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Gene Conversion in Steroid 21-Hydroxylase Genes

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Summary

The steroid 21-hydroxylase gene, CYP21B, encodes cytochrome P450c21, which mediates 21-hydroxylation. The gene is located about 30 kb downstream from pseudogene CYP21A. The CYP21A gene is homologous to the CYP21B gene but contains some mutations, including a C→T change which leads a termination codon, TAG, in the eighth exon. We found the same change in a mutant CYP21B gene isolated from a patient with 21-hydroxylase deficiency. Furthermore, a reciprocal change—i.e., a T→C change in the eighth exon of the CYP21A gene—was observed in the Japanese population and was associated with the two HLA haplotypes, HLA-B44-DRw13 and HLA-Bw46-DRw8. These changes may be considered the result of gene conversion-like events.

Introduction

Two steroid 21-hydroxylase genes (CYP21A and CYP21B) alternate in tandem with two genes for the fourth component of complement (C4A and C4B) on the short arm of chromosome 6 between the loci of HLA-B and HLA-DR (Carroll et al. 1985*a*; White et al. 1985). The CYP21B gene encodes an adrenal microsomal cytochrome P-450, which is specific for steroid 21-hydroxylation (P450c21). The CYP21A gene is homologous to the CYP21B gene in as many as 98% of the nucleotide sequences, but it is a pseudogene. The CYP21A gene contains an 8-bp deletion in the third exon, a 1-bp insertion in the seventh exon and a C→T transition in the eighth exon. These mutations generate frameshifts and a nonsense mutation, respectively, which would prevent synthesis of the functional protein (Higashi et al. 1986; White et al. 1986).

A defect of this protein would cause 21-hydroxylase deficiency, which is the most common cause of congenital adrenal hyperplasia (CAH). 21-Hydroxylase deficiency is an autosomal recessive defect in adrenal biosynthesis of cortisol and aldosterone, with resultant excess of androgen secretion. Because of the excess androgen, virilization is a prominent feature of the disorder (simple virilizing). In more severe cases, aldosterone biosynthesis is also impaired, and untreated individuals may die in the neonatal period from an inability to conserve serum electrolytes (salt wasting). Neonatal screening revealed that the incidence for the salt-wasting form of CAH was 1/10,000–1/16,000 births in most white populations, 1/16,000 births in Japanese, and 1/700 births in Yupik Eskimos in Alaska (Pang et al. 1988). The reason that the deleterious disease has been maintained in such a high frequency remains unknown.

For the molecular mechanism of 21-hydroxylase deficiency, White et al. (1984) suggested a deletion of the functional CYP21B gene in some patients with the HLA-Bw47-DR7 haplotype. These patients had no C4B protein and neither of the fragments—3.7-kb *Taq*I and 12-kb *Eco*RI—hybridizing to the cDNA probe encoding P450c21. It was then generally espoused from the analysis of conventional *Taq*I RFLPs that over 25% of patients have deletion of their functional CYP21B genes (White et al. 1987). Matteson et al. (1987), however, inferred from a family study that apparent deletions of the 3.7-kb *Taq*I fragment represented gene conversions, unequal crossovers, or polymorphisms—but not physical loss of the CYP21B genes. Donohoue et al. (1986) first proposed gene conversion as a possible cause of 21-hydroxylase deficiency. We have supported this

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proposal by partial sequencing of a CYP21B gene from a patient with CAH (Harada et al. 1987*a*), and this was further confirmed by complete analyses of mutant CYP21B genes (Amor et al. 1988; Globerman et al. 1988; Higashi et al. 1988*b;* Speiser et al. 1988).

We report here the complete nucleotide sequences of a CYP21B gene from a patient homozygous for HLA Bw75-C2C-BfS-C4A3-C4B1-HLA DRw9 by descent (Harada et al. 1987*b*), thus providing more convincing evidence of a gene conversion between an active CYP21B gene and a CYP21A gene. A family study of the patient suggested a polymorphism in the CYP21A gene. We therefore examined the organization of CYP21 genes and adjoining C4 genes by Southern blot analysis in Japanese individuals. This population study showed that the HLA-B7-DR1 haplotype had triplicated C4 and CYP21 genes and that part of the CYP21A gene was converted to the CYP21B in the two HLA haplotypes—HLA-B44-DRw13 and HLA-Bw46-DRw8—in Japanese.

