic hyperandrogenemia results in the disruption of the HPO axis and the development of a PCOS-like phenotype. Overall prognosis for fertility in oligo-ovulatory NCAH patients is good, and should not be any different from that of other hyperandrogenic women seeking ovulation induction.

Bibliografía


