Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 4552–4556, May 1993
Genetics

Mutations in the *CYP11B1* gene causing congenital adrenal hyperplasia and hypertension cluster in exons 6, 7, and 8

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Communicated by Seymour Lieberman, February 3, 1993

ABSTRACT **Steroid 11β-hydroxylase deficiency (failure to convert 11-deoxycortisol to cortisol) is the second most common cause of congenital adrenal hyperplasia and results in a hypertensive form of the disease. The 11β-hydroxylase enzyme is encoded by the *CYP11B1* gene on chromosome 8q22. Two mutations in *CYP11B1* have previously been reported in patients with 11β-hydroxylase deficiency—Arg-448 → His and a 2-bp insertion in codon 394. We now report eight previously uncharacterized mutations causing this disorder. Seven are point mutations (three nonsense and four missense) and one is a single base pair deletion causing a frameshift. We have used an *in vitro* transfection assay to show that all five known missense mutations causing 11β-hydroxylase deficiency abolish enzymatic activity. In principle, deletions of *CYP11B1* could be generated by unequal crossing-over between *CYP11B1* and the adjacent *CYP11B2* gene, but no such deletions were found among the deficiency alleles in this study. Seven of the 10 known mutations are clustered in exons 6–8, a nonrandom distribution within the gene. This may reflect the location of functionally important amino acid residues within the enzyme or an increased tendency to develop mutations within this region of the gene.**

Cortisol is synthesized from cholesterol in the zona fasciculata of the adrenal cortex in five enzymatic steps. These include cleavage of the cholesterol side chain to yield pregnenolone, 3β dehydrogenation to progesterone, and successive hydroxylations at the 17α, 21, and 11β positions. Inherited defects in any of these steps cause congenital adrenal hyperplasia, a disorder of cortisol biosynthesis. Greater than 90% of cases of congenital adrenal hyperplasia are due to a deficiency in steroid 21-hydroxylase activity (1), whereas most of the remaining cases (5–8%) are due to 11β-hydroxylase deficiency (2–4).

In both 21- and 11β-hydroxylase deficiency, the inability of the adrenal cortex to synthesize cortisol increases secretion of corticotropin, resulting in overproduction of steroid precursors that are shunted into the pathway for androgen biosynthesis. Female patients with this disorder are thus born with masculinized external genitalia, and affected individuals of both sexes undergo rapid somatic growth with premature epiphyseal closure, resulting in short adult stature. One of the precursors that can accumulate in 11β-hydroxylase deficiency is deoxycorticosterone, a steroid with mineralocorticoid (sodium-retaining) activity. Elevated levels of this hormone (and possibly its metabolites) may cause hypertension and hypokalemia. About two-thirds of untreated patients become hypertensive, sometimes early in life (5). This clinical feature distinguishes 11β-hydroxylase deficiency from 21-hydroxylase deficiency in which the inability to synthesize deoxycorticosterone, and subsequently aldosterone, causes renal salt wasting in the majority of patients.

Whereas the genetic basis of 21-hydroxylase deficiency has been studied extensively (reviewed in ref. 6), there have been few genetic studies of 11β-hydroxylase deficiency. There are two genes encoding 11β-hydroxylase isozymes, *CYP11B1* and *CYP11B2*, located on chromosome 8q22 (7–9). The encoded isozymes are mitochondrial cytochromes P-450 and are predicted to be 93% identical in amino acid sequence (8). CYP11B1 (P-450c11) is expressed at high levels in the normal adrenal gland (8, 10), has 11β-hydroxylase activity (conversion of 11-deoxycortisol to cortisol and deoxycorticosterone to corticosterone) (10, 11), and is regulated by corticotropin (10). CYP11B2 (P-450aldo, P-450cmo, P-450c18) is expressed at low levels in the normal adrenal gland but at higher levels in aldosterone-secreting tumors (10, 12), has 11β-hydroxylase, 18-hydroxylase, and 18-oxidase activities (conversion of deoxycorticosterone to aldosterone) (10, 11), and is regulated by angiotensin II (10). CYP11B2 has thus been referred to as "aldosterone synthase."

