Highly polymorphic *Xba*I RFLPs of the human 21-hydroxylase genes among Chinese

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Abstract

A highly polymorphic *Xba*I restriction site flanking the human 21-hydroxylase genes (21-OHA and 21-OHB) was found with the probe pC21/3c, the cDNA of the 21-hydroxylase gene. From the results of RFLP analysis of 10 subjects with congenital adrenal hyperplasia (CAH) owing to 21-hydroxylase deficiency and their parents, at least four polymorphic fragments (30 kb, 27 kb, 25 kb, and 15 kb) resulting from cleavage at the polymorphic endonuclease sites outside the genes were found, and at least 10 different polymorphic patterns were observed among Chinese. These results indicate that these polymorphic loci are very informative for prenatal diagnosis of 21-hydroxylase deficiency.

Material and methods

High molecular weight genomic DNA was prepared from 10 CAH patients and their parents. DNA (7 µg) was digested with restriction enzyme *Xba*I and restriction fragments were subjected to electrophoresis on 0.7% agarose gel, 1 v/cm, for 48 hours. The length of the fragments was estimated with molecular weight markers of lambda DNA digested with *Apa*I, *Nae*I, and *Xho*I. These DNA markers consist of the 38.5 kb, 33.5 kb, 28.5 kb, 20 kb, 15 kb, and 10 kb fragments. Blotting and hybridisation were carried out as reported elsewhere.⁷ The probe pC21/3c, provided by Professor White, contains an insert of about 2 kb, almost a full length cDNA encoding human 21-hydroxylase.⁸ The probe was labelled with ³²P-dATP by nick translation.

Results

Using DNA isolated from 10 CAH families, digested with *Xba*I, five individual *Xba*I hybridisation bands were found and each fragment was polymorphic. The length of these fragments was > 35 kb, ~ 30 kb, ~ 27 kb, ~ 25 kb, and 15 kb (figure). The frequencies of these fragments present in the parents were 13/20, 6/20, 13/20, 4/20, and 8/20. Because the high molecular weight of the > 35 kb fragment may represent underdigested DNA, this result was omitted from this study. Ten different polymorphic patterns were observed in the other four fragments (table 1).

Discussion

There are no *Xba*I sites in the region of either the 21-OHA gene or the 21-OHB gene. These polymorphic fragments of 30 kb, 27 kb, 25 kb, and 15 kb are obviously the result of cleavage at the *Xba*I site outside the 21-OH genes. In a preliminary report, Phillips *et al*⁹ described an *Xba*I RFLP and a lack of association between CAH and absent bands on Southern blotting, which may be the result of the high molecular weight of the *Xba*I RFLPs. Later, Matteson *et al*¹⁰ reported 28 kb, 24 kb, and 16 kb polymorphic fragments in the *Xba*I RFLP when hybridisation was carried out with the C4 probe pMC4/21. However, under careful electrophoretic conditions (1 v/cm, 48 hours) and with lambda DNA-*Apa*I, lambda DNA-*Nae*I, and lambda DNA-*Xho*I digest mixture as the molecular weight standard, the *Xba*I RFLPs we obtained are highly polymorphic. At least four polymorphic fragments (~ 30 kb, ~ 27 kb, ~ 25 kb, and 15 kb) were seen with frequencies ranging from 20% to 65%. Such a

Congenital adrenal hyperplasia (CAH), an autosomal recessive disorder that causes cortisol deficiency, is most commonly associated with 21-hydroxylase (21-OH) deficiency. There are two 21-OH genes (pseudogene 21-OHA and functional gene 21-OHB), each of which is located immediately adjacent to the 3' end of the two complement C4 genes in the class III region of the HLA complex.^{1,2} RFLPs have been found with *Eco*RI, *Hind*III, *Acc*I, *Nco*I, *Xba*I, and *Taq*I and one or two polymorphic fragments shown. The frequency of these polymorphisms is only about 20%.³⁻⁶ In this report, using *Xba*I, we found that among Chinese there are at least four polymorphic fragments resulting from cleavage at polymorphic sites outside the genes. These polymorphisms are very informative for genetic analysis.

