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MINIREVIEW

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The chimeric *CYP21P/CYP21* gene and 21-hydroxylase deficiency

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Abstract The chimeric *CYP21P*/*CYP21* gene is a consequence of a 26- or 32-kb deletion in the *C4*-*CYP21* repeat module of *CYP21P*, tenascin A (*XA*), serine/threonine nuclear protein kinase (*RP2*), and the *C4B* and *CYP21* genes in congenital adrenal hyperplasia (CAH) with steroid 21-hydroxylase deficiency. To date, there have been three distinct chimeras found in CAH patients in ethnic Chinese. Initiation for production of these molecules is proposed to be *chi*-like sequences and a minisatellite consensus existing in several noncoding regions in *CYP21* genes. These molecules have the 5' end of the *CYP21P*-specific sequence in common but differ in the 3' end of *CYP21*-specific genes. In addition, there appears to be a 3.2-kb fragment generated by *Taq* I digestion, which leads to allele dropout in PCR amplification for detecting the aberrant splicing site of the IVS2 −12A/C > G mutation at nucleotide (nt) 655 in the *CYP21* gene. Therefore, the chimeric *CYP21P*/*CYP21* cannot be detected by conventional methods. It has been demonstrated that a PCR product amplified with allele-specific primers covering *tenascin* B (*TNXB*) to the 5' end of the *CYP21* gene combined with Southern analysis by *Ase* I and *Nde* I digestion may be used for identifying the chimera in the *CYP21* gene.

Keywords Recombination · Deletion · *Chi*-like sequence · Minisatellite consensus sequence · Chimera · RCCX module · C4-CYP21 repeat module · *CYP21* · CAH

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Introduction

Congenital adrenal hyperplasia (CAH) is a common autosomal-recessive disorder caused mainly by defects in the steroid 21-hydroxylase (*CYP21*) gene. More than 90% of CAH cases are caused by mutation of the *CYP21* gene (White and Speiser 2000). To date, 72 different *CYP21* mutations have been reported (Human Gene Mutation Database 2003), among which 57 are spontaneous mutations. The remaining 15 mutations (Lee 2001) are believed to be products of intergenic recombinations of DNA sequences between the *CYP21* gene and the highly homologous *CYP21P* pseudogene. These mutations can be identified by allele-specific oligonucleotide (ASO) (Speiser et al. 1994) and reverse dot-blot hybridizations (Yang et al. 2001), direct DNA sequencing (Tajima et al. 1993), PCR/ligase (Day et al. 1995), and the amplification-created restriction site (ACRS) method (Lee et al. 1996). For some unknown defective loci somewhere within the *CYP21* gene, SSCP (Tajima et al. 1993; Lee et al. 1998) and denatured gradient gel electrophoresis (DGGE) (Ohlsson et al. 1999) may be applied to possibly find novel mutations in the *CYP21* gene. However, all these applications for mutational detection should be carried out using the functional *CYP21* gene. Therefore, the primary PCR product of the *CYP21* gene has to be prepared by differential PCR amplification to eliminate any *CYP21P* contamination prior to mutational analysis. Most primary PCR products are derived by multiple allele-specific primer sets to generate the *CYP21* gene in two PCR fragments using two-to-three-step PCR amplification (Day et al. 1996; Ordonez-Sanchez et al. 1998). For convenience and for amplification efficiency, a single, complete *CYP21*-specific amplification performed in a single reaction mixture is used (Lee et al. 1996). This PCR product contains the entire *CYP21* gene, which can be cloned and characterized by an expression analysis (Lee and Chang 2001).

Identification of the 30-kb gross gene deletion (White et al. 1984) encompassing the *C4A* and *CYP21* genes in the