The only missense mutation reported to be associated with 11β-hydroxylase deficiency is a single base substitution in exon 8 of *CYP11B1* that changes codon 448 from CGC (arginine) to CAC (histidine) (R448H) (13). R448H is present on the majority of mutant alleles in Jews from Morocco, among whom 11β-hydroxylase deficiency occurs relatively frequently (≈1/5000 births). R448H is expected to have an adverse effect on enzymatic activity because the mutation lies within a conserved "heme binding" peptide, which contains the cysteine residue that is presumed to constitute the fifth ligand to the iron atom of the heme prosthetic group. The equivalent of R448 is conserved in every known eukaryotic P-450 (see references in refs. 14 and 15) but is a histidine at the analogous position in *Pseudomonas putida* P-450cam (14, 16). The functional effect of the R448H mutation on P-450c11 enzymatic activity has not previously been studied *in vitro*. Using a transient transfection assay, we now confirm that R448H abolishes 11β-hydroxylase activity. The other previously described mutation, found in a Turkish patient (17), was a 2-bp insertion in codon 394, leading to premature termination of the open reading frame and a presumably nonfunctional product.

In this paper, we describe eight additional mutations causing 11β-hydroxylase deficiency. None of the patients in which these mutations were identified was of Moroccan or Turkish ancestry. Of the eight mutations we found, four are missense mutations, three are nonsense mutations, and one is a single base pair deletion in exon 2, which causes a frameshift and premature termination of the protein. Each of the missense mutations was shown by *in vitro* transfection studies to abolish 11β-hydroxylase activity. Most of the point mutations lie within the region of the gene containing exons 6–8, suggesting that this region may contain a higher pro-

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portion of residues essential for the 11-hydroxylase activity of the enzyme and/or that this region is subject to a higher mutation rate.

MATERIALS AND METHODS

Patients. The first four patients studied were likely to carry homozygous mutations. Patient 1 was a Lebanese Arab whose parents were first cousins once removed. The parents of patients 2a and 2b were not known to be related but were both Indian Sikhs. Patient 3 was an American Black whose father was unknown. In addition to 11β-hydroxylase deficiency, this patient had dysmorphic facial features, cleft lip and palate, club feet, valvular and infundibular pulmonic stenosis, and attention deficit disorder, raising the possibility of consanguineous parentage. The parents of patients 4a and 4b were Yemenite first cousins. Clinical data on all patients are presented in Table 1.

DNA Amplification and Sequence Analysis. DNA was prepared from peripheral blood leukocytes and exons 1–2, 3–5, and 6–9 of *CYP11B1* were amplified from 500-ng samples of DNA as described (13). When difficulties were encountered in the amplification of exons 6–9, this region was instead amplified in two halves by using the exon 8 primers AGCGCTGGGGGTTATAGCG (antisense) and TACTCTCTGGGTCGCAACCCC (sense) to amplify exons 6–8 and 8–9, respectively. Amplified gene fragments were purified by agarose gel electrophoresis and recovery on NA45 paper (Schleicher & Schuell), and the exons of each PCR product were sequenced directly with the primers described (13), modified T7 DNA polymerase (Sequenase II, United States Biochemical), and templates denatured in the presence of dimethyl sulfoxide (18).

Allele-Specific Oligonucleotide Hybridization. Exon 6–8 PCR products were amplified from the genomic DNA prepared from members of family 1, subjected to electrophoresis through 1% agarose, and transferred to two nylon membranes (Micron Separations, Westboro, MA) using a sandwich blot technique (19). Membranes were irradiated with 1200 J of UV light and then hybridized with ^{32}P-labeled oligonucleotides corresponding to the normal antisense (AGCACCCACCGCAAGGTCT) or mutant sense (AGACCTTGC<u>A</u>GTGGGTGCT) R374Q sequences. Hybridization conditions were overnight incubation at 42°C in the presence of 1 M NaCl/0.1 M sodium citrate/0.5% SDS/0.25% nonfat dried milk powder. Membranes were washed in 1 M NaCl/0.1 M sodium citrate/0.5% SDS, with a wash temperature of 58°C for the normal probe and of 55°C for the mutant probe, and were then exposed to Kodak XAR film.