Material and Methods

Southern Blot Analysis

High-molecular-weight DNAs were isolated from peripheral blood leukocytes or from Epstein-Barr virus–transformed cell lines and were treated with restriction endonucleases before preparation of Southern blots on GeneScreen Plus (New England Nuclear, Boston). These blots were hybridized with radiolabeled DNA probes for 40 h at 42°C in 50% formamide, 0.1% Denhardt's, 0.75 M sodium chloride, 0.05 M NaH_2PO_4, 5 mM EDTA, 5% dextran, 1% sodium laurylsulfate and were washed twice for 5 min each at room temperature in 0.36 M sodium chloride, 0.02 M NaH_2PO_4, 2 mM EDTA and once for 15 min at 65°C in 0.36 M sodium chloride, 0.02 M NaH_2PO_4, 2mM EDTA, 0.5% sodium laurylsulfate before the autoradiography. The hybridization probes used were (1) pC21/3c (White et al. 1985), provided by P. C. White, a plasmid which contained a 2-kb cDNA encoding P450c21, (2) a *Kpn*I-*Bam*HI fragment of 0.5 kb from the 5′ portion cDNA encoding human C4 (pAT-A) (Belt et al. 1984), supplied by the Gene Bank of Japan, and (3) *Pst*-0.6B, the 0.6-kb *Pst*I fragment of the cloned CYP21B gene from a patient. The nucleotide sequences of the fragment were identical to those of the reported CYP21B genes (Higashi et al. 1986; White et al. 1986; Rodrigues et al. 1987). The probes were radiolabeled with [α-^{32}P] dCTP, by using a random priming method (Feinberg and Vogelstein 1984).

Isolation and Analysis of Cloned DNA

A genomic library was constructed by a method described elsewhere (Harada et al. 1987*a*), using phage vector Charon4A and *Eco*RI-digested DNA from a patient with 21-hydroxylase deficiency. The library was screened with the probe, pC21/3c, by the plaque hybridization method (Maniatis et al. 1982). Phage DNAs were prepared from several positive plaques and were subcloned into plasmid vector PTZ18R or 19R. Nucleotide sequences were determined by the dideoxy chain-termination method (Sanger et al. 1977) and, in some parts, by the chemical degradation method (Maxam and Gilbert 1977).

HLA Typing and Statistical Analysis

HLA-B and -DR typing was performed by standard microlymphocytotoxicity methods (Terasaki et al. 1978). Statistical analysis was performed following the method described by Mittal (1976).

Cell Lines

Lymphoblastoid cell lines established from homozygous typing cells NAK, PGF, TOK, FJO, WY49, TS10, YT, HHK, MIK, MANN, BEILER, SID, Hi-2, TAK, PMGO75, and LD541265 were distributed in the course of Ninth and Tenth International HLA Workshops.

Results

Southern Blot Analysis of a Patient and Her Parents

We analyzed a patient who suffered from the salt-wasting form of 21-hydroxylase deficiency. The patient was homozygous for the HLA Bw75-C2C-BfS-C4A3-C4B1-HLA DRw9 haplotype (Harada et al. 1987*a*) and came from a consanguineous marriage; the grandfather of her mother and the grand-grandmother of her father were siblings. We presumed the homozygosity of the HLA region corresponded to a homozygosity of the linked CYP21B gene. First, Southern blot analysis was performed using DNA samples from both the patient and her parents (fig. 1). When DNA samples were digested by restriction endonuclease *Taq*I and hybridized with pC21/3c encoding P450c21, the CYP21 genes were identified by the 3.7-kb (CYP21B) and 3.2-kb (CYP21A) fragments (White et al. 1984). Both the 3.7- and the 3.2-kb bands were observed, and the two bands were of equal relative intensity in the patient and her parents. No obvious changes were observed in the homozygous CYP21B genes of the patient, and further analyses were performed.

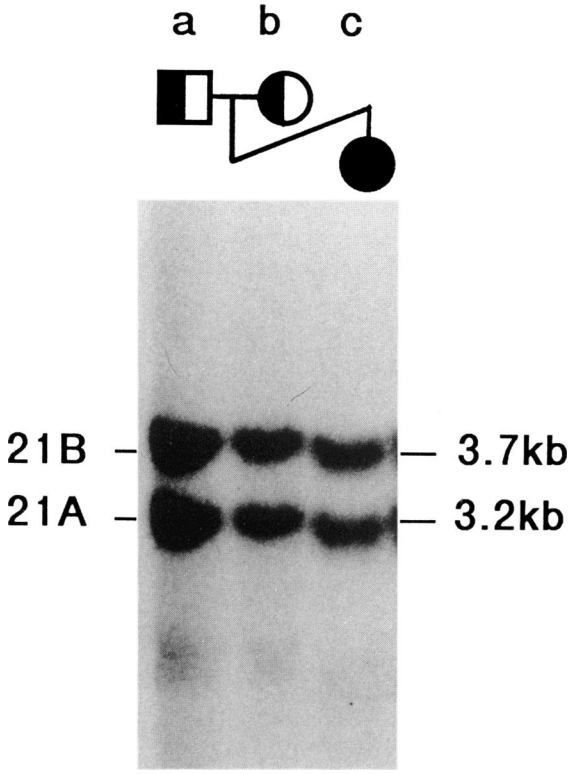

Figure 1 *Taq*I restriction-fragment patterns of the CYP21 genes. pC21/3c served as the DNA probe. Samples are from the father (a), the mother (b), and the patient (c).

