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Genetics

P450XXI (steroid 21-hydroxylase) gene deletions are not found in family studies of congenital adrenal hyperplasia

(cytochrome P-450/mutation/hormone/genetic disease)

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ABSTRACT **Congenital adrenal hyperplasia (CAH) is a common genetic disorder due to defective 21-hydroxylation of steroid hormones. The human P450XXIA2 gene encodes cytochrome P450c21 [steroid 21-monooxygenase (steroid 21-hydroxylase), EC 1.14.99.10], which mediates 21-hydroxylation. The P450XXIA2 gene may be distinguished from the duplicated P450XXIA1 pseudogene by cleavage with the restriction endonuclease *Taq* I, with the XXIA2 gene characterized by a 3.7-kilobase (kb) fragment and the XXIA1 pseudogene characterized by a 3.2-kb fragment. Restriction endonuclease mapping by several laboratories has suggested that deletion of the P450XXIA2 gene occurs in about 25% of patients with CAH, as their genomic DNA lacks detectable 3.7-kb *Taq* I fragments. We have cloned human P450c21 cDNA and used it to study genomic DNA prepared from 51 persons in 10 families, each of which includes 2 or more persons with CAH. After *Taq* I digestion, apparent deletions are seen in 7 of the 20 alleles of the probands; using *Eco*RI, apparent deletions are seen in 9 of the 20 alleles. However, the apparently deleted alleles seen with *Taq* I do not coincide with those seen with *Eco*RI. Furthermore, studies with *Bgl* II, *Eco*RI, *Kpn* I, and *Xba* I yield normal patterns with at least two enzymes in all cases. Since all probands yielded normal patterns with at least two of the five enzymes used, we conclude that the P450XXIA2 gene "deletions" widely reported in CAH patients probably represent gene conversions, unequal crossovers, or polymorphisms rather than simple gene deletions.**

Congenital adrenal hyperplasia (CAH) is a group of inborn errors of cortisol biosynthesis. Deficient cortisol secretion stimulates excess corticotropin, leading to the accumulation of precursor steroids proximal to the disordered enzyme (1). In most enzymatic lesions this accumulation results in excess androgen production, causing virilization of affected newborns. About two-thirds of the cases have an associated mineralocorticoid defect leading to impaired renal tubular resorption of sodium ("salt losing") that can result in shock and death (1).

CAH has an estimated frequency of 1 per 5000 births (1). Over 90% of CAH cases are due to deficient 21-hydroxylase activity [steroid 21-monooxygenase (steroid 21-hydroxylase), EC 1.14.99.10]. This activity is mediated by a specific cytochrome P450 enzyme, termed P450c21, which receives electrons from NADPH by way of a flavoprotein (2, 3). Another steroidogenic enzyme, P450c17 (17α-hydroxylase/17,20-lyase), employs the same flavoprotein but remains active; hence, patients with deficient 21-hydroxylase activity must have lesions in the gene encoding P450c21. The gene encoding P450c21 lies within the human HLA major histocompatibility locus on the short arm of chromosome 6 (4, 5). As a result, different clinical forms of deficient 21-hydroxylase activity are in linkage disequilibrium with certain HLA haplotypes; hence, linkage analysis using HLA antigens has been used for prenatal detection of CAH (reviewed in ref. 1).

There are two genes for P450c21, termed P450XXIA1 and P450XXIA2 (6). They are about 30 kilobases (kb) apart, each lying just 3′ to the C4A and C4B genes encoding the fourth component of complement (5, 7–11) (Fig. 1). The P450XXI genes each span about 3.4 kb and contain 10 exons (9–11). Although the two genes are highly homologous, only the human P450XXIA2 gene is functional, whereas the P450XXIA1 gene is nonfunctional due to an 8-base-pair (bp) deletion in exon 3 (9, 11). For simplicity, these genes are referred to by their trivial names P450c21A (nonfunctional gene) and P450c21B (functional gene). Apparent deletions of the P450c21B gene, evidenced by absent 3.7-kb *Taq* I and 12-kb *Eco*RI genomic fragments, were reported by White *et al.* (7, 8) in patients having salt-losing 21-hydroxylase deficiency and HLA *Bw47-C4A Q0* (null) haplotypes. Carroll *et al.* (5, 12), however, assign the P450c21A gene to this 12-kb *Eco*RI fragment. Consistent with this, Donohoue *et al.* (14) reported absence of the 3.7-kb *Taq* I fragment but presence of the 12-kb *Eco*RI fragment in a patient with CAH. Among 18 persons lacking C4 activity (homozygous C4 null alleles), 2 had apparent P450c21A gene deletions but none had P450c21B gene deletions based on *Taq* I digests (13). Schneider *et al.* (15) used *Taq* I digests alone to study the C4 and P450c21 alleles in 8 subjects with salt-losing 21-hydroxylase deficiency, finding apparent deletions in 10 of the 16 P450c21B alleles examined. Among 20 unselected patients with CAH, Rumsby *et al.* (12) found one homozygote and seven heterozygotes for P450c21 "gene deletions" (i.e., 9 of 40 alleles). Werkmeister *et al.* (16) similarly reported 10 of 44 alleles "deleted" in CAH patients.