Mutagenesis and Subcloning of Constructs. The missense mutations were introduced into normal *CYP11B1* cDNA by PCR using oligonucleotides containing the desired change (20). Briefly, DNA from plasmid pCMV4-B1 (10) was amplified with *Pyrococcus furiosis* DNA polymerase (Stratagene) in two overlapping segments, each using one oligonucleotide corresponding to either the 5′ or 3′ end of the *CYP11B1* coding sequence and one containing the desired mutant sequence. The 5′ and 3′ amplified segments were then gel purified, combined in a single reaction mixture, denatured, annealed, and extended to produce a full-length molecule containing the mutant sequence, which was then amplified by using the primers from the 5′ and 3′ ends of the coding sequence. Restriction sites were included in these flanking primers to facilitate cloning into the expression vector pCMV4 (21). The complete sequence of each construct was checked to ensure that no unwanted mutations had been introduced by the PCR.

Transient Transfection Assays. Transfections were performed with Lipofectin reagent (GIBCO/BRL) as described (10) except that 5 μg of each of the pCMV4-B1 or mutant constructs was used along with 1 μg of the pCMV4-HAD and pCMV4-HAR constructs (22). The latter constructs contain cDNAs encoding human adrenodoxin and human adrenodoxin reductase, respectively. Enzymatic activity in the transfected cells was determined as described (10), except that the cells were incubated with a lower concentration of substrate (0.1 μM 11-deoxy[^{3}H]cortisol), and conversion to [^{3}H]cortisol was assessed by autoradiography of the thin-layer chromatograms after enhancement with Resolution TLC (EM Corp., Chestnut Hill, MA).

RESULTS

Identification of Mutations Associated with 11β-Hydroxylase Deficiency. Exons of the *CYP11B1* genes of patients with 11β-hydroxylase deficiency were amplified by PCR and sequenced directly. The absence of any gross deletions or rearrangements in either copy of the gene was confirmed in all patients by Southern blot analysis (data not shown) or by demonstration of carrier status in both parents. Five of the mutations were found in homozygous form: CGG (Arg-374) to CAG (Gln; R374Q) in patient 1, CAG (Gln-338) to TAG (Stop; Q338X) in patients 2a and 2b, CAG (Gln-356) to TAG (Stop; Q356X) in patient 3, ACG (Thr-318) to ATG (Met; T318M) in patient 4a, and GTG (Val-441) to GGG (Gly; V441G) in patient 5. Patient 6 was found to be heterozygous for two different mutations: AAG (Lys-174) to TAG (Stop; K174X) and CGA (Arg-384) to CAA (Gln; R384Q). A single

Table 1. Characteristics of patients with 11β-hydroxylase deficiency

Patient	Mutation	Sex	Prader stage	Bone age/ actual age, yr	BP, mmHg (age, yr)	Serum steroids, ng/dl				Urine THS, mg per 24 h
						S	DOC	Δ4	T	
1	R374Q	F	V	5.5/2.5	130/80* (2)	—	—	204	56	6.2
2a	Q338X	F	IV	12/8	110/60 (6)	>2,000	—	—	77	0.3
2b	Q338X	F	IV	9/6	—	1,830	—	—	117	—
3	Q356X	M	PA	15/10	150/94* (9)	4,290	265	675	209	—
4a	T318M	F	III	2.3/2.7	90/64 (1)	—	2013	817	64	1.3
4b	T318M	F	IV	—	88/78*†	—	525	4042	689	0.9
5	V441G	M	PA	9.5/3.2	130/80* (3)	24,289	2037	1570	95	—
6	K174X/R384Q	F	IV	8.8/6.3	110/65 (2)	—	209	482	64	—
7	ΔC32/?	M	PA	7/1.5	—	11,138	—	1296	146	—
8‡	R448H									

PA, precocious adrenarche; BP, blood pressure. Hormone abbreviations (with normal laboratory values for prepubertal children in parentheses) are as follows: S, 11-deoxycortisol (15–170 ng/dl); DOC, 11-deoxycorticosterone (3–17 ng/dl); Δ4, androstenedione (5–149 ng/dl); T, testosterone (0–60 ng/dl); THS, urinary tetrahydrodeoxycortisol (<0.05 mg per 24 h).