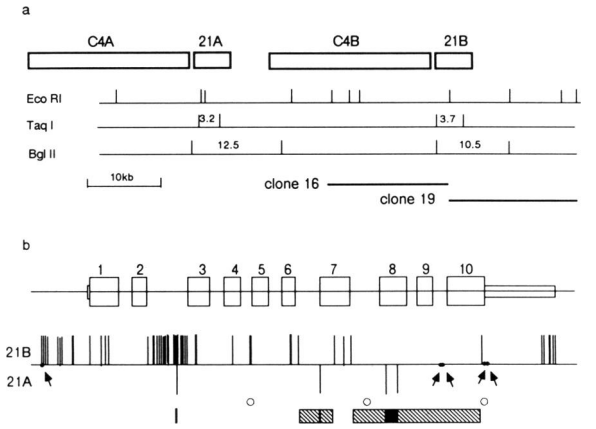

Figure 2 Structure of CYP21 and C4 genes of the patient. *a,* Clones containing CYP21B gene. The relative size and location of the C4 and CYP21 genes are shown at the top. Map and restriction-endonuclease sites are drawn in the middle. The size and location of clones 16 and 19 are shown at the bottom. *b,* Structure of the mutant CYP21B gene. The positions at which the CYP21A gene differed from the nucleotide of the CYP21B gene are marked by bars beneath the exon-intron structure. Bars on a line indicate nucleotides in this patient which correspond to those of the CYP21B gene, while bars under the line indicate the nucleotides which correspond to those of the CYP21A gene. The positions marked by arrows are point mutations. The locations of sequences exhibiting a 90% homology to the human minisatellite repeat sequence are marked by open circles. The minimum area of gene conversion is indicated by the blackened box, and the maximum area of conversion is indicated by the hatched box.

Mutations in the CYP21B Gene in the Patient

To detect mutations in the homozygous CYP21B genes, we constructed a genomic library from the patient. Using pC21/3c as a probe, we isolated from the genomic phage library six phage clones containing *Eco*RI fragments more than 10 kb. From these six, two clones harboring the CYP21B gene were chosen (fig. 2*a*) for further analyses. Clone 19 was identified as containing the 3′ portion of the CYP21B gene because this clone did not hybridize with pAT-A for the C4 probe. Clone 16 was identified as containing the 5′ portion of the CYP21B gene by a physical map of *Eco*RI, *Taq*I, and *Pvu*II enzyme sites, because these enzymes could be used to distinguish the CYP21B gene from the CYP21A gene in this patient. These two clones were subcloned and sequenced.

Nine nucleotide changes were observed in the mutant CYP21B gene of the patient (table 1), compared with the sequences of the heretofore reported CYP21B genes (Higashi et al. 1986*b;* White et al. 1986; Rodrigues et al. 1987). In figure 2*b,* nine changes are indicated by four bars under a line and by five positions marked by arrows. The mutant CYP21B gene was a mosaic of the CYP21A and the CYP21B genes. Of these changes, four were in the exons, one was in the 5′ flanking region, three were in the introns, and one was in the 3′ untranslated region. The nucleotide change in the 5′ flanking region was an insertion of G at nucleotide position −333. At nucleotide position 598 in the second intron, the observed G→A transition corresponded to the nucleotide sequence of the CYP21A gene. The other two changes in the intron were deletions of G at nucleotide positions 2400 and 2405, in the ninth intron. One of the four changes in exons (i.e., the C→G transition at nucleotide position 1590 in the seventh exon) was a silent mutation, while the others—one in the tenth exon and two in the eighth exon—were not.

There were two C→T changes in the eighth exon, at 1998 and 2112. A C→T change at 1998 leads a premature termination codon, TAG, in codon 319. This change was predicted to cause premature termination

Table 1

Nucleotide Changes in the Patient

Position	Location	Mutant B	CYP21B	CYP21A
−333	5′ Region	TGGGGT	TGGGT	TGGGT
598	Intron 2	TTGAGG	TTGGGG	TTGAGG
1590	Codon 249	CTG(Leu)	CTC(Leu)	CTG(Leu)
1998	Codon 319	TAG(END)	CAG(Gln)	TAG(END)
2112	Codon 357	TGG(Trp)	CGG(Arg)	TGG(Trp)
2400–2401	Intron 9	CCCCGT	CCCCGCT	CCCCGCT
2405–2406	Intron 9	CCGTG	CCGCTG	CCGCTG
2701	Codon 494	AGC(Ser)	AAC(Asn)	AAC(Asn)
2721	3′ Region	CCAAT	CCGAT	CCGAT