To determine the relative frequency of deletions versus nondeletional gene mutations ablating 21-hydroxylase activity, we cloned a human adrenal P450c21 cDNA and used this, in conjunction with a murine C4 cDNA clone, to study the restriction patterns in DNA from 51 persons from 10 families, wherein each family had at least 2 persons affected with CAH. Of the 20 P450c21B alleles from the 10 family probands, digestion with *Taq* I indicated that 7 P450c21B alleles appeared to be deleted. However, more extensive analysis with *Bgl* II, *Eco*RI, *Kpn* I, and *Xba* I indicated that other mutations causing polymorphisms or "gene conversion" events may account for these apparent deletions of the P450c21B gene. Thus, our data, in contradistinction to earlier studies (7, 8, 12, 13, 15, 16), show that simple deletions of the P450c21B gene are not a common cause of salt-losing 21-hydroxylase deficiency and suggest that the salt-losing

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Abbreviation: CAH, congenital adrenal hyperplasia.

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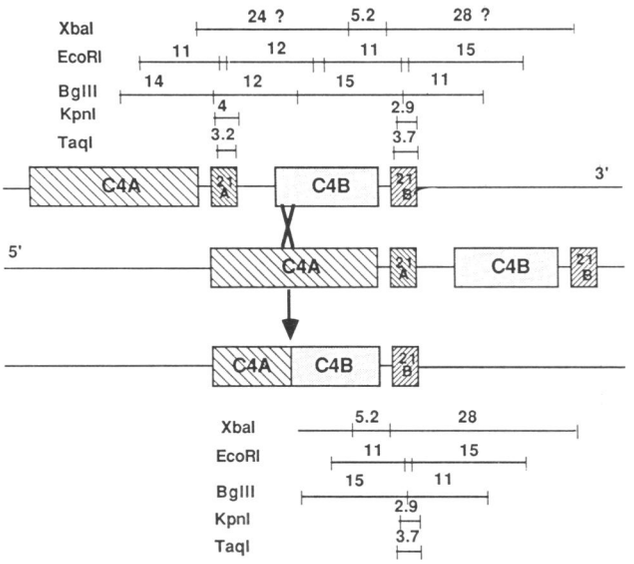

FIG. 1. Map of the P450c21 gene locus. (*Top*) Restriction endonuclease sites (from refs. 5, 8, 12, 13). (*Middle*) Scale drawing of the C4 and P450c21 genes showing an unequal crossover event between the C4B and C4A genes compatible with the findings in our subjects 1-6 and 10-2 (see Fig. 3). (*Bottom*) Restriction endonuclease sites resulting from the proposed crossover event.

CAH phenotype is due to many different lesions in the P450c21B gene.

MATERIALS AND METHODS

Our human adrenal cDNA library in λgt10 (17–19) was probed with a ^{32}P-labeled 1141-bp *Eco*RI fragment of the bovine P450c21 gene in plasmid pUC/1.2 (20). About 1.2×10^6 plaques were screened, yielding 5 positives. The DNA from the phage containing the longest cloned insert was subcloned into pUC18 (phac21-1) and sequenced as described (10, 17–19).