*Blood pressure > 95th percentile.

†Blood pressure measured in the newborn period.

‡See ref. 13 for details.

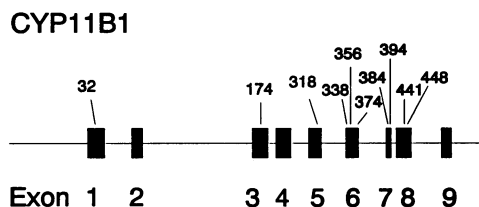

FIG. 1. Diagram of the *CYP11B1* gene showing positions of all mutations known to cause 11β-hydroxylase deficiency. Seven mutations (T318M, Q338X, Q356X, R374Q, i394, V441G, and R448H) were found in homozygous form. The remaining three mutations (ΔC32, K174X, and R384Q) were in heterozygous form. Mutations cluster in the region of exons 6, 7, and 8.

heterozygous mutation, a deletion of a C in codon 32 (ΔC32) leading to a frameshift, was identified in patient 7. The absence of any other changes in the coding sequence of the *CYP11B1* gene in this individual could indicate the presence of a second mutation within an intron. The mutations in this and previous studies (13, 17) seem to cluster in exons 6, 7, and 8 (Fig. 1). By a simple simulation study, in which mutations were assigned randomly in proportion to the size of the exons, we found that the probability that the observed clustering is a chance occurrence is <4%. If only point mutations are considered, this probability is reduced to 2.1%.

Prenatal Diagnosis. A prenatal diagnosis in family 1 was performed by allele-specific oligonucleotide hybridization to amplified DNA. Both parents were heterozygous for R374Q, the proband was homozygous and the fetus was heterozygous (Fig. 2). A prenatal diagnosis was performed in family 2 by direct sequence analysis of amplified DNA, and this fetus was found to be homozygous unaffected (data not shown). The latter diagnosis has been confirmed by the birth of an unaffected male, while the outcome of the former pregnancy is not yet available.

Functional Effects of Mutations. The missense mutations T318M, R374Q, R384Q, V441G, and R448H were introduced into *CYP11B1* cDNA, subcloned into the eukaryotic expression vector pCMV4 (21), and transfected into COS-1 cells. Cells transfected with the expression plasmid containing the wild-type sequence (pCMV4-B1) were able to convert 11-deoxycortisol to cortisol, some of which was subsequently converted to cortisone by an 11-dehydrogenase activity intrinsic to the cells (10). In contrast, cells transfected with plasmids containing any of the mutant sequences produced no detectable cortisol by autoradiography or scanning of TLC plates (Fig. 3), thus explaining the genetic basis of the deficiency in these patients. Transfections were unnecessary for mutations ΔC32, K174X, Q338X, and Q356X, all of which lead to truncated and presumably nonfunctional proteins. The success of all transfections was confirmed by detection of full-length CYP11B1 mRNA on a Northern blot (Fig. 3).

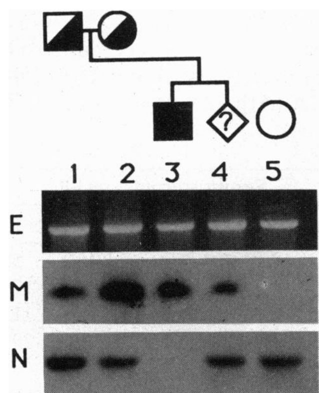

FIG. 2. Prenatal diagnosis performed by allele-specific hybridization. Half-open symbols represent parents of patient 1, who were heterozygous for the R374Q mutation. The proband is represented by the solid square, the fetus is indicated by the question mark, and the open circle represents an unaffected unrelated female. E, ethidium bromide-stained PCR products, spanning exons 6–8; M, Southern blot probed with the oligonucleotide containing the mutant sequence; N, blot that was probed with the oligonucleotide containing the normal sequence. Fetus is heterozygous for the mutant allele and so is predicted to be unaffected.