NOTE.—The number of nucleotide positions differs from those reported because of deletions and insertions of nucleotides. Codon number corresponds to that given by Rodrigues et al. (1987).

of translation before the conserved "heme-binding" region of the P450 polypeptide, which would result in a completely nonfunctional enzyme. Thus, it was suggested that this change was a critical change of the mutant CYP21B gene and that this patient suffered from the disease because of having the mutant CYP21B genes homozygously. Because the C→T changes at nucleotide positions 1998 and 2112 are normally present in the CYP21A pseudogene but not in the CYP21B gene, it seemed possible that they had been transferred to the CYP21B gene by a gene conversion-like event. It was suggested that these two changes had arisen in a single conversion, because it was unlikely that two independent gene conversion-like events had occurred in this limited area. The minimum and maximum areas of the gene conversion in the CYP21B gene were those denoted in figure 2*b* by a blackened box and a hatched box, respectively.

There were three sequence stretches homologous to the human minisatellite repeat sequence (HMR: GGGCAGGAXG) in the fifth exon (from position 1117; TGGCAGGACG), the seventh intron (from position 1958; GGGGCAGGACT), and the 3′ untranslated region (from position 2710; GGGGCAGGACC). The HMR sequence was postulated to provide a recombination signal promoting a formation of minisatellites (Jeffreys et al. 1985) and may play a role in the gene conversion of the CYP21 genes.

The A→G change was found in the tenth exon at codon 494 converting ^{494}Asn in the normal CYP21B gene to ^{494}Ser. The same mutation was reported in the CYP21B gene from a patient with the salt-wasting form of the disease (Rodrigues et al. 1987). We also found the mutation in a patient with the simple virilizing form of the disease (K. Urabe, A. Kimura, F. Harada, and T. Sasazuki, unpublished observation). This amino acid substitution may affect the function of 21-hydroxylase, because these changes were independently detected in three patients, although it might merely represent a polymorphism of 21-hydroxylase.

PstI RFLP Analysis of the Patient and Her Parents

The C→T change in the eighth exon creates a premature termination and destroys a *Pst*I enzyme site (CTGCAG→CTGTAG) in the mutant CYP21B genes of the patient. Thus, the genomic probe *Pst*-0.6B was used for Southern blot analysis to confirm the homozygosity of the C→T change in the CYP21B gene of the patient (fig. 3). The mutant CYP21B gene as well as the CYP21A gene would be characterized by the presence of the 0.9-kb *Pst*I fragment, while the normal CYP21B gene should represent the 0.6-kb *Pst*I fragment. The patient lacked the 0.6-kb *Pst*I fragment (figure 3, lane c), indicating that the C→T changes in the mutant CYP21B genes were homozygous. Because her parents should have a mutant CYP21B gene on one of their alleles, we expected they would represent a 3:1 ratio of 0.9- and 0.6-kb *Pst*I fragments. A densitometric measurement revealed that the intensity of the 0.9-kb band of her mother (fig. 3, lane b) was approximately three times (3.6:1) that of the 0.6 kb band. However, her father had 0.9- and 0.6-kb *Pst*I bands of equal intensity (fig. 3, lane a). Because the paternity was supported from the HLA analysis and from an analysis of neuraminidase activity (Harada et al. 1987*b*), this observation suggested that one of the CYP21A genes of her

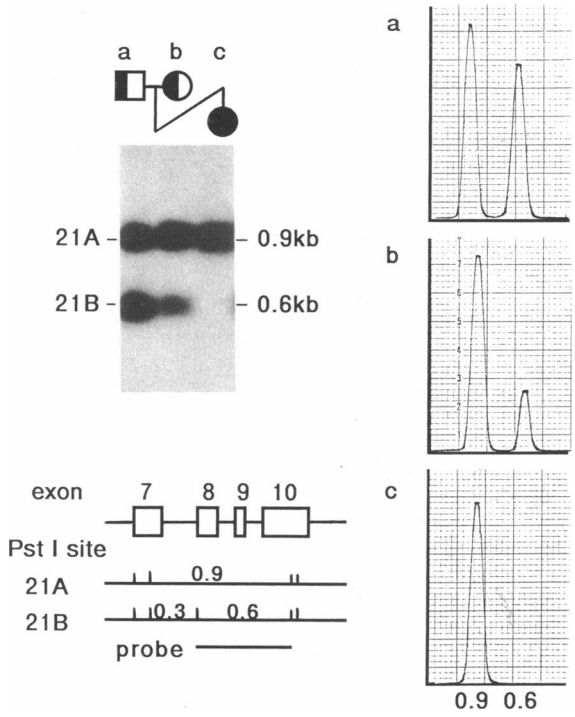

Figure 3 *PstI* RFLPs and their densitometric patterns in the family of the patient. Data are from the father (a), the mother (b), and the patient (c). *PstI* restriction-endonuclease sites in both CYP21A and CYP21B, as well as a probe named *Pst*-0.6B, were placed under the autoradiogram. The densitometric patterns are on the right.