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*Xba*I restriction fragments of 21-OH gene(s) in Chinese population (DNA fragment sizes (in kb) are marked on the left of the gel).

Table 1 *XbaI* polymorphic fragments of 10 CAH families.

No		Size (kb)				
		> 35*	30	27	25	15
1	P	+		+		
	F	+		+		+
	M	+				+
2	P	+	+			+
	F	+		+		
	M		+		+	
3	P	+	+		+	
	F	+		+	+	
	M	+	+			+
4	P		+	+		
	F		+			+
	M		+	+		
5	P	+		+		
	F	+		+		
	M	+	+	+		
6	P	+		+		
	F			+		+
	M	+		+		+
7	P	+			+	+
	F	+		+		+
	M	+			+	
8	P		+		+	
	F				+	
	M	+	+			
9	P		+	+		
	F	+		+		
	M		+	+		
10	P	+		+		+
	F	+		+		
	M			+		+
Frequency (F + M)		13/20 (65%)	6/20 (30%)	13/20 (65%)	4/20 (20%)	8/20 (40%)

P = patient, F = father, M = mother.
*Owing to underdigestion by *XbaI*, the > 35 kb fragment may be false positive.

significant difference in the *XbaI* RFLPs might be because *XbaI* fragments are larger than other restriction fragments, such as *EcoRI*, *HindIII*, *AccI*, and *NcoI*. These fragments might also happen to be contained in a highly polymorphic region.

Previous studies indicate that gene conversion occurs with high frequency between the 21-OHA and 21-OHB genes¹⁰⁻¹² and this may be responsible for the relatively high degree of *XbaI* polymorphism shown in our results. The highly polymorphic fragments are very informative for prenatal diagnosis of 21-hydroxylase deficiency, making them some of the best markers for the genetic study of this disease. In our study of the 10 CAH families, some fragments were observed in the father or mother but not in their offspring (the proband). These fragments must be linked to normal genes. If the fetal material in the next pregnancy does not have these fragments, the fetus would be affected. Accordingly, three families (1 to 3) are fully informative for prenatal diagnosis, and four families (4 to 7) are 50% informative. For instance, in family 2, the 27 kb and 25 kb fragments are present in both the father and the mother but not in the patient, so these two fragments must be linked to normal genes and can provide information from both the father and mother. There are two subjects (No 4-M and No 8-F) in these 10 CAH families who have only one *XbaI* fragment. No 4-M has the 27 kb fragment while No 8-F has the 25 kb fragment. No homozygous deletion of the 21-OHA or 21-OHB gene was found in these two subjects according to our previous study with *TaqI*,¹³ so we propose

Table 2 The relationship of *XbaI* fragments to the 21-hydroxylase genes.

	Fragments (kb)				
	> 35	30	27	25	15
21-OHA	Unknown	+	+	+	+
21-OHB	Unknown	+	+	+	Unknown

that the 27 kb and 25 kb fragments are associated with both the 21-OHA and 21-OHB genes. Also, according to the study with *TaqI*, no deletions or duplications were found in family 2; the patient inherited both the 21-OHA and 21-OHB genes from the mother. In this study, with *XbaI*, the patient inherited only the 30 kb fragment from the mother, so this fragment is also associated with both the 21-OHA and 21-OHB genes. In family 7, the 15 kb fragment of the patient presumably came from the father. In the study with *TaqI*, the father of this family is a deletion heterozygote for the 21-OHB gene and the patient inherited only the 21-OHA gene from the father. Therefore, we propose that the 15 kb fragment may contain the 21-OHA gene and may or may not contain the 21-OHB gene (table 2).

It should be noted that the > 35 kb band may be caused by underdigestion of the DNA in the present experimental conditions. Only part of the samples with this band was digested with a large amount of enzyme overnight and analysed repeatedly. This band appeared in some samples but not in others. The nature of this > 35 kb band needs further study.

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