We studied DNA from 51 members of 10 families, including 22 persons having deficient 21-hydroxylase activity evidenced by low neonatal plasma cortisol and excess plasma 17-hydroxyprogesterone. All were salt-losers, requiring replacement with both glucocorticoids and mineralocorticoids. The zygosities of clinically unaffected family members were determined by HLA typing using the microcytotoxicity test and standard sera (21), and some families were confirmed hormonally. DNA from peripheral blood was digested with *Bgl* II, *Eco*RI, *Kpn* I, *Taq* I, and *Xba* I as specified by the suppliers. DNA was electrophoresed through 0.8–1.0% (wt/vol) agarose and then transferred to nitrocellulose. The blots were probed with ^{32}P-labeled cDNA inserts, washed, and autoradiographed (22). The probes used were the 1229-bp cDNA from phac21-1 and the 1500-bp murine C4 cDNA clone pMC/21 (23), generously provided by Tomasso Meo (Institut Pasteur, Paris). The relative intensities of the autoradiographic bands in the *Taq* I blots of DNA from affected individuals were confirmed by laser densitometry. Linkage analysis was carried out using the method of maximum likelihood (24) and the computer program LIPED (25).

RESULTS

cDNA Sequence. The cDNA insert cloned in phac21-1 was completely sequenced on both strands. It encodes amino acids 265–494 plus the complete 3′ untranslated region of P450c21 (Fig. 2). There are only 2 nucleotide differences between our sequence and that of Higashi *et al.* (9) but there are 15 differences with the sequence of White *et al.* (11).

P450c21 Gene. The autoradiographic patterns of DNA from the 10 families, obtained after digestion with *Bgl* II, *Eco*RI, *Taq* I, *Kpn* I, or *Xba* I and hybridization to the human P450c21 probe, are shown in Fig. 3. After digestion with *Eco*RI, absent or decreased hybridization of the 12-kb fragment was observed in the affected children in pedigrees 4, 5, 6, 7, and 9 (Fig. 3), suggesting deletions of their P450c21B genes (7, 8) or P450c21A genes (5, 12). However, similar patterns indicating homozygous "deletions" were also seen in four nonaffected individuals (1-6, 4-5, 5-4, and 10-2) (Fig. 3) in four different families. Thus, absence of the 12-kb *Eco*RI band does not correlate with either absence of the P450c21B gene or deficient 21-hydroxylase activity.

The patterns obtained following digestion with *Taq* I show reduced or absent 3.7-kb fragments in samples from the affected children in pedigrees 1, 4, 6, 8, 9, and 10 but not in the unaffected individuals in pedigrees 1, 4, 5, and 10 who lack the 12-kb *Eco*RI fragment. Affected children in pedigree 8 had "deletion" patterns after *Taq* I digestion but normal patterns after *Eco*RI digestion. Thus, the samples (1-6, 4-3, 4-4, 4-5, 5-2, 5-3, 5-4, 9-2, 9-3, 9-4, and 10-2) from all five pedigrees that had homozygous absent 12-kb *Eco*RI fragments had normal *Taq* I patterns. Conversely, samples 8-2 and 8-4 showed homozygous absent 3.7-kb *Taq* I fragments but had a normal *Eco*RI pattern. These data indicate that the 12-kb *Eco*RI-fragment contains the P450c21A gene, as suggested by Carroll *et al.* (5, 12), and not the P450c21B gene, as suggested by White *et al.* (7, 8).

To clarify the discrepant results with *Taq* I and *Eco*RI, we examined the patterns obtained following digestion with *Bgl* II, *Kpn* I, and *Xba* I. These data showed many additional inconsistencies. For example, samples from affected individuals in pedigrees 5, 6, and 9 had a reduction in the 12-kb fragments following *Bgl* II digestion, indicating apparent deletion of the P450c21A gene rather than deletion of the P450c21B gene (5, 12). Nonaffected individuals 1-6 and 10-2 had normal 3.2-kb *Taq* I bands, suggesting retention of the P450c21A genes, but absent 12-kb fragments after *Eco*RI or *Bgl* II digestion indicate apparent deletions of these same P450c21A genes. Conversely, affected individuals 8-2 and 8-4