father either had a *PstI* site in the eighth exon—i.e., a C residue was present at the site in the CYP21A gene—or, although unlikely, had at least two normal CYP21B genes on the other normal allele. As the C residue is normally present at the position in the CYP21B gene, it seemed, according to the former possibility, that the C residue had been transferred, by a gene conversion-like event, from the CYP21B gene to the CYP21A gene. We will call the CYP21A gene carrying the *PstI* site in the eighth exon—i.e., the CYP21A gene without the termination codon in the eighth exon—the "converted CYP21A gene." HLA analysis of the family suggested that the converted CYP21A gene is linked to the HLA-B44-DRw13 haplotype, as shown in figure 4.

Gene Organization of C4 and CYP21 Genes

To confirm that the HLA-B44-DRw13 haplotype carries the converted CYP21A gene and to reveal whether another haplotype would carry this converted CYP21A gene, we performed *TaqI* and *PstI* RFLP analyses on DNA samples of lymphoblastoid cell lines (LCLs) from homozygous typing cells (fig. 5). Because the CYP21A

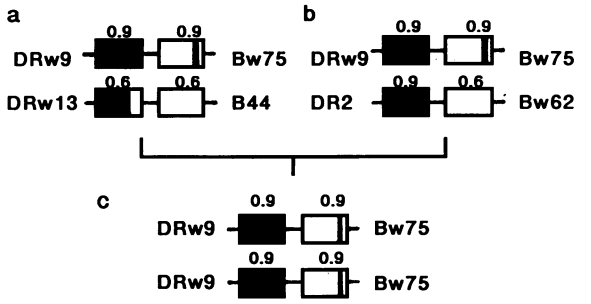

Figure 4 Model of the structure of both CYP21 genes in the family of the patient. Data are from the father (a), the mother (b), and the patient (c). Their HLA haplotypes are also shown. Blackened and open rectangles represent the standard CYP21A and CYP21B genes, respectively. *PstI* fragment sizes of 0.9 and 0.6 kb are indicated on the rectangles.

and CYP21B genes are located adjacent to C4A and C4B genes, respectively, analyses of the C4 genes were also performed. DNA samples from LCLs homozygous for specific haplotypes of caucasoid origin were also examined: WT49 (HLA-B17-DR3), HHK (HLA-B7-DR13), MANN (HLA-B44-DR7), PMGO75 (HLA-B14-DR1), and CD541265 (HLA-B8-DR3). HLA-B44-DR7 (fig. 5, lane 10), HLA-B14-DR1 (fig. 5, lane 15), and HLA-B8-DR3 (fig. 5, lane 16) haplotypes are known to be associated with specific organization of C4 and CYP21 genes, as shown in figure 5 (White et al. 1984; Carroll et al. 1985*b*; Garlepp et al. 1986). When *TaqI* RFLP analyses were carried out using the pC21/3c and the 5′portion of pAT-A (see Material and Methods) as probes, the C4A gene represented a 7-kb fragment, whereas the three fragments (6.4 kb, 6.0 kb, and 5.4 kb) represented different alleles of the C4B gene (fig. 5*a*). The 6.4-kb fragment was generated from a deletion of the C4A gene in the case of HLA-B8-DR3 haplotype (Schneider et al. 1986). The 6.0-kb (C4B long) and 5.4-kb (C4B short) fragments corresponded to the two alleles of C4B as described by Schneider et al. (1986). The CYP21 genes were identified by the 3.2-kb (CYP21A) and 3.7-kb (CYP21B) fragments (White et al. 1984).

The intensities of both C4A:C4B and CYP21A:CYP21B were 1:1 in LCLs of Japanese-specific haplotypes, except for the LCL NAK. NAK, which is homozygous for the HLA-B7-DR1 haplotype, had a 1:2 ratio for the intensity of 3.7- and 3.2-kb *TaqI* fragments and had an increased intensity of the 6.0-kb fragment of the C4B long allele. These observations suggested that NAK carried an extra CYP21A gene and an extra C4B gene. Similar observation was reported in the HLA B14-

