Heterozygote Detection in Congenital Adrenal Hyperplasia

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Detection of heterozygote carriers for congenital adrenal hyperplasia by use of a modified tetracosactide (a synthetic corticotropin) stimulation test with prior overnight dexamethasone suppression proved to have a diagnostic accuracy of 95%. Discrimination of heterozygotes from normals was best when we used a criterion based on the ratios of 17α-hydroxyprogesterone to cortisol at baseline and at 30 min after intravenous administration of 250 μg of tetracosactide.

Additional Keyphrases: steroids • tetracosactide • hirsutism • polycystic ovary syndrome • genetics • discriminant analysis

Congenital adrenal hyperplasia, an inborn error of metabolism, is transmitted as an autosomal recessive by a gene located on chromosome 6, which codes for an enzyme (21-hydroxysteroid dehydrogenase, EC 1.1.1.151) required along the major biosynthetic pathway to cortisol (1). The frequency of homozygous individuals varies from 1/490 to 1/67,000 in different communities, suggesting heterozygote carrier frequencies of 1/20 to 1/250.

Detection of heterozygotes is of clinical importance, both for genetic counseling and in evaluation of women with idiopathic hirsutism or polycystic ovary syndrome (2). Attempts to demonstrate biochemical markers for the carrier state in the 1950s by using adrenal stimulation and measuring urinary steroid metabolites were only partly successful. Since the advent of radioimmunoassay for 17α-hydroxyprogesterone (17OHP), normals have been better differentiated from heterozygotes on the basis of their blood concentrations of 17OHP after stimulation with corticotropin (3–9). Basal 17OHP values do not differentiate reliably between normal individuals and heterozygotes or even some homozygotes for a mild, variant type of 21-hydroxylase deficiency (2). Weil et al. (6) recently showed that suppression of endogenous corticotropin, by administration of a small dose of dexamethasone the night before testing, improved detection sensitivity. We have modified the conventional short stimulation test to include tetracosactide (Synacthen; Ciba-Geigy) and demonstrate that, with the use of dexamethasone, 95% diagnostic accuracy is possible, based only on the ratios of 17OHP to cortisol at zero time and 30 min after corticotropin administration.

Materials and Methods

Subjects. Parents of children in four families with at least one member having homozygous 21-hydroxylase-deficient congenital adrenal hyperplasia were recruited for the study. These parents were presumed to be obligate heterozygotes because HLA typing did not exclude paternity in any family. One father was not available for testing.

The control subjects, without known endocrine disorders, were seven men and six nonhirsute women with regular, ovulatory menses. All women were studied in the follicular phase of the menstrual cycle; none was using oral contraceptives.

Subjects took 1 mg of dexamethasone at 2200 to 2400 hours the night before study. Between 0800 and 0900 hours the following morning, a cannula was inserted into a vein in the forearm and, after a control sample was collected, 250 μg of tetracosactide was injected intravenously. Further blood specimens were collected into heparinized tubes at 30, 60, 90, and 120 min later. After centrifugation and separation, plasma was stored at −20 °C until assay. Subjects who reported not taking dexamethasone correctly were excluded, or the test was repeated.

Assays. Plasma cortisol and 17OHP were measured in duplicate in each specimen, and all specimens from each subject were assayed together. Plasma cortisol was assayed with a commercial radioimmunoassay kit (Diagnostic Products Corp., Los Angeles, CA 90045) without prior extraction or chromatography. Antibody cross reactivity was 3.6% for cortisol precursors (progesterone, 17OHP, cortisol, 11-deoxycortisol) and the detection limit was 15 nmol/L. Coefficients of variation were 5% within assay and 12% between assay for high (1180 nmol/L), medium (384 nmol/L), and low (83 nmol/L) concentrations of pooled quality-control sera.