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265                 270                 280
Ala Gln Pro Ser Met Glu Glu Gly Ser Gly Gln Leu Leu Glu Gly His Val His Met Ala
GCG CAG CCG AGC ATG GAA GAG GGC TCT GGA CAG CTC CTG GAA GGG CAC GTG CAC ATG GCT
                    290                 300
Ala Val Asp Leu Leu Ile Gly Gly Thr Glu Thr Thr Ala Asn Thr Leu Ser Trp Ala Val
GCA GTG GAC CTC CTG ATC GGT GGC ACT GAG ACC ACA GCA AAC ACC CTC TCC TGG GCC GTG
                    310                 320
Val Phe Leu Leu His His Leu Glu Ile Gln Gln Arg Leu Gln Glu Glu Leu Asp His Glu
GTT TTT TTG CTT CAC CAC CTT GAG ATT CAG CAG CGA CTG CAG GAG GAG CTA GAC CAC GAA
                    330                 340
Leu Gly Pro Gly Ala Ser Ser Ser Arg Val Pro Tyr Lys Asp Arg Ala Arg Leu Pro Leu
CTG GGC CCT GGT GCC TCC AGC TCC CGG GTC CCC TAC AAG GAC CGT GCA CGG CTG CCC TTG
                    350                 360
Leu Asn Ala Thr Ile Ala Glu Val Leu Arg Leu Arg Pro Val Val Pro Leu Ala Leu Pro
CTC ATT GCC ACC ATC GCC GAG GTG CTG CGC CTG CGG CCC GTT GTG CCC TTA GCC TTG CCC
                    370                 380
His Arg Thr Thr Arg Pro Ser Ser Ile Ser Gly Tyr Asp Ile Pro Glu Gly Thr Val Ile
CAC CGC ACC ACA CGG CCC AGC AGC ATC TCC GGC TAC GAC ATC CCT GAG GGC ACA GTC ATC
                    390                 400
Ile Pro Asn Leu Gln Gly Ala His Leu Asp Glu Thr Val Trp Glu Arg Pro His Glu Phe
ATT CCG AAC CTC CAA GGC GCC CAC CTG GAT GAG ACG GTC TGG GAG AGG CCA CAT GAG TTC
                    410                 420
Trp Pro Asp Arg Phe Leu Glu Pro Gly Lys Asn Ser Arg Ala Leu Ala Phe Gly Cys Gly
TGG CCT GAT CGC TTC CTG GAG CCA GGC AAG AAC TCC AGA GCT CTG GCC TTC GGC TGC GGT
                    430                 440
Ala Arg Val Cys Leu Gly Glu Pro Leu Ala Arg Leu Glu Leu Phe Val Val Leu Thr Arg
GCC CGC GTG TGC CTG GGC GAG CCG CTG GCG CGC CTG GAG CTC TTC GTG GTG CTG ACC CGA
                    450                 460
Leu Leu Gln Ala Phe Thr Leu Leu Pro Ser Gly Asp Ala Leu Pro Ser Leu Gln Pro Leu
CTG CTG CAG GCC TTC ACG CTG CTG CCC TCC GGG GAC GCC CTG CCC TCC CTG CAG CCC CTG
                    470                 480
Pro His Cys Ser Val Ile Leu Lys Met Gln Pro Phe Gln Val Arg Leu Gln Pro Arg Gly
CCC CAC TGC AGT GTC ATC CTC AAG ATG CAG CCT TTC CAA GTG CGC CTG CAG CCC CGC GGC
                    490
Met Gly Ala His Ser Pro Gly Gln Asn Gln OP
ATG GGG GCC CAC AGC CCG GGC CAG AAC CAG TGA TGGGGCAGGACCGATGCCAGCCGGGTAGCTCAGTTT

CTCCTTTATTGCTCCTGTACGAACCCCTCCCCTCCCCCCTGTAAACACAGTGCTGCGAGATCGCTGGCAGAGAAGGCTTC

CTCCAGCGGCTGGGTGGTGAAGGACCCTGGCTCTTCTCTCGGGGCGACCCCTCAGTGCTCGGCAGTCATACTGGGGTGCG

AGAGAGGTGGGCAGCAGCTCAGCCTCCCCCCGCTGGGGAGCGAAAGTTTCTTGGTCTCAGCTTCATTTCCGTGAAGGGCA

CCGAGAACTCGAAGCCCTTCCAGTGGTACCAGCTCACTCCCTGGGAAAGGGCTTGTCAAGAGAGAGTCAAAGCCGGATGT

CCCATCTGCTCTTCCCGTTCCCCTTAAGGAGGTAGCTCCCAGCACTCAACCAACCTCCCCGCAGAGCTCCCTTCCTGACC

CTCCGCTGCAGAGGATTGAGGCTTAATTCTGAGCTGGCCCTTTCCAGCCAATAAATCAACTCCAGCTCCCTCTGCGAGGC

TGGCATGATTCTTCCATTTC(poly A)
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FIG. 2. Sequence of phac21-1. Note the AATAAA sequence 51 bases from the poly(A) region.