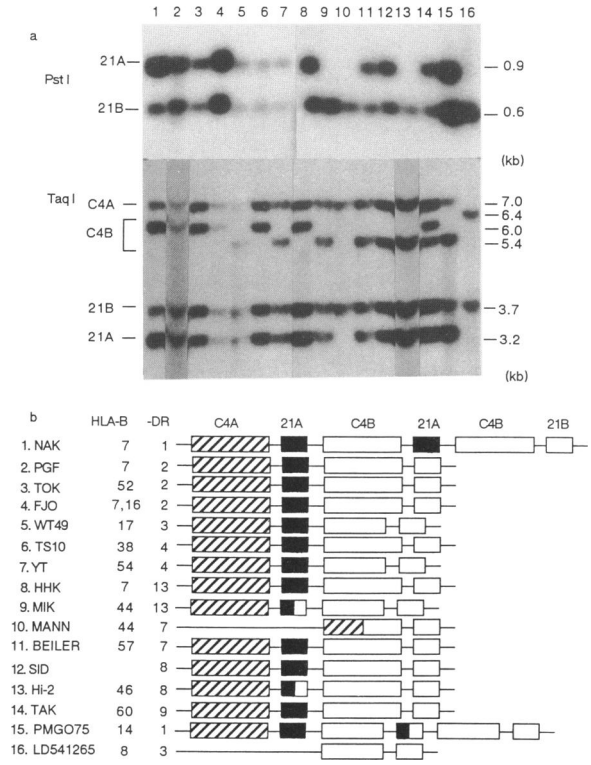

Figure 5 Gene organization of C4 and CYP21 in homozygous typing cells. The numbers 1–16 in panels *a* and *b* represent the LCLs homozygous for specific haplotypes, as follows: 1 = NAK (HLA-B7-DR1); 2 = PGF (HLA-B7-DR2); 3 = TOK (HLA-B52-DR2); 4 = FJO (HLA-B7/16-DR2); 5 = WT49 (HLA-B17-DR3); 6 = TS10 (HLA-B38-DR4); 7 = YT (HLA-B54-DR4); 8 = HHK (HLA-B7-DR13); 9 = MIK (HLA-44-DR13); 10 = MANN (HLA-B44-DR7); 11 = BEILER (HLA-B57-DR7); 12 = SID (HLA-DR8); 13 = Hi-2 (HLA-B46-DR8); 14 = TAK (HLA-B60-DR9); 15 = PGMGO745 (HLA-B14-DR1); 16 = LD541265 (HLA-B8-DR3). *a*, *PstI* and *TaqI* restriction-fragment patterns of C4 and CYP21 genes. *b*, Schema of C4 and CYP21 genes. Hatched, blackened, large open, and small open squares represent C4A, CYP21A, C4B, and CYP21B genes, respectively. Half-blackened/half-open squares represent gene-converted CYP21A genes. These organizations were drawn on the basis of the observation in fig. *5a*, except for MANN, for which the 3′-C4A-CYP21A-5′C4B genes are deleted (Schneider et al. 1986).

DR1 haplotype (fig. 5, lane 17) of caucasoid origin (Garlepp et al. 1986).

As shown in figure *5a*, no 0.9-kb *PstI* fragment was observed in MIK (HLA-B44-DRw13) (lane 9) or in Hi-2 (HLA-Bw46-DRw8) (lane 13). Because there was no obvious deletion of the CYP21 genes of these LCLs in the *TaqI* RFLP analysis, the presence of the *PstI* site in the eighth exon in the CYP21A gene suggested that these two cell lines had the converted CYP21A genes.

RFLP Analyses in Healthy Individuals in the Japanese Population

Because the converted CYP21A gene was thought to be linked to the HLA-B44-DRw13 haplotype and the HLA-Bw46-DRw8 haplotype in Japanese, *TaqI*, *BglII*, and *PstI* RFLP analyses were performed in 156 healthy unrelated individuals, using pC21/3c and *Pst*-0.6B as probes, to demonstrate a statistical association between an HLA antigen and the structure of the CYP21 genes. Of these 156, data on 16 individuals are shown in figure 6. Most of the individuals with HLA-B44-DRw13 and HLA-Bw46-DRw8 haplotypes have increased intensities of the 0.6-kb *PstI* fragments, while they have 3.7- and 3.2-kb *TaqI* fragments of equal intensity and have 12.5- and 10.5-kb *BglII* fragments of equal intensity (fig. 6, lanes 4–9). Linkage disequilibria of the increased intensity of the 0.6-kb band were observed with HLA-B44 (t = 2.96), with HLA-DRw13 (t = 2.68), and with HLA-Bw46 (t = 2.23). We have not done family studies to corroborate that these two haplotypes have the converted CYP21A genes; however, population studies suggest the presence of the converted CYP21A gene closely linked to specific HLA haplotypes. Most of the individuals with the HLA-B7-DR1 haplotype have increased intensities of the 3.2-kb *TaqI*, 0.9-kb *PstI*, and 12.5-kb *BglII* fragments (fig. 6, lanes 1–3). Significant linkage disequilibria of the increased intensity of the 0.9-kb *PstI* fragment with HLA-B7 (t = 3.66) and with HLA-DR1 (t = 4.22) were observed. These results suggested that the triplicated C4-CYP21 organization was associated with the HLA-B7-DR1 haplotype in the Japanese population.

