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RESTRICTION FRAGMENT LENGTH POLYMORPHISMS ON THE q24-q28 REGION OF X CHROMOSOME AMONG JAPANESE POPULATION

Takashi TAGA,[1] Wataru SHIRAHASE,[1] Morimi SHIMADA,[1]
Kiyoshi KUROKAWA,[2] and Hisao UEYAMA[2,*]

Departments of [1]Pediatrics and [2]Medical Biochemistry, Shiga University of Medical Science, Seta, Otsu 520-21, Japan

Summary Restriction fragment length polymorphisms were studied among the Japanese population using 12 polymorphic DNA probes on the q24-q28 region of X chromosome. The frequency distribution for probes p22-33, p482.6a, p43-15, 52A, pPM101, cX33.2 and cpx234, was the same as that for Caucasians, and that for probes 4D-8 and St14-1 (*Msp*I) was slightly different ($p<0.05$). However, it was quite different ($p<0.01$) for probes p114.12, St14-1 (*Taq*I), 36B-2 and MN12. Probe p114.12 showed no *Hind* III polymorphism for the Japanese people. On the contrary, probe MN12, which has a low PIC value (0.15) for Caucasians, was found to be useful for Japanese (PIC value=0.50). These results suggest that 7 DNA probes (p482.6a, p43-15, 52A, St14-1, p114.12 (*Bcl*I), 36B-2 and MN12) are useful ($PIC>0.42$) for linkage analysis of X-linked disease in Japan.

Key Words RFLP, X chromosome

INTRODUCTION

Restriction fragment length polymorphisms (RFLPs) are powerful tools for carrier detection and prenatal diagnosis of inherited diseases, especially of genetic disorders with unidentified biochemical lesions such as Huntington's disease and cystic fibrosis (Guesella *et al.*, 1983; Tsui *et al.*, 1985).

We have several families of adrenoleukodystrophy (ALD), which shows an X-linked inheritance. The disease is one of the most frequent neurodegenerative disorders, and its defective gene is localized on the distal end of the long arm of X chromosome (Migeon *et al.*, 1981). The chromosomal region is very important, because the affected gene of quite a few heritable disorders is known to be around

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* To whom correspondence should be addressed.

Xq26-Xq28, *e.g.*, hemophilia A and B, the fragile X mental retardation syndrome, G6PD deficiency, Lesch-Nyhan syndrome, color blindness and so on. Many polymorphic DNA probes have been reported on the X chromosome, but the usefulness of the probes for genetic linkage analysis is determined mainly by their frequency of RFLPs, which may be different among human races as described previously (Antonarakis *et al.*, 1985; Waincoat *et al.*, 1986; Paul *et al.*, 1987). In this communication, we present frequencies of RFLPs among the Japanese population for 12 probes on the q24-q28 region of X chromosome.

MATERIALS AND METHODS

High-molecular-weight DNA was extracted and purified from peripheral blood leukocytes of unrelated Japanese individuals. The DNA (5 μg each) was digested with appropriate restriction enzymes, and then separated by gel electrophoresis on 0.7 or 1.0% agarose in TAE buffer (0.04 M Tris-acetate–2 mM EDTA, pH 7.8), and transferred to a nitrocellulose membrane filter (NitroPlus 2000, Micron Separations Inc.) (Southern, 1975) using Vacuum Blotting System (LKB 2016 VacuGene, Pharmacia). The DNA probes were labeled by using Random Primer DNA Labeling Kit (Takara, Japan) and [α-^{32}P]dCTP(ICN) (final specific activity=10^9 dpm/μg). Hybridization was carried out as described (Wion *et al.*, 1986), and then the filter was washed briefly in 6×SSC (20×SSC: 3 M NaCl–0.3 M sodium citrate), once in 2×SSC containing 0.1% sodium dodecyl sulfate (SDS) for 30 min at room temperature, and twice in 0.1×SSC containing 0.1% SDS for 60 min at 65°C. Autoradiography was carried out at −20°C for 1–4 days.

The DNA probes used were kindly supplied by Dr. J.L. Mandel (St14-1), Dr. K.E. Davies (MN12) and Dr. P.L. Pearson (cX33.2). The other probes were obtained from American Type Culture Collection (ATCC). The probe p482.6a contains a 1.1 kb *Eco*RI/*Sst*I fragment derived from the original 9.6 kb insert in p482.6 (Wion *et al.*, 1986). The probe 36B-2 (2.2 kb *Hin*dIII fragment) is next to the probe 6A-1 on the human genome and can detect the same *Taq*I polymorphism as 6A-1 without a constant band of 3.5 kb. The probe pPM101 contains a 2.3 kb *Eco*RI fragment derived from the original 18.0 kb insert in 07–03.

