

RESTRICTION FRAGMENT LENGTH
POLYMORPHISMS OF THE CYP11B1 GENE
IN THE JAPANESE POPULATION

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Summary Restriction fragment length polymorphisms (RFLPs) of the CYP11B1 gene were studied in Japanese using cDNA clone P450c11 as a probe. Genomic DNAs from 60 unrelated Japanese individuals were digested with 8 different restriction enzymes and analyzed by Southern blot hybridization. Two RFLPs were detected in *MspI* digests of the DNA. One (A) was characterized by polymorphic bands at 3.4 and 2.5 kilobasepairs (kb) and the other (B) by polymorphic bands at 1.7 and 1.2 kb. The third RFLP was observed in *PvuII*-digested samples and was polymorphic at 5.8 and 4.0 kb bands. Two of the three RFLPs found, RFLP (A) and (C), have not been described in the only previous report which was based on Caucasian samples. We also examined the RFLPs of a 3 generation family of 11 β -hydroxylase deficiency caused by an abnormality of the CYP11B1 gene. All the family members were homozygous in all three RFLPs and was thus not informative.

Key Words CYP11B1, RFLP, congenital adrenal hyperplasia, ethnic group

INTRODUCTION

Steroid 11 β -hydroxylase (P450c11) is a mitochondrial cytochrome P450 enzyme necessary for cortisol biosynthesis. The CYP11B1 gene which codes the P450c11

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enzyme is located along with the highly homologous CYP11B2 gene on chromosome 8q22 (Chua *et al.*, 1987; Mornet *et al.*, 1989).

Deficiency of 11 β -hydroxylase causes a hypertensive form of congenital adrenal hyperplasia. Classic 11 β -hydroxylase (P450c11) deficiency accounts for 5–8% of cases of congenital adrenal hyperplasia in the Caucasian population (Zachmann *et al.*, 1983) but only 1% in the Japanese population (Suwa, 1980). The difference in the frequency may be due to the difference of the genetic background in the two ethnic groups.

Globerman *et al.* (1988) described a restriction fragment length polymorphism (RFLP) of the CYP11B1 locus. They based their study on Caucasian samples but it is unknown whether RFLP applies to other ethnic groups. We attempted to analyze the RFLPs of CYP11B1 in the Japanese population using 8 different restriction enzymes including the one described by Globerman *et al.* (1988).

MATERIALS AND METHODS

High molecularweight DNA was extracted from peripheral blood leukocytes of 60 unrelated Japanese individuals and 7 members of a 3-generation family with 11 β -hydroxylase deficiency. The DNA (4 μ g each) was digested with one of 8 restriction enzymes (*Bam*HI, *Bgl*III, *Eco*RI, *Hind*III, *Msp*I, *Pst*I, *Pvu*II, or *Taq*I (New England Biolabs)) and separated by electrophoresis on a 0.7% agarose gel in TBE buffer (89 mM Tris-borate, 2 mM EDTA, pH 8.0). The DNA samples were then transferred to a nitrocellulose membrane filter (Hybond-C, Amersham) by blotting (Southern, 1975). The P450c11 cDNA probe was labeled by using a nick translation kit (Bethesda Research Laboratories, Gaithersburg, MD) and [α -³²P]dCTP (3,000 Ci/mmol; Dupont-New England Nuclear, Boston, MA). Pre-hybridization of the filters, hybridization to the ³²P-labeled P450c11 cDNA probe and posthybridization washes followed the procedure of Sambrook *et al.* (1989). Hybridizing bands were detected by autoradiography at –80°C using Fuji AIF FX film (Fuji Photo Film, Kanagawa, Jpn.) and Dupont Lightning Plus intensifying screens (DuPont, Wilmington, DE).

The P450c11 cDNA probe, clone pH11F2, was kindly supplied by Dr. White.

RESULTS AND DISCUSSION

P450c11 cDNA revealed two RFLPs when digested with *Msp*I. RFLP (A) consisted of 3.4 kb (A1) and 2.5 kb (A2) bands. RFLP (B) consisted of 1.7 kb (B1) and 1.2 kb (B2) bands (Fig. 1). RFLP (A) in 60 unrelated Japanese individuals showed genotype A1A1 in 17 individuals (28%), A1A2 in 36 cases (60%) and A2A2 in 7 (12%). RFLP (B) in 60 unrelated Japanese individuals showed genotype B1B2 in 10 cases (17%) and B2B2 in 50 (83%). B1B1 was not observed.