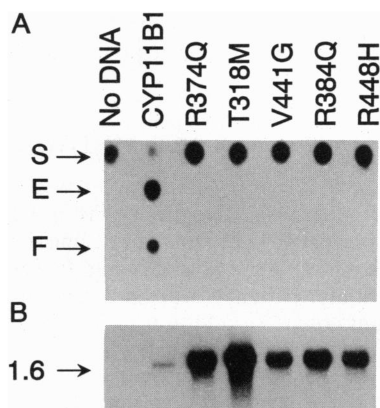

FIG. 3. Enzymatic activity of cells transfected with normal and mutant *CYP11B1* cDNA constructs. (*A*) COS-1 cells were transfected with 1 μg of pCMV4-ADX; 1 μg of pCMV4-HAR; and either no DNA, 5 μg of pCMV4-B1, or 5 μg of pCMV4-B1 carrying the mutations indicated. Transfected cells were then incubated with 0.1 μM 11-deoxy[^{3}H]cortisol and the resulting metabolites were identified by thin-layer chromatography and autoradiography. Positions of steroids are marked on the autoradiogram as follows: S, 11-deoxycortisol; F, cortisol; E, cortisone (which is produced from cortisol by an 11β-hydroxysteroid dehydrogenase activity intrinsic to COS-1 cells). (*B*) CYP11B1 RNA transcripts from each transfection were detected by Northern blot analysis as described in ref. 22. Lanes are as indicated in *A*.

DISCUSSION

Clustering of Mutations Causing 11β-Hydroxylase Deficiency. It is notable that most (7/10) of the mutations of *CYP11B1* identified thus far are clustered in exons 6, 7, and 8, which together contain only 30% of the coding sequence of the gene. Two possible explanations for this observation are that mutations in this region are more likely to have deleterious effects on enzymatic activity and thus to be ascertained in patients with 11β-hydroxylase deficiency or that this region is unusually prone to develop mutations for some reason(s).

Human, bovine, mouse, and rat 11β-hydroxylase enzymes are identical at 70% of their amino acids in exons 6, 7, and 8, compared with 44% in the remaining exons, suggesting that this region contains a preponderance of residues essential to the function of the enzyme. Exon 8 contains a stretch of residues which surround a cysteine (C450 in CYP11B1) that is absolutely conserved in all P-450 enzymes and is the fifth ligand to the heme iron. Two missense mutations causing 11β-hydroxylase deficiency (V441G and R448H) lie in this area. Another mutation (R374Q) occurs within a second conserved area (residues 362–375) that has been proposed to be involved in substrate binding (23). In the related enzyme, cholesterol desmolase, two other residues within this region were recently shown to be critical for binding to the accessory electron transport protein adrenodoxin (24). T318 and R384, the other residues in which we found missense mutations, are not located within regions of known function but are also highly conserved among members of the P-450 family (14).

Although the presence of four of five missense mutations in highly conserved regions of the enzyme is consistent with ascertainment bias as a reason for the observed distribution of mutations, two other facts argue against this being the sole explanation. First, three of five nonsense and frameshift mutations causing 11β-hydroxylase deficiency are also found in exons 6–8, although such mutations should completely prevent synthesis of a functional enzyme in any location within the gene. Second, mutations in the *CYP21* and *CYP17* genes, which cause two other forms of congenital adrenal hyperplasia, are not similarly clustered.

Molecular Mechanisms Generating Mutations in *CYP11B1*. Because 21- and 11β-hydroxylase deficiencies account for the vast majority of cases of congenital adrenal hyperplasia, whereas cholesterol desmolase and 17α-hydroxylase deficiencies are rare, it is reasonable to ask whether the first two disorders have something in common that distinguishes them from the latter two deficiencies. One such obvious feature is that 21- and 11β-hydroxylase, but not the other two enzymes, are encoded by duplicated genes. *CYP11B1* and *CYP11B2* are located ≈40 kb apart (25, 26), with *CYP11B1* to the right of *CYP11B2* if the genes are pictured as being transcribed from left to right (25, 27). This arrangement is similar to that of the human 21-hydroxylase genes, where the *CYP21* active gene is to the right and ≈30 kb distant from the *CYP21P* pseudogene (the intervening distance is occupied by the complement *C4B* gene). Virtually all mutations causing 21-hydroxylase deficiency are due to recombinations between *CYP21* and *CYP21P*. About 20% of these result in deletion of *CYP21* sequences due to unequal meiotic crossing-over between *CYP21* and *CYP21P*. The remainder are apparent gene conversions in which deleterious mutations are transferred from *CYP21P* to *CYP21* (2, 28, 29). Thus, it initially seemed possible that similar recombinations between *CYP11B1* and *CYP11B2* might be found in 11β-hydroxylase deficiency.