RESULTS AND DISCUSSION

Table 1 summarizes the results of RFLP analysis with 12 DNA probes. We analyzed three other probes (cx38.1, pλ2.7, and DX13), which are not included in the table. Probe cx38.1 seemed to contain repeated sequences, and probe pλ2.7 gave no *Eco*RI polymorphisms. The frequency of RFLP detected with DX13 has been reported by Suzuki *et al.* (1988), and we obtained about the same frequency (5.4 kb:2.0 kb=27:73).

In the case of probe p482.6, which is derived from coagulation factor VIII

Table 1. RFLP for DNA probes on the q24-q28 region of X chromosome.

Probe	Restriction enzyme		Japanese		Previous reports	
			Size (kb)	Frequency (n)	Size (kb)	Frequency
p43-15	*Bgl*II		9.5	0.32 (23)	9.5	0.19
			6.0	0.68 (49)	6.0	0.81
p22-33	*Taq*I		20.0	0.13 (2)	20.0	0.17
			11.0	0.87 (42)	11.0	0.83
36B-2	*Taq*I		7.0	0.52 (26)	7.0	0.33
			5.0	0.48 (24)	5.0	0.67
cpX234	*Taq*I		3.2	0.20 (10)	3.2	0.17
			1.1	0.80 (40)	1.1	0.83
52A	*Taq*I		1.6	0.69 (33)	1.6	0.50
			1.3	0.31 (15)	1.3	0.50
pPM101	*Ban*II		4.2	0.10 (5)	4.2	0.19
			2.4	0.90 (47)	2.4	0.81
4D-8	*Msp*I		25.0	0.94 (47)	25.0	0.82
			7.8	0.06 (3)	7.8	0.18
p114.12	*Bcl*I		1.2	0.33 (16)	1.2	0.59
			0.9	0.67 (32)	0.9	0.41
	*Hin*dIII		2.7	1.00 (98)	2.7	0.87
					2.6	0.13
St14-1	*Msp*I		2.3	0.56 (30)	2.0	0.70
			1.8	0.44 (24)	1.6	0.30
	*Taq*I	1	6.6	0.00 (0)	6.6	0.01
		2	5.4, 5.2	0.00 (0)	5.4, 5.2	0.04
		3	4.8	0.02 (1)	4.8	0.12
		4	4.5	0.15 (8)	4.5	0.36
		5, 6	4.3, 4.1	0.06 (3)	4.1, 4.0	0.21
		7	3.9	0.37 (20)	3.9	0.11
		8	3.4	0.40 (22)	3.4	0.16
	*Taq*I	α	5.5	0.52 (28)	5.5	0.92
		β	4.1	0.48 (26)	4.1, 1.4	0.08
p482.6a	*Xba*I/*Kpn*I		6.2	0.33 (18)	6.2	0.41
			4.8	0.67 (36)	4.8	0.59
MN12	*Bgl*I		10.0	0.46 (24)	10.0	0.08
			8.0	0.54 (28)	8.0	0.92
cX33.2	*Apa*L1		12.0	0.26 (14)	12.0	0.36
			8.3	0.74 (39)	8.3	0.64

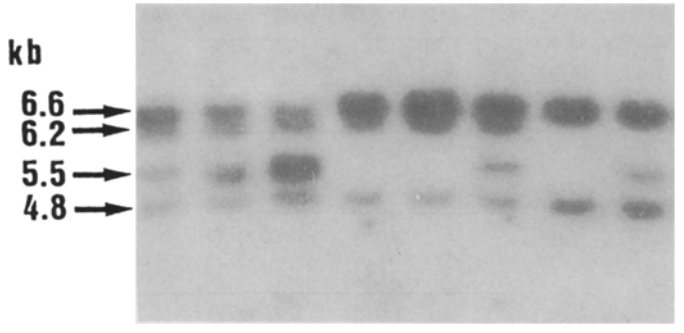

Fig. 1. Polymorphic pattern of *Xba*I/*Kpn*I fragments hybridizing to the p482.6a probe. Japanese (female) DNAs were digested with *Xba*I and *Kpn*I and analyzed by blot hybridization. In addition to the polymorphic bands (6.2 kb and 4.8 kb) and the constant (for Caucasians) band (6.6 kb), a 5.5 kb band is seen in some Japanese.