C4-CYP21 repeat module was controversial in the past (Miller 1988; White et al. 1988; Morel et al. 1989), even though the deletion had been demonstrated by pulse field electrophoresis (Collier et al. 1989). The ambiguity was mainly caused by the lack of detailed information on the extent of gene conversion or deletion in the C4-CYP21 repeat module or RCCX module (Shen et al. 1994) in chromosome 6p21.3. This module includes *CYP21P, XA* (Gitelman et al. 1992), the serine/threonine nuclear protein kinase *RP* (Yang et al. 1998), *C4*, *CYP21*, and *tenascin* B (*TNXB*) (Bristow et al. 1993) genes (Fig. 1A). The C4 protein is coded by two genes, *C4A* and *C4B*. Long gene (20.4 kb) or short gene (14.1 kb) of the *C4* is due to the presence of an endogenous retrovirus sequence (6.7 kb), HERV-K (C4), in intron 9 (Yu 1991). *TNXB*, in the downstream *CYP21* gene, is partially duplicated in the downstream *CYP21P* gene, where it is termed *XA* gene (*TNXA*). Both *XA* and *TNXB* are transcribed on the opposite strand. The *RP* gene contains two duplicated genes, *RP*1 and *RP*2. The *RP*2 gene is truncated and corresponding to *RP*1 adjacent to *TNXA* (Yang et al. 1999). These tandemly arranged genes, *CYP21P-XA-RP2-C4B-CYP21-TNXB*, are designated as the RCCX module. At present, such a deletion is considered to result from an unequal crossover in meiosis occurring in 20% of alleles in most populations (White and Speiser 2000). However, the population frequency is dependent on the population studied (White and Speiser 2000). Three reports (Levo and Partanen 1997; Koppens et al. 2000; L'Allemand et al. 2000) pointed out that such a gross 30-kb deletion consisted of a fused *CYP21* gene, with its 5' and 3' ends corresponding to *CYP21P* and *CYP21* respectively, and the product appearing as a 3.2-kb *Taq* I fragment in Southern blot analysis. In a recent study (Lee et al. 2003a), it demonstrated that such a 30-kb gene deletion in fact is a chimeric *CYP21P*/*CYP21* formation caused by multiple gene deletions, including *XA*, *RP2*, and *C4B*, and between unequal parts of the *CYP21P* and *CYP21* genes in the C4-CYP21 repeat module (Fig. 1A). Such a gene deletion or gene conversion is traditionally detected by Southern blotting with multiple isotope-labeled probes and RFLP analysis; *Taq* I generates the 3.7-kb (functional) and 3.2-kb (pseudogene) fragments, while *Bgl* II produces the 11-kb (functional) and 12-kb (pseudogene) fragments. These two approaches to fragment analysis have been used since 1984 (White et al. 1984; Donohoue et al. 1989; Koppens et al. 2000; L'Allemand et al. 2000). However, the method is laborious and indirect, and densitometric screening of fragments is error prone, although a nonisotopic Southern procedure was later described (Krone et al. 1998).

Causes of the formation of the chimeric *CYP21P/CYP21* gene

Basically, the chimera features fused genes. In the case of P450c21, chimeric *CYP21P*/*CYP21* is formed by recombination of the duplicated *CYP21P* and *CYP21* genes, but the cause of formation is unclear. However, several possibilities have been proposed:

Proximity to the human major histocompatibility complex (MHC)

In order to increase diversity of transplantation antigens and to maximize versatility of immune responses in humans, there is a high recombination rate in the MHC of the HLA complex. Both the *CYP21P* and *CYP21* genes are on chromosome 6p21.3, adjacent to and alternating with the *C4A* and *C4B* genes encoding the fourth components of the serum complement (White et al. 1984), located within the HLA complex. As a result, the frequency of recombination events between these two genes is relatively high in human populations.