Unequal crossovers involving *CYP11B1* and *CYP11B2* should yield two daughter chromosomes. One should have a genetic duplication and carry three genes: *CYP11B2*, a hybrid gene with 5′ and 3′ ends corresponding to *CYP11B1* and *CYP11B2*, respectively, and *CYP11B1*. The hybrid gene should be expressed at high levels in the zona fasciculata due to the presence of *CYP11B1* regulatory sequences, but, depending on the position of the crossover, the encoded enzyme might have the 18-hydroxylase and 18-oxidase activities normally present only in *CYP11B2*. Hybrid genes of this type have been observed in patients with a condition termed glucocorticoid suppressible hyperaldosteronism (25–27), a rare autosomal dominant form of inherited hypertension.

The other daughter chromosome of the unequal crossover has a deletion and carries a single *CYP11B* gene with 5′ and 3′ ends corresponding to *CYP11B2* and *CYP11B1*, respectively. This hybrid gene, like *CYP11B2*, should be expressed only at very low levels in the zona glomerulosa, the normal site of aldosterone synthesis. Even if the encoded enzyme were active, the low level of expression should cause the chromosome carrying the deletion to function as an 11β-hydroxylase deficiency allele. However, no such hybrid genes have been detected, either in 11β-hydroxylase patients or in normal control individuals studied by Southern blot analysis (unpublished observations). This implies either that such deletions are rare (consistent with the apparent rarity of glucocorticoid-suppressible hyperaldosteronism) or that levels of expression of the single remaining *CYP11B* gene are in fact high enough that 11β-hydroxylase deficiency does not occur.

Apparent gene conversions that transfer sequences from *CYP11B2* to *CYP11B1* would not be expected to have an adverse effect on enzymatic activity because *CYP11B2* (unlike *CYP21P*) normally encodes an active enzyme. Sufficiently large transfers might confer 18-hydroxylase and 18-oxidase activity upon CYP11B1, again leading to glucocorticoid-suppressible hyperaldosteronism. Thus far, such a rearrangement has not been observed. Conversely, transfers of sequences from *CYP11B1* to *CYP11B2* might destroy the 18-hydroxylase and 18-oxidase activity of the CYP11B2 enzyme, leading to an inability to synthesize aldosterone (a condition also termed corticosterone methyloxidase II deficiency). Such an apparent gene conversion (V386A) has been found to segregate with this disorder (in combination with another mutation in exon 3 of *CYP11B2*, R181W) in several affected kindreds (22).

If *CYP11B1*–*CYP11B2* recombinations are not a significant cause of 11β-hydroxylase deficiency alleles, what other mechanisms could account for the high frequency of these alleles relative to those causing cholesterol desmolase or 17α-hydroxylase deficiencies and also contribute to the apparent clustering of mutations in exons 6–8?

Four of the eight point mutations that we found—T318M, R374Q, R384Q, and R448H—are CpG → TpG transitions; in the cases of R374Q, R384Q, and R448H, this has occurred on the noncoding strand. CpG → TpG is the most frequent type of point mutation in humans (30) and is thought to arise as a result of deamination of ^{Me}CpG. Notably, 49% (23/47) of all CpG dinucleotides within the *CYP11B1* coding sequence are located in exons 6–8, suggesting that CpG → TpG mutations should indeed occur relatively frequently in this region of the gene. The proportion (≈3%) of CpG dinucleotides in *CYP11B1* is also higher than that in the entire genome (1.5%) (30) or in the *CYP17* and *CYP11A* genes encoding 17α-hydroxylase and cholesterol desmolase (2%). Mutation of CpG therefore represents a plausible mechanism for increased frequency of mutation of *CYP11B1*.