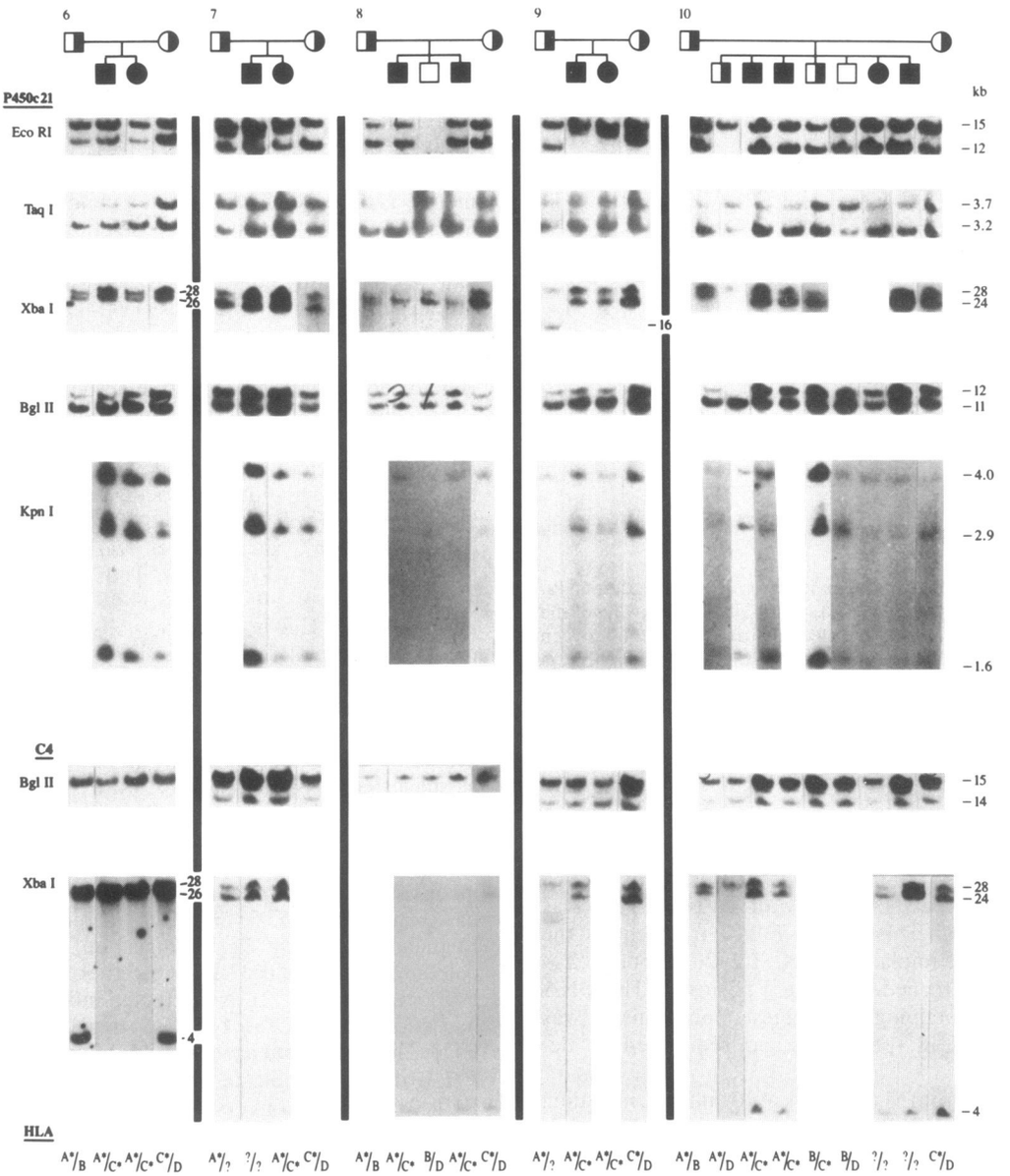
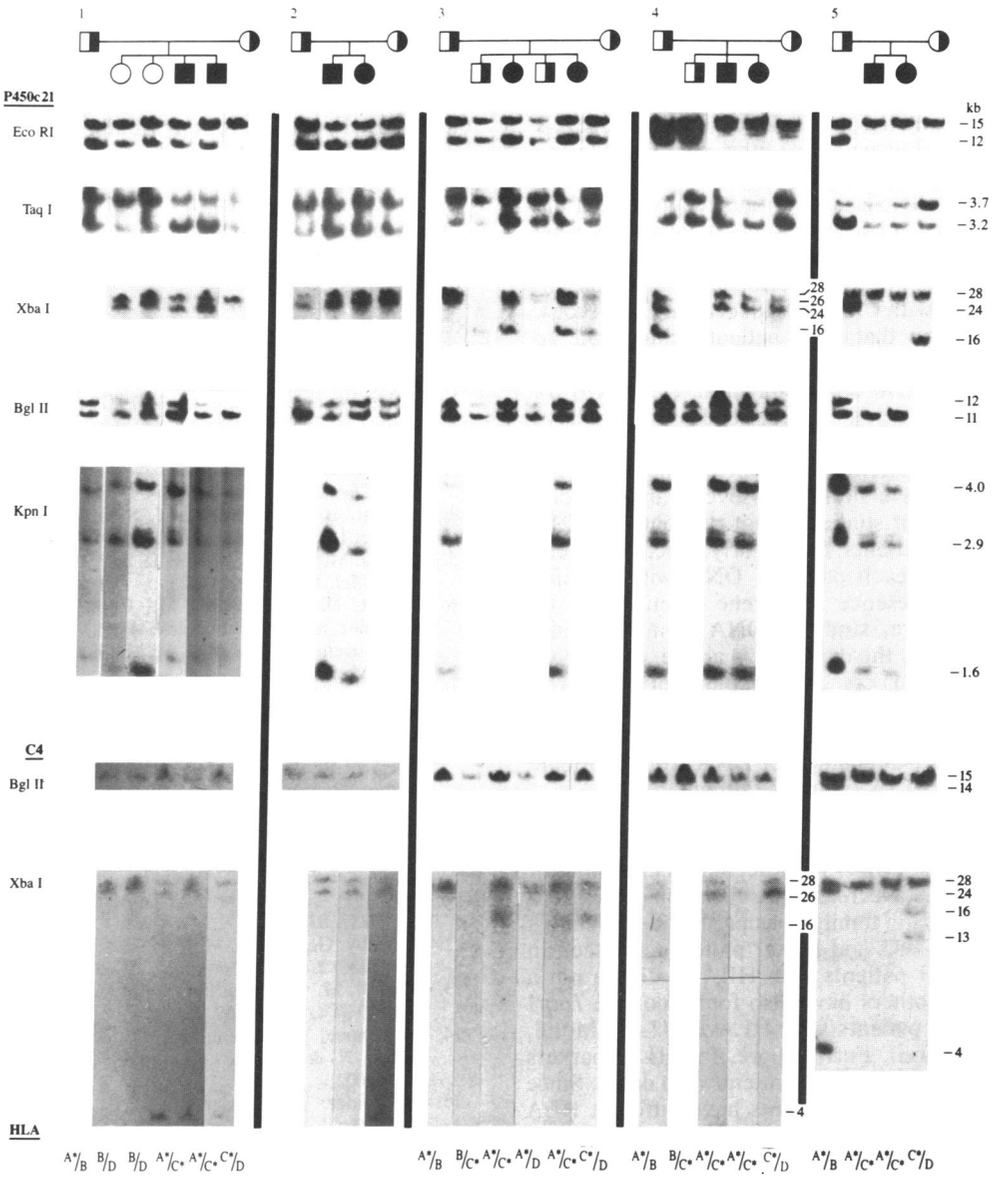