Plasma 17OHP was measured by radioimmunoassay by a modification of Vining’s (10) method for testosterone. We diluted 25 μL of serum to 500 μL with distilled water and applied it to a kieselguhr minicolumn (Extrelut; E. Merck, Darmstadt, F.R.G.). We eluted 17OHP directly into assay tubes with four 0.7-mL aliquots of diethyl ether and, after evaporation of the organic solvent, reconstituted the extract in assay buffer. Analytical recovery of tracer 17OHP exceeded 95%; consequently, we made no correction for procedural losses. Antibody (P17-43) to 3-oxime–bovine serum albumin conjugate was from Endocrine Sciences, Tarzana, CA 91356; it had cross reactivities of 5% with 17-hydroxypregnenolone and 1% with cortisol and cortisol precursors. CVs were 10% within assay and 15% between assay for high (19.6 nmol/L) and normal (6.6 nmol/L) concentrations in pooled quality-control sera. The detection limit was 20 pmol per tube.

Data analysis. Data analysis included the raw data—i.e., plasma cortisol at 0 (F0), 30 (F1), 60 (F2), 90 (F3), and 120 (F4) min after corticotropin injection—as well as plasma 17OHP at the same times (S0, S1, S2, S3, S4). In addition a series of derived parameters was constructed:

- ratio of 17OHP to cortisol (R0, R1, R2, R3, R4)
- areas under curves for 17OHP, cortisol, and their ratio (AUCS, AUCP, AUCR)
- sum of responses for 17OHP and cortisol (e.g., S = S0 + S1 + S2 + S3 + S4)
- rate of increase of 17OHP, cortisol, and their ratio at each time, in comparison with the basal values

Statistical analysis was by Student’s unpaired t-test after confirmation of underlying normal distribution. Stepwise linear discriminant analysis was performed with the BMDP-7M program implemented on a DIGITAL 11/70 computer (11). The stepwise method allowed the use of an unusually large number of parameters (10 raw data and 16 derived parameters) for the number of subjects studied. In this
analysis, a variable is selected at each step based on F-tests. A high F-value for a variable indicates that it has apparent discriminatory power between the groups being classified. At each step, the variable with the highest F-value is selected and added to the discriminant function until a step is reached where no variable has a suitably high F-value and the stepwise procedure stops. In effect, the 20 subjects are tested sequentially against single variables rather than against the 26 variables simultaneously. The discriminant function is formed from a weighted linear combination of the variables selected, evaluated for each case, and the percentage of correct classifications noted.

Results

Because there were no significant differences (p > 0.15) between males and females (follicular phase) in any of the assayed parameters, their results were pooled for subsequent analysis into groups of controls and heterozygotes.

Basal plasma cortisol was appropriately suppressed after dexamethasone in all subjects, and cortisol levels did not differ between controls and heterozygotes at any time tested (p > 0.15). In contrast, at each time heterozygotes had greater mean concentrations of 17OHP as well as greater values for all the derived parameters that included 17OHP measurements (p < 0.05) (Table 1).

Despite the differences as groups, there was still some overlap between groups and for any given time, and we could not with any single parameter completely separate the two groups. Thus we used stepwise linear discriminant analysis to determine whether discrimination between the groups was better with a combination of raw data and (or) derived parameters, and to decide which set of data parameters was the most decisive in making such a distinction. Using all the data (raw and derived parameters) as single parameters, we obtained a diagnostic accuracy of 75–85%; the misclassifications were mostly heterozygotes incorrectly assigned as normals (false negatives for the test). Using all the data together as a starting point, we correctly classified 95% of the subjects, misclassifying only one heterozygote as normal. The optimal discriminant function was $D = 3.4 - 12.2(R0) - 187.9(R1)$ (with $R0$ and $R1$ as previously defined); the values of this function for each subject are illustrated in Figure 1.

Discussion

The prevalence of women with mild homozygous or severe heterozygous congenital adrenal hyperplasia among those who present with idiopathic hirsutism or polycystic ovarian syndrome is estimated from rare (12) to 42% (13), with an overall weighted estimate of 6 to 12% (2). The ability to detect heterozygotes with reliability gives some assurance that this type of test may be sensitive enough to diagnose underlying adrenal steroidogenic defects in women with these common disorders. In addition, a test for heterozygous state is useful in genetic counseling of affected homozygotes who, with appropriate treatment, are potentially fertile. Treated homozygotes have either a 50% or no chance of having affected children, according to whether a spouse is a heterozygous carrier.