▶

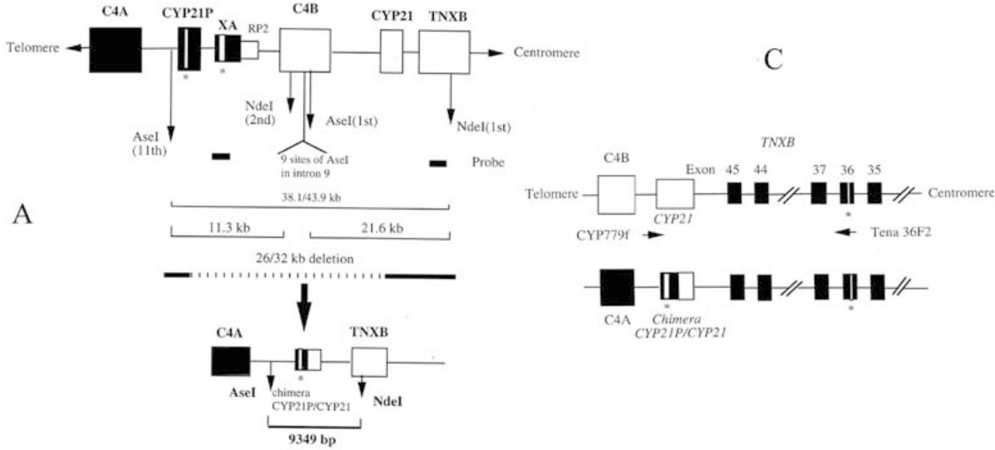
Fig. 1 A Strategy for identification of the chimeric *CYP21P*/*CYP21* in the RCCX module of the human histocompatibility (MHC) class III gene. The structure of the normal gene is shown in the *white box*; the *black box* represents a nonfunctional gene (*CYP21P* and *XA*) or a different functional gene (*C4A*). The deleted region of the RCCX module is indicated by a *solid line*, which represents a 26- or 32-kb deletion as described in the text published previously (Lee et al. 2003a). The chimeric *CYP21P*/*CYP21* is indicated by a *half-white, half-black box*. A 120-bp deletion in exon 36 of the *TNXA* gene and 707–714delGAGACTAC in the *CYP21P* gene are marked with an *asterisk (*)*. *Vertical arrows* show the location of the restriction endonuclease cleavages. Deletion of the *XA*, *RP2*, and *C4B* genes led to the formation of a chimeric *CYP21P*/*CYP21* gene in a 9.3-kb fragment digested by *AseI* and *NdeI* endonucleases by Southern analysis, which was described previously (Lee et al. 2003a). **B** Restriction analysis of the amplification-created restriction site (ACRS) amplification product (Lee et al. 1996) and the map for detection of mutational loci in three CAH carriers with distinct chimeric *CYP21P*/*CYP21*. The three distinct chimeras identified were designated *CH-1*, *CH-2*, and *CH-3*. ACRS analysis was carried out on 2.5% Metaphor (FMC Bioproducts, USA). The ACRS primers used for the detection of P30L (*a*), IVS2 −12A/C > G (*b1*), I172 N (*c*), I236 N (*d*), V281L (*g*), Q318X (*j1*), and R356 W (*j2*) have been described previously (Lee et al. 1996; 2002). On the gel, *lanes 2, 6, 10, 14, 18, 22, and 25* were a carrier with *CH*-1. *Lanes 3, 7, 11, 15, 19, 23, and 26* were a carrier with *CH*-2. *Lanes 4, 8, 12, 16, 20, 24, and 27* were a carrier with *CH*-3. Each amplification product was either untreated ("–" *lanes*) or treated ("+") with an appropriate restriction enzyme (*RE*) (Lee et al. 1996). The marker used was a 100-bp molecular ladder (*lane "mk"*). The schematic representation is a result of three identified chimeras detected by the ACRS method. Exons for the *CYP21P* and *CYP21* genes are shown as *solid blocks* and *open boxes* respectively. The *vertical arrow* indicates the position of the locus corresponding to the 2.5% metaphor analysis. A *horizontal arrow* indicates the position of the *chi*-like sequence and the minisatellite consensus. **C** A schematic diagram of the strategy for PCR amplification of a 6.2-kb fragment by allele-specific primers (CYP779f/Tena 36F2) encompassing the *TNXB* gene to the *CYP21* gene (Lee et al. 2003a, 2003b). The structure of the *CYP21* and C4B genes is shown by a *white box*, while the *black box* represents the *C4A* gene and exons of the *TNXB* gene. The chimeric *CYP21P*/*CYP21* gene is indicated as described in *A*. *Horizontal arrows* represent the direction and location of the primers CYP779f and Tena36F2. Primer Tena36F2 is located in exon 36 of the *TNXB* gene in which a 120-bp fragment deleted in *TNXA* is marked by an *asterisk (*)*