FIG. 3. Pedigrees of the families studied. Squares and circles represent males and females, respectively; open symbols, solid symbols, and half-open/half-solid symbols represent homozygous normals, homozygous affected individuals (21-hydroxylase deficiency), and heterozygotes, respectively. Family numbers are indicated across the top. In the text, individuals are referred to by family number followed by their position in the pedigree (or autoradiograms) from left to right (e.g., the mother in family 1 is designated as 1-6). Autoradiogram patterns of DNA blots from each individual after digestion with the various restriction endonucleases (left margin) and hybridization to the P450c21 probe (top) or the C4 probe (bottom) are shown under each individual's pedigree symbol. *Eco*RI, *Kpn* I, and *Taq* I studies were also done with the C4 probe yielding identical patterns in all subjects (not shown). The HLA haplotypes for each individual are indicated at the bottom of the figure. B and D designate the normal alleles; A* and C* designate alleles linked to CAH. *(Figure continued on following page.)*

had normal patterns after *Bgl* II digestion but had patterns indicating homozygous P450c21B gene deletions after *Taq* I digestion. The patterns following *Kpn* I digestion were normal in all affected individuals except in families 8 and 10, which have absent and decreased amounts, respectively, of the 2.9-kb signal corresponding to the P450c21B gene (13). DNA digested with *Xba* I yielded 24- and 28-kb fragments in most families. Families 3, 4, 5, and 9 also showed a 16-kb fragment; this may represent an *Xba* I polymorphism as this band is also seen in some controls (26). The significance of the absent 24-kb bands in affected individuals 5-2 and 5-3 and of the diminished 28-kb bands in affected individuals 8-2 and 8-4 is unknown, as the *Xba* I map of this locus is ambiguous.