Discussion

Gene conversion refers to a nonreciprocal recombination event in which a segment of one gene replaces the corresponding segment of a related gene (Baltimore 1981), and it was originally defined in fungi. A similar event has been proposed, from nucleotide sequences, for other species. In mammalian species, gene conversion has been shown to occur in cultured cells (Liskay and Stachelek 1983), and the occurrence of such homogenizing gene conversion-like events in the human β-globin genes (Michelson and Orkin 1983), human fetal γ-globin genes (Slightom et al. 1980), oxytocin-vasopressin genes (Ruppert et al. 1984), and immunoglobulin (Yamawaki-Kataoka et al. 1982) gene families has been suggested from comparisons of DNA sequences from the genes within multigene families.

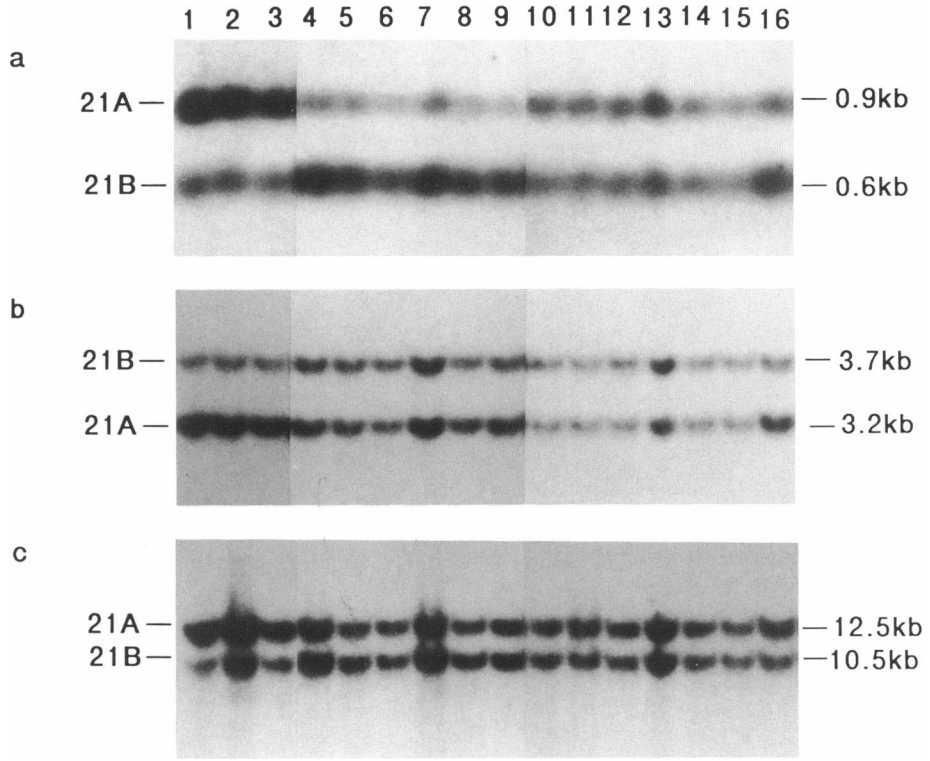

Figure 6 *Pst*I, *Taq*I, and *Bgl*II RFLPs of CYP21 genes in healthy Japanese individuals. Lane 1–16 represent the individuals with HLA-B-DR haplotypes, as follows: 1 = HLA-B7/- - DR1/-; 2 = HLA-B7/w62 -DR1/w9; 3 = HLA-B7/w59 -DR1/4.1; 4 = HLA-B44/w61 -DR2/w13; 5 = HLA-Bw51/44 -DR5/w13; 6 = HLA-B44/w67 -DRw8.1/w13; 7 = HLA-Bw46/- -DR4/w8; 8 = HLA-Bw46/w59 -DRw8/9; 9 = HLA-Bw46/51 -DR4/w8; 10 = HLA-Bw52/w62 -DR2/4; 11 = HLA-Bw61/TS1 -DR4/w9; 12 = HLA-Bw52/w60 -DR2/w9; 13 = HLA-B35/- -DR4.2/-; 14 = HLA-Bw52/37 -DR2/w10; 15 = HLA-B35/w48 -DR4/w14; 16 = HLA-B51/- - DR4.1/-. *Pst*I, *Taq*I, and *Bgl*II restriction-fragment patterns are shown in panels *a*, *b*, and *c*, respectively.