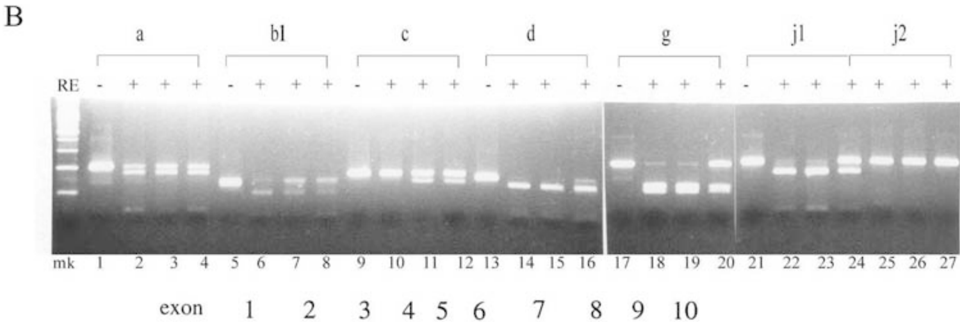
Homologous recombination

Because the exon sequence of the *CYP21P* pseudogene shares 98% nucleotide sequence homology with functional *CYP21* (Higashi et al. 1986; White et al. 1986), homologous recombination of misalignment may occur at meiosis (Tusie-Luna and White 1995). It has been noted that the region between intron 2 and the 3' end of exon 3 in *CYP21* is considered a hotspot for recombinations and microconversions (Tusie-Luna and White 1995).

Initiation by the existence of a *chi*-like sequence and tandem-repetitive minisatellite consensus

Sequences such as *chi* (GCTGGTGG; Smith et al. 1981) and the tandem-repetitive minisatellite consensus





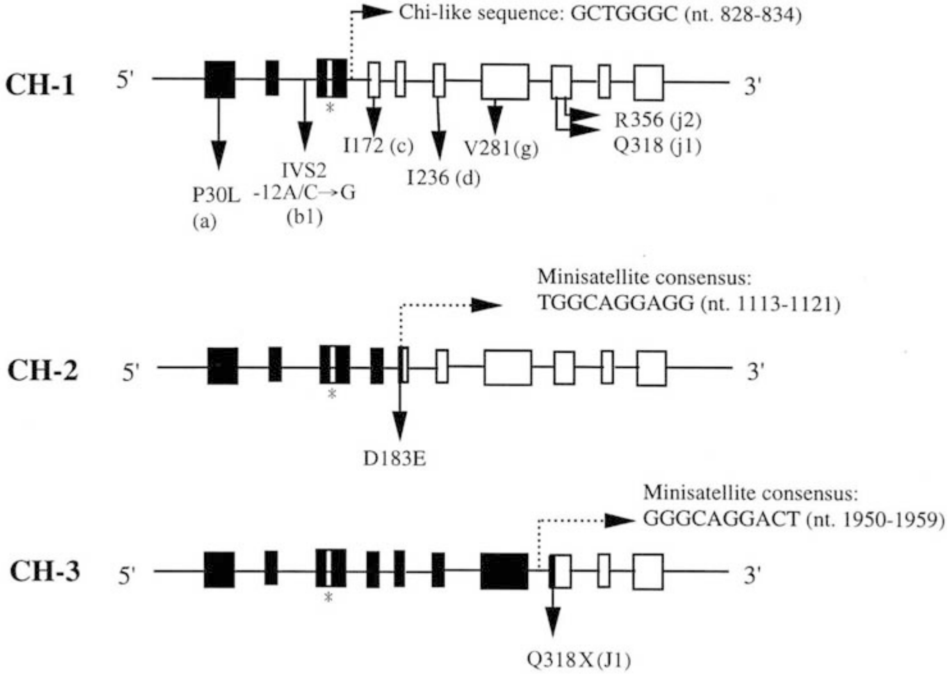


Table 1 Distribution of the *chi*-like sequence and minisatellite consensus in the *CYP21P* and *CYP21* genes

Initiator for the chimera	*CYP21P* and *CYP21*	
	Location	Sequence (nt)[a]
Chi sequence: GCTGGTGG		
Chi-like sequences		
GCTGGGGC	5' end	−54 to −48
GCTGGGGG	IVS2	393–400
GCTGGTCT	IVS2	461–468
GCTGGAGG	IVS2	649–642
GCTGGGCT	IVS3	828–835
GCTGGGGG	IVS4	1062–1055
GCTGGGGT	IVS5	1264–1271
GCTGTGGG	IVS6	1449–1441
GCTGGAGG	IVS7	1921–1928
GCTGCTGG	IVS7	1943–1950
GCTGGGTG	3' end	2830–2838
Minisatellite consensus:		
GGGCAGGAXG		
TGGCAGGACG	IVS4	1113–1121
GGGCAGGACT	IVS7	1950–1959
GGGCAGGACC	3' end	2710–2719