One RFLP (RFLP(C)) was detected with polymorphic bands of 5.8 and 4.0

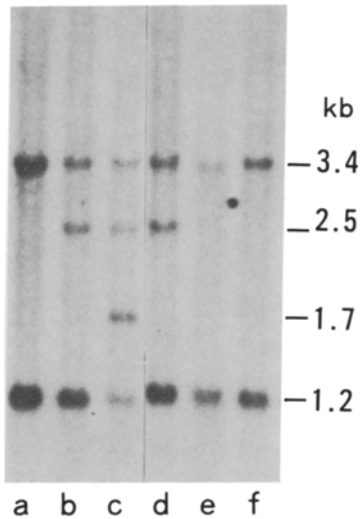


Fig. 1.

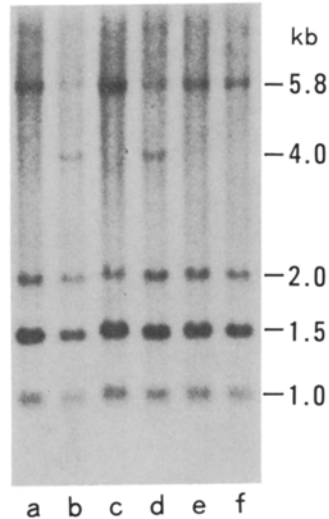


Fig. 2.

Fig. 1. RFLPs detected by *Msp*I digests. Genomic DNA obtained from peripheral blood was digested with *Msp*I and hybridized with 32 P-labeled P450c11 cDNA. Two RFLPs were detected. One was characterized by polymorphic bands at 3.4 and 2.5 kb (RFLP (A)) and the other by bands at 1.7 and 1.2 kb (RFLP (B)). Lanes a–d show data from normal individuals and lanes e–f from patients with 11 β -hydroxylase deficiency.

Fig. 2. RFLP(C) detected by *Pvu*II digestion. Genomic DNA prepared from peripheral blood was digested with *Pvu*II and hybridized with 32 P-labeled P450c11 cDNA. The RFLP was characterized by polymorphic bands at 5.8 and 4.0 kb. Lanes a–d show data from normal individuals and lanes e–f from patients with 11 β -hydroxylase deficiency.

kb (Fig. 2) when digested with *Pvu*II. Of 60 unrelated Japanese individuals, 44 (73%) were homozygous for the 5.8 kb band and 16 (27%) were heterozygous. No homozygote for the 4.0 kb band was detected. The observed frequencies of individual genotypes did not differ significantly from those expected from Hardy-Weinberg equilibrium in all three RFLPs. We did not find any RFLPs with the restriction enzymes *Bam*HI, *Bgl*II, *Eco*RI, *Hind*III, *Pst*I, and *Taq*I.

We examined a family with 11 β -hydroxylase deficiency for the three RFLPs which were found above (A, B, and C). All members were homozygous in all the RFLPs. This information thus was not informative in genetic counselling (Fig. 3).

Out of the three RFLPs described in the present study, only RFLP (B) has been described in the literature (Globerman *et al.*, 1988). Their Caucasian samples did not show RFLP (A). It is possible that they did not find RFLP (C) because they simply did not try *Pvu*II. There is a difference in the frequency of 11 β -hydroxylase deficiency caused by the P450c11 abnormality in the Caucasian and Japanese populations. Classic 11 β -hydroxylase deficiency comprises 5–8% of cases of con-

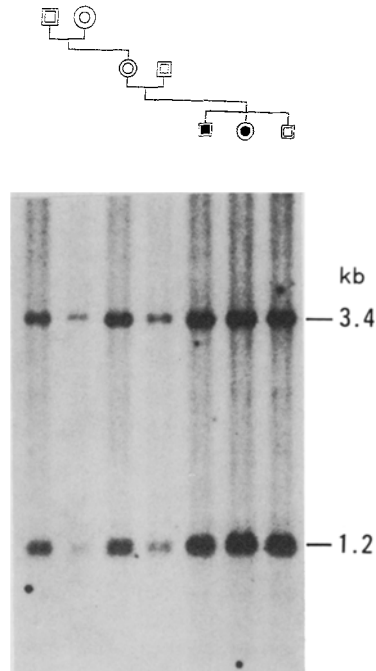


Fig. 3. RFLP pattern by *Msp*I digestion in a family with 11 β -hydroxylase deficiency. All members were homozygous for both the RFLP(A) and (B) loci.

genital adrenal hyperplasia in the Caucasian population (Zachmann *et al.*, 1983) but only 1% in the Japanese population (Suwa, 1980). The difference of RFLPs may reflect a difference in the 11 β -hydroxylase region between the two races. Further studies are needed to clarify the cause of the ethnic difference in the frequency of 11 β -hydroxylase deficiency.

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