The patterns seen after *Taq* I digestion suggest the affected individuals in family 4 are heterozygous and those in family 8 are homozygous for deletions of the P450c21B gene. However, in both families, at least two other restriction endonucleases (family 4, *Kpn* I and *Bgl* II; family 8, *Bgl* II and *Eco*RI) generated normal "nondeletion" patterns for both genes. Samples from individuals in pedigrees 1, 4, 6, 9, and 10 appeared to be heterozygous and those from family 8 appeared to be homozygous for P450c21B deletions following digestion of their DNA with *Taq* I, but in these same samples the *Bgl* II or *Eco*RI patterns indicated heterozygosity or homozygosity for P450c21A "deletions." Thus, 7 of the 20 P450c21B alleles from the CAH probands appeared to carry deletions following single digestions with *Taq* I, as reported (12, 13, 15, 16), but in each case the *Bgl* II and *Eco*RI patterns suggested that the P450c21A gene rather than the P450c21B gene is "deleted." Interestingly, 4 nonaffected individuals (1-6, 4-5, 5-4, and 10-2) also appeared to be homozygous for P450c21A gene "deletions" after *Eco*RI digestion.



The haplotypes are shown below:

	1	3	4	5	6	7	8	9	10
*A**	*A24 B18*	*A24 B7*	*A30/31 Bw60*	*A3 B27*	*A19 Bw60*	*A3 B5*	*A2 B18*	*A2 Bw47*	*A3 Bw47*
B	*?B7*	*A1 B8*	*A11 B22*	*A32 B5*	*A1 B35*	*A2 B5*	*A3 B7*	*A24 B5*	*A3 B7*
*C**	*A2 B15*	*A2 B44*	*A32 B17*	*A1 B8*	*A3 B7*	*A31 B12*	*A32 B40*	*A24 B5*	*A2 B5*
D	*A30/31 B18*	*A1 B8*	*A1 B15*	*A29 B44*	*A23 B21*	*A2 B12*	*A3 B12*	*A24 B27*	*A32 B16*

C4 Gene. The C4 genes were studied in samples from all 10 families. Following digestion with *Bgl* II, affected individuals in pedigrees 1, 2, 3, 4, 5, 6, and 8 had absent 14-kb fragments and those in families 7, 9, and 10 had decreased 14-kb fragments, corresponding to the C4A gene (5, 12). Following *Xba* I digestion, hybridization to the C4 probe revealed the same bands seen with the P450c21 probe plus an additional 4-kb band seen in families 1, 2, 5, 6, 8, and 10 (Fig. 3). This observation agrees with the restriction map of White *et al.* (8), which shows the 3′ end of the C4A gene, the P450c21A gene, and the 5′ end of the C4B gene all reside on a single large *Xba* I fragment >20 kb.

When linkage studies were done using *Bgl* II, *Eco*RI, *Kpn* I, *Taq* I, or *Xba* I, both P450c21B alleles could be detected in affected individuals in pedigrees 5, 6 and 8. In pedigrees 1, 2, 3, 4, 9, and 10, only one P450c21B allele could be detected. Thus, the segregation pattern of 12 of 20 CAH alleles can be detected by restriction analysis. Using these DNA fragments as markers, no recombinants were seen between DNA fragments containing P450c21 or C4 sequences versus the CAH phenotype or HLA haplotypes in 15 informative meioses. The corresponding logarithm of odds (lod score) is 4.52 at $\Theta = 0$ (24).