[a] Based on Higashi et al. (1986)

(GGGCAGGAXG; Jeffreys et al. 1985) are important for genetic recombination in eukaryotic cells. These sequences may be recognition sites for the recombinase complex responsible for gene recombination. It has been pointed out that the *chi*-like sequence GCTGGGG is present several times in the *CYP21P* and *CYP21* gene (Table 1). Most of them appear in intron sequence. Two distinct areas of nucleotides (nt), 470–999 and 1375–1993 (Higashi et al. 1986), of *CYP21* genes have been proposed to be related to crossover regions (Donohoue et al. 1989). On the other hand, there are three minisatellite consensus sequences located in the *CYP21* gene at nt 1113–1121 (IVS4), 1950–1959 (IVS7), and the 3' untranslated region from nt 2710–2719 (Urabe et al. 1990) (Table 1). One report suggested unequal crossover in exon 7 sequences (Chu et al. 1992).

Types of chimeric *CYP21P/CYP21*

There have been various studies (White et al. 1988; Sinnott et al. 1990; Levo and Partanen 1997; Koppens et al. 2000) indicating hybrid genes with the 30-kb deletion between *CYP21P* and *CYP21* genes in Caucasians. Several studies (Lee et al. 2002; Lee et al. 2003a) have shown that there are three distinct chimeric *CYP21P*/*CYP21*s in the *CYP21* gene in the ethnic Chinese population in Taiwan. These three molecules are designated *CH-1*, *CH-2,* and *CH-3* (Fig. 1B) (Table 2). Results indicated that *CH-1* has an identical sequence to the *CYP21P* gene from exons 1–3 without the mutation at I172 N (nt 999) (Fig. 1B) (Table 2), *CH-2* is identical to the *CYP21P* gene from exons 1–5 of D183E (nt 1117), and *CH-3* is identical in sequence to the *CYP21P* gene from exons 1–8 of Q318X (nt 1994) without the R356 W mutation (Fig. 1B) (Table 2) (Lee et al. 2002; Lee et al. 2003a). In addition to these, the three chimeric *CYP21P*/*CYP21*s possess identical 5' ends of the *CYP21P* gene (Table 2) (Lee et al. 2002; Lee et al. 2003a). Obviously, structural differences of the three chimeras show that these molecules are composed of unequal sequences between the *CYP21P* and *CYP21* genes leading to the production of a variety of structural arrangements.

Properties of the chimeric *CYP21P/CYP21* gene

Allele dropout in PCR amplification for detecting the IVS2 −12A/C > G mutation

As previously reported (Day et al. 1996), the allele dropout that occurs in the detection of the IVS2 −12A/C > G mutation at nt 655 (Higashi et al 1986) is due to preferential amplification of DNA segments and is an artifact caused by polymerase (Schulze et al. 1998). This leads to an excessively high frequency of apparent homozygosity and genotyping discordance between parents and probands. A previous study (Lee et al. 2000) pointed out that allele dropout was associated with unequal amplification in the presence of the chimeric *CYP21P*/*CYP21*, which lacks a specific primer for amplification during analysis of the mutation IVS2 −12A/C > G. In addition, a recent study (Lee et al. 2003b) found that a haplotype of the *CYP21* gene with the mutations of IVS2 −12A/C > G and 707–714delGAGACTAC (Higashi et al. 1986) is a case of gene deletion including the *CYP21P*, *XA*, *RP2*, and *C4B* genes in the C4-CYP21 repeat module. This finding further confirms that allele PCR dropout in previous PCR analyses was in fact caused by the presence of the 5' end region of the *CYP21P* sequence, which is consistent with our previous suggestion (Lee et al. 2000). Since an antisense primer anchored at nt 707–714 of *CYP21* has been used in most primary PCR amplifications (Tajima et al. 1993; Day et al. 1996; Koppens et al. 2000) to eliminate *CYP21P* contamination, it may have led to PCR dropout in detecting *CYP21* mutations of IVS2–12A/C > G combined with707–714delGAGACTAC and the chimeric *CYP21P*/*CYP21*.