Genes that are actively transcribed in germ cells or early embryos are thought to promote an increased frequency of mutations in the surrounding DNA due to changes in the local chromatin structure (31). Evaluation of the role of this mechanism in generating mutations causing congenital adrenal hyperplasia awaits information regarding the genetic surroundings of the genes for the steroidogenic enzymes, which is known at present only for *CYP21*. It is also possible that cholesterol desmolase and 17α-hydroxylase deficiency are rare because they adversely affect fetal survival by interfering with estrogen biosynthesis by the fetoplacental unit.

Spectrum of Phenotypic Effects in 11β-Hydroxylase Deficiency. All of the missense mutations abolished 11β-hydroxylase activity. The encoded proteins in patients with nonsense or frameshift mutations should be truncated and would not be expected to have any enzymatic activity due to the lack of both the heme binding peptide and the putative steroid binding site. Nevertheless, there is variation in the severity of the phenotypic effects in different patients. It was previously noted that there was no consistent correlation between the severity of hypertension and degree of virilization in the individuals homozygous for R448H and that there was substantial variation among these patients in biochemical parameters such as plasma levels of the 11β-hydroxylase substrates deoxycortisol and deoxycorticosterone and urinary excretion of tetrahydrodeoxycortisol (a principal metabolite of 11-deoxycortisol) (13). Similar variation in phenotype is observed among the patients in the present study (Table 1). For example, patient 1, a genetic female homozygous for R374Q, had apparently male external genitalia except for hypospadias and was raised as a male. Similar patients have been noted among Moroccan Jews, most of whom are homozygous for R448H. In contrast, patients 2a and 2b, females who were homozygous for a nonsense mutation of *CYP11B1* that would be expected to encode a truncated protein without enzymatic activity had milder virilization of the external genitalia; each

had an enlarged clitoris and bifid labioscrotal folds. In the other affected sib pair (patients 4a and 4b) carrying the mutation T318M, degree of genital ambiguity, basal hormonal levels, and blood pressure differed.

Thus, phenotypic variations observed among the patients studied here as well as individuals homozygous for R448H (13) must be governed by factors outside the *CYP11B1* locus.

Strategy for Prenatal Diagnosis. One of the most distressing signs of congenital adrenal hyperplasia due either to 21- or 11β-hydroxylase deficiency is prenatal virilization of affected females, leading to the development of ambiguous genitalia. Experience with 21-hydroxylase deficiency has shown that administration of dexamethasone to the mother of an affected fetus is effective in suppressing the fetal adrenal cortex, thus preventing excessive secretion of androgens and permitting normal development of female external genitalia (32–35). Although experience is lacking, a similar approach should be effective for female fetuses affected with 11β-hydroxylase deficiency. To be most effective, therapy should be started as soon as pregnancy is recognized. Subsequent prenatal diagnosis, using DNA prepared from chorionic villus samples taken at 10 weeks, would allow therapy to be quickly terminated if the fetus were found to be unaffected or male.

Except in specific ethnic groups such as Jews from Morocco (about 13% of the Israeli population) it seems likely that most patients will carry unique mutations, and it will be of interest to catalog these new mutations. If analysis of large numbers of patients is required, it may be possible to scan for mutations by using single-stranded conformation polymorphisms or heteroduplex analysis. However, at present, it seems that prenatal diagnosis can best be accomplished by direct PCR amplification and sequence analysis as reported here.

We thank Professor D. P. Davies, Professor M. Mikkelsen, Dr. K. W. Kastrup, and J. Haverstick for bringing patients to our attention, and Professor S. Ulick for urinary tetrahydrodeoxycortisol measurements done on patients 4a and 4b. This work was supported by Grants DK37867 and DK42169 from the National Institutes of Health. K.M.C. was supported by the Klosk Foundation and P.C.W. is an Irma T. Hirschl Trust Scholar.

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