DISCUSSION

The phac21-1 sequence (Fig. 2) contains two nucleotide differences compared to the corresponding region of the gene sequence reported by Higashi *et al.* (9); the C to G change in the second base of codon 426 changes proline to arginine. Similar comparison to the sequence reported by White *et al.* (11) reveals 15 nucleotide differences; also, we find the poly(A) track begins 30 bases further downstream. Although variations in poly(A) addition sites are common and of unknown significance, the multiple differences between the sequences we and Higashi *et al.* (9) obtained to that obtained by White *et al.* (11) are more difficult to explain. Extensive

polymorphism arising from genetic drift could account for many such differences, but the large number observed (1.2%) suggests that some may represent sequencing errors.

Based primarily on the absence of 3.7-kb *Taq* I fragments, other groups have estimated that deletion of the P450c21B gene occurs in 25–40% of patients with severe, salt-losing CAH (7, 8, 12, 13, 15, 16). Most of these studies show that only one 3.7-kb *Taq* I allele is absent, thus requiring that the remaining 3.7-kb *Taq* I allele represents a P450c21B gene carrying another grossly deleterious (point) mutation. Our data are not inconsistent with the association of an absent 3.7-kb *Taq* I fragment with CAH or with certain HLA types. However, our data show that every patient lacking a 3.7-kb *Taq* I fragment has a normal-sized P450c21B gene following digestion with at least two other enzymes. Therefore, we suggest that gross gene deletions described by others have not occurred in our 10 families and that the absent 3.7-kb *Taq* I fragments are due to restriction fragment length polymorphisms (RFLPs), gene conversions, crossovers, and/or polymorphisms. Although our studies do not rule out P450c21B gene deletions in other patients, they clearly indicate the need for extensive study of each patient's DNA with multiple enzymes before the presence of a gene deletion can be determined. Furthermore, study of DNA from all family members is needed since the data shown above, our unpublished studies with normal persons, and studies of normals by others (27) indicate that RFLPs are common in this locus. These studies are especially important in establishing the diagnoses of CAH by examination of DNA from chorionic villus biopsies, where HLA typing is difficult.

The HLA *Bw47* allele is reported to be highly linked to P450c21B deletions (7, 8). The patients in families 9 and 10 who are heterozygous for *Bw47* have decreased intensities of their 3.7-kb *Taq* I bands. Decreases are also seen in the 12-kb *Eco*RI and *Bgl* II bands in family 9, as predicted by White's model, but normal *Eco*RI and *Bgl* II patterns are seen in family 10. Thus, CAH patients with HLA *Bw47* are not a homogeneous group; others have also found normal *Taq* I patterns in two CAH patients with HLA *Bw47* (Y. Morel, personal communication). Furthermore, the HLA markers linked to *Bw47* in White's and our patients also differ. Since all of the CAH alleles in our patients have different HLA haplotypes, the P450c21 gene lesions may differ in all of the alleles of our probands.

Thus, our findings agree with a recent report of Donohoue *et al.* (14) in which CAH subjects had only 3.2-kb *Taq* I fragments and lacked 5′ P450c21B sequences but had both normal *Eco*RI fragments. The mechanism hypothesized to explain this inconsistency was a "gene conversion event" in which the sequences of the active P450c21B gene are mutated or converted, partially or completely, to become homologous to the inactive P450c21A gene (14). Though the mechanism of such gene conversion is unknown, this phenomenon has been described in other human gene systems, such as the fetal globin and immunoglobulin genes (28, 29). An alternative explanation is that repeated cycles of unequal crossing-over between P450c21 and C4 alleles produce fusions, duplications, and/or deletions that alter the restriction sites within or flanking the P450c21 genes. Following such rearrangements some restriction patterns may be normal whereas others are altered depending on the point of crossover. Such an unequal crossover event could yield a C4A/C4B P450c21B product having the restriction patterns seen in individuals 1-6 and 10-2 (Fig. 3)—i.e., retained 11-kb *Bgl* II and 3.7-kb *Taq* I but absent 12-kb *Eco*RI and 24-kb *Xba* I fragments (Fig. 1 *Bottom*) (8, 14, 15). The reciprocal product C4A-[P450c21A]-C4A-[P450c21A]-C4B-[P450c21B] is compatible with the C4A duplications suggested from protein studies of Raum *et al.* (30) and is similar to C4A duplications detected by Schneider *et al.* (15). Thus, our data indicate that P450c21 gene deletions reported by others (7, 8, 12, 13, 15, 16) were due in part to incorrect or incomplete mapping and in part to gene conversions, crossovers, polymorphisms, and point mutations. Furthermore, our finding of different HLA haplotypes with each CAH allele suggests that P450XXI is a highly allelic locus.

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