Production of a 3.2-kb fragment by *Taq* I digestion

Since three chimeric *CYP21P*/*CYP21* genes contain an identical sequence in the 5' end of *CYP21P* (Table 2) resulting in a frameshift of the sequence upstream of *CYP21P*, each of the chimeras has the base T at nt −209 replaced by C (Higashi et al. 1986) (Table 2), producing a *Taq* I restriction site in these defective *CYP21* alleles and subsequently leading to a 3.2-kb *Taq* I fragment (Fig. 2A). Therefore, the assumption that the 3.2-kb fragment from conventional Southern analysis is a deletion product of the *CYP21* gene is incorrect.

Table 2 Sequence analysis of the three chimeric *CYP21P*/*CYP21* genes from ethnic Chinese congenital adrenal hyperplasia (CAH) patients. *nt* nucleotide, *aa* amino acid, *Del* deletion

Sequence[a] (nt/aa) of	*CYP21*	*CYP21P*	Types of chimeric gene		
			CH-1	*CH-2*	*CH-3*
nt −306	G	C	C	C	C
−294/−293	T/A	C/C	C/C	C/C	C/C
−282	A	G	G	G	G
−209	T	C	C	C	C
−198	C	T	T	T	T
−188/−189	-	+T	+T	+T	+T
−126	C	T	T	T	T
−113	G	A	A	A	A
−110	T	C	C	C	C
−103	A	G	G	G	G
−4	C	T	T	T	T
P10	P	L	L	L	L
P30	P	L	L	L	L
L39	L(TTG)	L (CTG)	L(CTG)	L(CTG)	L(CTG)
P45	P(CCA)	P(CCC)	P(CCC)	P(CCC)	P(CCC)
nt 395	T	C	C	T	C
431/432	-	+TGTT	+TGTT	+TGTT	+TGTT
442	T	G	G	G	G
470	A	G	G	G	G
478	A	G	G	G	G
486	A	G	G	G	G
502	A	G	A	G	G
513	T	G	G	G	G
524	T	C	C	C	C
530	A	T	T	T	T
540/541	-	+TCC	+TCC	+TCC	+TCC
546	C	A	A	A	A
572–574	GGT	TCA	TCA	TCA	TCA
577	G	A	A	A	A
580/581	G/A	A/G	A/G	A/G	A/G
589	G	T	T	T	T
594	G	A	A	A	A
601	C	A	A	A	A
620	A	G	G	G	G
624	G	T	T	T	T
629/630	C/A	G/G	G/G	G/G	G/G
620	A	G	G	G	G
655	A/C	G	G	G	G
S108	S(TCC)	S(TCG)	S(TCG)	S(TCG)	S(TCG)
G110	G(GGA)	G110delGA	G110delGA	G110delGA	G110delGA
D111	D	Del	Del	Del	Del
Y112	Y	Del	Del	Del	Del
S113	S(TCC)	S(TCT)	S(TCT)	S(TCT)	S(TCT)
nt 860	C	C	T[b]	C	C
886–890	CCGCT	CCGCT	CCCGCT[c]	CCCGCT	CCCGCT
I172	I	N	I	N	N
nt 1104	C	A	C	C	A
1111	T	C	T	C	C
D183	D(GAC)	E(GAG)	D(GAC)	E(GAG)[d]	GAG
D234	D(GAT)	D(GAC)	D(GAT)	D(GAT)	GAC
I236	I	N	I	I	N
V237	V	E	V	V	E
M239	M	K	M	M	K
nt 1420/1421	AC	GT	AC	AC	GT
L248	L(CTC)	L(CTG)	CTC	CTC	CTG
V281	V	L	V	V	L
F306–L307	-	insT	-	-	insT
nt 1789	G	C	G	G	C
Q318X	Q(CAG)	X(TAG)	Q	Q	X[e]
R356W	R	W	W	W	W

[a] Based on Higashi et al. (1986) [b] T base and [c] C insertion are present in the normal *CYP21* gene as reported by White et al. (1986) [d] C–G and [e] C–T base changes may be the breakpoint sites for *CH-2* and *CH-3* respectively