Several previous studies have reported the use of stimulation with corticotropin in conjunction with measurement of 17OHP responses and obtained diagnostic accuracies of between 50 and 90% in detection of heterozygotes (2–9). Results have been best when prior overnight dexamethasone administration is included (6, 7) and when the early (30 min) rather than later adrenal responses to corticotropin are considered (7). Our data confirm that the combination of these two stratagems can provide a simple and nearly completely effective discrimination.

Plasma 17OHP is a steroid precursor produced from both the adrenal gland and gonads. Diurnal and menstrual periodicity of its circulating concentrations reflect these independent contributions. Therefore, to minimize background "noise" in 17OHP determinations, both the time of day and menstrual phase (preferably follicular) must be standardized; under these circumstances the subjects form more homogeneous groups, making the diagnostic value of provocative testing more effective. Serum cortisol has a highly variable pattern in humans, owing to diurnal, pulsatile, and stress-induced patterns of corticotropin secretion. In addition to the random nature of variation from the latter two modes, there is considerable between-subject variation in times of peak and troughs of the diurnal pattern. Suppression of corticotropin by dexamethasone minimizes the background between-subject variation in adrenal steroid output, thus increasing the discriminatory power of the provocative tests by

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**Table 1. Serum Cortisol and 17OHP Responses (nmol/L) to Tetracosactide in Controls (n = 13) and Heterozygous Subjects (n = 7)**

<table>
<thead>
<tr>
<th></th>
<th>0</th>
<th>30</th>
<th>60</th>
<th>90</th>
<th>120</th>
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<tr>
<td></td>
<td>x ± SE</td>
<td>Range</td>
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<td>Range</td>
<td>x ± SE</td>
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<tr>
<td>Cortisol</td>
<td></td>
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<tr>
<td>Control</td>
<td>47 ± 3</td>
<td>34-62</td>
<td>554 ± 28</td>
<td>428-751</td>
<td>696 ± 38</td>
</tr>
<tr>
<td>Hetero-</td>
<td>45 ± 7</td>
<td>25-66</td>
<td>586 ± 33</td>
<td>464-732</td>
<td>729 ± 46</td>
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<tr>
<td>zygote</td>
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<tr>
<td>17OHP&lt;sup&gt;a&lt;/sup&gt; Control</td>
<td>2.10 ± 0.58</td>
<td>0.3-4.5</td>
<td>5.07 ± 0.31</td>
<td>3.6-7.6</td>
<td>5.45 ± 0.28</td>
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<tr>
<td>Hetero-</td>
<td>5.19 ± 1.10</td>
<td>1.3-8.8</td>
<td>12.6 ± 2.45</td>
<td>7.0-17.0</td>
<td>13.06 ± 2.50</td>
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<sup>a</sup> All comparisons: controls vs heterozygotes, not significant. <sup>b</sup> All comparisons: controls vs heterozygotes, p < 0.001.

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**Fig. 1. Values of the discriminant function giving best separation between controls and heterozygous subjects**

$D = 3.4 - 12.2(R0) - 187.9(R1)$ with $R0 = 17OHP/$cortisol (baseline) and $R1 = 17OHP/$cortisol (30 min post-corticotropin)

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ensuring a more uniform starting point.

By these means the comparison of pre- and post-corticotropin-stimulated steroid concentrations allows direct focus on adrenal steroidogenesis with minimum background "noise" and constant gonadal contribution to 17OHP values. Under these conditions, the ratio of 17OHP to 11-deoxycortisol (the immediate product of adrenal 21-hydroxylation of 17OHP) might be even more effective in the diagnosis of the heterozygous state.

In conclusion, our data show that a modified tetracosactide test with the addition of overnight dexamethasone suppression achieves a high degree (95%) of diagnostic accuracy in classification of normals from heterozygotes based on radioimmunoassay of cortisol and 17OHP in the basal and one post-corticotropin-stimulated (30 min) blood sample. This simple test should prove useful in the evaluation of women with idiopathic hirsutism or polycystic ovary syndrome for the detection of underlying adrenal steroid biosynthetic defects.

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References