A single C4 gene and a single CYP21 gene carried on about 30 kb of DNA were thought to be duplicated before mammalian radiation, resulting in the present arrangement of two CYP21 genes alternating with two C4 genes (Carroll et al. 1985*a*; White et al. 1985). Duplicated CYP21 genes then were thought to diverge into a functional CYP21B gene and a pseudogene (CYP21A) in humans because of the impropriety of the existence of two functional CYP21 genes (Harada et al. 1987*a*). There is still a 98% identity, in base sequence, between the two CYP21 genes. It is, therefore, possible to assume that gene conversion has taken place between the CYP21A gene and the CYP21B gene, to maintain this great degree of homology. Consequently, if any mutations found in the CYP21A gene would be introduced into the CYP21B gene by gene conversion, both would become inactive and result in 21-hydroxylase deficiency. Donohoue et al. (1986) first proposed gene conversion as a possible cause of 21-hydroxylase deficiency. We have demonstrated that a gene conversion-like event could explain, from a partial nucleotide sequencing of a mutant CYP21B gene (Harada et al. 1987*a*), the molecular mechanism of 21-hydroxylase deficiency.

In the present work, we have sequenced completely the CYP21B gene from another patient with 21-hydroxylase deficiency. The CYP21B gene was indistinguishable from the normal one by conventional *Taq*I RFLP in the patient. We found the two nucleotide changes (C→T) in the eighth exon, one of which leads a premature termination codon. Because these changes are normally present in the CYP21A pseudogene, it might be transferred to the CYP21B gene by a gene conversion-like event. The exact area of gene conversion was not determined because of extremely high sequence homology between CYP21A and CYP21B genes; however, a region of more than 114 bp was presumed to be converted in the mutant CYP21B gene (fig. 2 and table 1). Recently the mutation at the same position in the eighth exon has been reported by other workers (Globerman et al. 1988). They reported the mutation generating a

premature termination codon at 318; the mutation corresponded to that at codon 319 in our sequence. The difference in codon number was due to one leucine insertion at codon 6 in the mutant CYP21B gene analyzed here. This insertion was thought to be a polymorphism of P450c21 because it was also found in the normal CYP21B gene (Rodrigues et al. 1987). Although the critical changes were the same in their case and in ours, they found only one nucleotide change in the eighth exon in their case – and therefore the possibility remained that the change in their case could be a point mutation rather than an actual gene conversion. On the other hand, we found in the eighth exon two nucleotide changes which suggested strongly a gene conversion in the CYP21B gene. Globerman et al. also demonstrated by oligonucleotide probe that three other patients had the same mutation at the position. Thus, the area of gene conversion in some of their patients might be the same as that in ours. At any rate, it is presumed that the gene conversion event in the eighth exon caused the disease in some patients. Morel et al. (1989) sorted 116 patients' alleles into five easily distinguished haplotypes based on blots of DNA digested with *Taq*I and *Bgl*II. Among five haplotypes, haplotype I (76/116 = 65.6%) was indistinguishable from normal and was thought to bear very small lesions, presumably point mutations. Haplotype IV (13/116 = 11.2%) may represent a gene conversion. The mutant CYP21B which we have sequenced belongs to haplotype I; however, it contained a mutation caused by a gene conversion-like event. Therefore it is probable that haplotype I includes mutations by gene conversion-like events. In addition, other examples of gene conversions causing 21-hydroxylase deficiency have been demonstrated by sequence analyses (Amor et al. 1988; Higashi et al. 1988*b*; Speiser et al. 1988). It is thus becoming convinced that a majority of the disease is caused by gene conversions, and it is assumed that the appreciable frequency of gene conversion can explain the relatively high frequency of the deleterious CYP21 gene in the human population.

The converted CYP21A gene was found to be associated with the HLA-B44-DRw13 and HLA-Bw46-DRw8 haplotypes in Japanese. But there was not a tight linkage between the specific HLA haplotype and the converted CYP21A gene, because converted CYP21A genes were also observed in individuals with other haplotypes. Higashi et al. (1988*a*) also indicated by using oligonucleotide probes that four of eight healthy individuals had a recombinant CYP21A gene presumably generated from a gene conversion or a crossing-over. As Miller (1988) has pointed out, we are now in the situation that "normal" (wild-type) loci cannot be easily defined. More extensive studies on these loci will be necessary to define an actual wild type in a large population.

Sequence analysis of the adjoining C4 genes has suggested that gene conversion-like events could occur between C4B and C4A genes (Yu and Campbell 1987). Therefore the gene conversion mechanism seems to serve for increasing the polymorphisms of C4, as well as those of HLA class I and II antigens (Evans et al. 1982; Mellor et al. 1983; Seemann et al. 1986). Thus, gene conversion events seem to occur rather frequently in the HLA region, a fact which may influence structures and the functions of the genes within the region.

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