Production of a 9.3-kb fragment resulting from deletion of the *TNXA, RP2,* and *C4B* genes

In the traditional Southern blot analysis, *Taq* I generates a 3.7-kb (functional) and a 3.2-kb (pseudogene) fragment, and *Bgl* II produces an 11-kb (functional) and a 12-kb (pseudogene) fragment, which cannot indicate detailed information on the extent of gene conversion or deletion in the RCCX module. Therefore, a study (Lee et al. 2003a) using two restriction endonucleases, *Ase* I and *Nde* I, with a single probe was used to identify the interchange region. Results revealed that deletion of the *TNXA, RP2,* and *C4B* genes (Fig. 1A) leads to formation of the chimeric *CYP21P/CYP21* in a 9.3-kb fragment (Fig. 2B, lane M). This improves the detection protocol for gene deletions and conversions in the RCCX module (Fig. 1A). Given the potential for variations and a lack of information about the *C4B* gene (Yu 1991) in Chinese populations, the size of the RCCX modular deletion may either be 26 or 32 kb long (Fig. 1A) (Lee et al. 2003a).

Biological activity of the chimeric *CYP21P/CYP21*

Because the 5' end of the three different chimeric *CYP21P/CYP21* genes have identical sequences with that of *CYP21P* (Table 2), nucleotide substitutions at these sites from *CYP21P*- to *CYP21*-specific sequences at nt −103, −110, and −123 (Higashi et al. 1986) cause a five-fold decrease in transcriptional activity and in the ability of the promoter to bind with the Sp1 protein (Chang and Chung 1995). At nt 655 (Higashi et al. 1986)

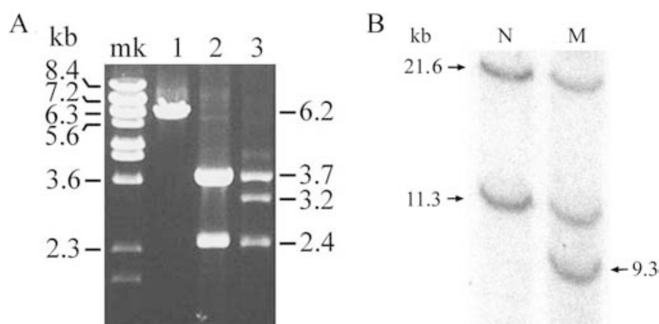


Fig. 2 A Analysis of the 6.2-kb PCR product by *Taq* I digestion on a 0.65% agarose gel. *Lane 1*: the 6.2-kb PCR product amplified with paired primers Tena36F2/CYP779f from a normal individual. *Lanes 2 and 3* are the 6.2-kb PCR product digested by *Taq* I from a normal individual and a congenital adrenal hyperplasia (CAH) patient with *CH-1* chimera in one of the chromosomes respectively. *"mk"* was a Lambda DNA-BstE II-digested molecular marker (New England BioLabs, Beverly, MA, USA). **B** Southern blot analysis of *Ase* I and *Nde* I digestion of genomic DNA. The hybridization probe used consisted of the 2271-bp PCR product derived using the paired primers Tena36F2/Tena43R (Lee et al. 2003a). A normal individual (*lane N*) had two fragments of 11.3 and 21.6 kb, and one patient with a *CH-1* chimera in one of the chromosomes (*lane M*) had an additional 9.3-kb fragment as well as having the 11.3- and 21.6-kb fragments

of the IVS2 -12A/C > G mutation, the aberrant splicing site results in very low but measurable enzyme levels (Higashi et al. 1988a). In addition, deletion of 707–714delGAGACTAC leads to a frameshift mutation, which forms a TGA stop codon downstream at nt 830 (Higashi et al. 1986) and produces a truncated protein in translation. Taken together, these three kinds of chimeric *CYP21P/CYP21* have no steroid 21-hydroxylase activity. However, the hybrid gene reported by the study (L'Allemand et al. 2000) without these two mutations had low 21-hydroxylase activity with a defect in P30L.

Identification of the chimeric *CYP21P/CYP21* gene

Identification of gene deletions and conversions is still being studied by Southern blot analysis with oligonucleotides (Higashi et al 1988b; Donohoue et al. 1989; Helmberg et al. 1992; Tusie-Luna and White 1995) or DNA probes (Krone et al. 1998; Koppens et al. 2000; L'Allemand et al. 2000) for hybridization. There are two established strategies of PCR amplification for identifying the *CYP21* gene:

1. Mixed-primer amplification of the 3.5-kb PCR product: To collect the chimeric *CYP21P/CYP21*, three primers (Table 3) containing a 3'-specific primer for the *CYP21* gene and two different 5'-primers for both *CYP21* and *CYP21P* used to amplify the normal *CYP21*, and the chimera have been successfully used for CAH diagnosis (Lee et al. 2000; 2002). The 3.5-kb fragments (Lee et al. 2000; 2002) covering sequences of the *CYP21* gene from −313 to 3170 (Higashi et al. 1986) were generated. The PCR products were analyzed using the ACRS primer for mutational analysis (Fig. 1B) (Lee et al. 1996).
2. The 6.2-kb PCR product encompassing the *TNXB* gene to the 3'-end of the *CYP21* gene: In order to understand the status of the *CYP21* gene within the RCCX module after gene deletion or conversion, a strategy of using a 6.2-kb PCR product amplified with locus-specific primers from the *tenascin* (*TNXB*) gene to the 5' end of *CYP21P* or *CYP21* has been successfully achieved (Figs. 1C, 2A) (Lee et al. 2003a,2003b). The sense primer located in *tenascin* B (*TNXB*) containing a 120-bp sequence deleted from *tenascin* A (*TNXA*) and an antisense primer in the 5' end of the *CYP21P* and *CYP21* genes were used (Table 3) (Fig. 1C). The 6.2-kb PCR product was further identified either by the ACRS primer to detect *CYP21* mutations (Fig. 1B) (Lee et al.1996) or by *Taq* I digestion directly analyzed on an agarose gel to evaluate the 3.2- and 3.7-kb fragments (Fig. 2A).

Conclusions

Questions regarding gross gene deletion of 30 kb encompassing the *C4A* and *CYP21* genes have arisen

Table 3 Primers for the amplification of chimeric *CYP21P/CYP21* gene

Primer	Sequence (5' > 3')	Location	Specificity
Mixed primers for 3.5-kb PCR product amplification[a]			
21BF1	CCCAGGTGGGGGCGGACACTA	−314 to −294[b]	*CYP21*
21AF1	CCCAGGTCGGGGCGGACACCC	−315 to −295	*CYP21P*
21BR	AATTAAGCCTCAATCCTCTGCAGCG	3177–3153	*CYP21*
Primers for 6.2-kb PCR product amplification[c]			
Tena 36F2	AGGCGCTCGCTATGAGGTGAC	81,255–81,275[d]	*TNXB*
CYP779f	AGGTGGGCTGTTTCCTTTCA	87,443–87,463	*CYP21P/CYP21*

[a] Protocol for the amplification of 3.5-kb PCR product was previously reported (Lee et al. 2000; Lee 2001) [b] Based on that published by Higashi et al. (1986) [c] Protocol for the amplification of the 6.2-kb (Tena36F2/CYP779f) PCR product was previously published (Lee et al. 2003b) [d] Based on GenBank accession no. AL049547

because there is no information on the deleted region in the RCCX module. The chimeric *CYP21P/CYP21* is a consequence of the deletion of the complete *XA*, *RP2*, and *C4B,* and partial *CYP21P/CYP21* genes of 26 or 32 kb, depending on whether *C4B* is a long or short gene. Therefore, presentation of a 3.2-kb fragment produced by *Taq* I digestion is not only a single *CYP21* deletion but there is also a chimera included. A previous strategy (Krone et al. 1998) using the *Taq* I restriction enzyme was applied to specifically disrupt the *CYP21P* gene for identification of the *CYP21* mutation, which may possibly cause a loss of amplification of the chimera. Results of a recent study (Lee et al. 2003b) indicated that the haplotype of the *CYP21* allele with the mutation IVS2–12A/C > G combined with 707–714delGAGACTAC is not a chimeric *CYP21P/CYP21* gene but does show a 3.2-kb fragment by *Taq* I digestion. Therefore, we believe that the diversity of haplotypes of the *CYP21* allele with the 3.2-kb fragment may frequently occur in CAH patients with steroid 21-hydroxylase deficiency.

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