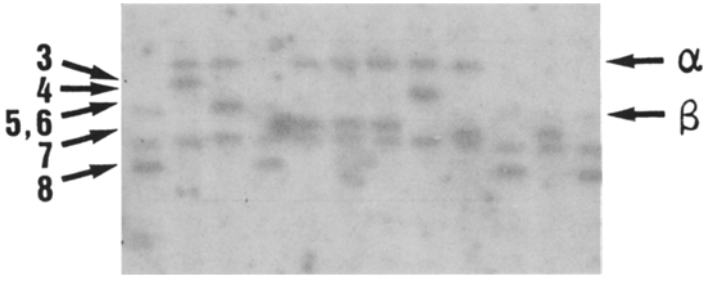

Fig. 2. Polymorphic pattern of *Taq*I fragments hybridizing to the St14-1 probe. Japanese (male) DNAs were digested with *Taq*I and analyzed by blot hybridization. There is a tight association between alleles 8 and β.

gene, a 5.5 kb band was found in about a half of the *Xba*I/*Kpn*I-digested DNAs we examined (Fig. 1). The previous study (Wion *et al.*, 1986) did not refer to this band but to the 6.6 kb band, the origin of which is unknown. About 30% of the males showed both bands, about 60% showed only the 6.6 kb band, and the others showed only the 5.5 kb band. Females showed more intense signals than males. If we assume that these bands are from X chromosome, then the calculated frequencies among females are 63, 36 and 1%, respectively; these are comparable with the actual frequencies (55, 44 and 5%, respectively). Therefore, these hybridizing regions may also be derived from the X chromosome. Wion *et al.* (1986) reported that no examples were found of the combination of large *Bcl*I allele (1.2 kb detected with p114.12) and small *Xba*I/*Kpn*I allele (4.8 kb detected with p482.6a). We did not detect this combination, either.

DNA probes, the RFLP frequencies of which showed a marked difference from those reported for Caucasians, are as follows: p114.12, St14-1, 36B-2, and MN12.

p114.12: The *Hin*dIII polymorphism was not observed for the probe p114.12; only the 2.7 kb band was found in our study in contrast to two bands in the previous report (2.7 and 2.6 kb). Therefore, the DNA probe p114.12, derived from coagulation factor VIII gene, may be useless for the Japanese. It is, however, still useful for detecting *Bcl*I polymorphisms, as suggested previously (Antonarakis *et al.*, 1985; Suzuki *et al.*, 1988). The RFLP frequencies reported by them (1.2 kb: 0.9 kb=26:74) and this communication (1.2 kb:0.9 kb=33:67) are the same.

St14-1: The fragment sizes were slightly different for the probe St14-1 (2.3 kb and 1.8 kb in *Msp*I RFLP, and 4.3 kb and 4.1 kb in *Taq*I RFLP) from those reported previously (Oberle *et al.*, 1985). Some differences were also observed in the incidence of each polymorphic band detected by St14-1. Suzuki *et al.* (1988) used the St14 probe on the Japanese population, but they did not refer to α and β alleles. We report here the incidence for St14-1 RFLPs (alleles 1–8, and α and β). Japanese showed much higher frequencies of alleles 8 and β than Caucasians (Table 1). Mandel *et al.* (1986) suggested that allele β is specifically associated with allele 8. We agree with their proposal; all the X chromosome having allele 8 had allele β in our study (Fig. 2). The original RFLP probe St14 (9.3 kb fragment) detects 5 *Msp*I alleles (Oberle *et al.*, 1985), but St14-1 we used (3 kb fragment) can detect only *Msp*I alleles 3 and 4. *Taq*I alleles 4 and 7 were suggested to be associated with *Msp*I allele 3 and *Taq*I allele 8 to be associated with *Msp*I allele 4. We confirmed that all the X chromosomes having *Taq*I allele 8 have *Msp*I allele 4.

MN12: The frequency was also significantly different from that reported previously for probe MN12. The PIC value is quite high (0.50) for Japanese population, but is very low for Caucasians (0.15).

Among the probes we used, the expected frequency of heterozygosity among the Japanese females is high (43–50%) with 36B-2, p114.12 (*Bcl*I), p43-15, St14-1, 52A, p482.6 and MN12. St14-1 is especially useful as in Caucasians, the frequency being 68%. Other DNA probes on the distal end of X chromosome such as p1.8 (coagulation factor VIII gene-derived) and DX13 (both localized to q28) are reported to be not so useful (frequency of heterozygosity=18–32%, Suzuki *et al.*, 1988). Moreover, there is no RFLP in Japanese for HPRT gene (localized to q26) or coagulation factor IX gene (located to q26.3-q27.2) in Japanese (Kojima *et al.*, 1987). Therefore, those DNA probes listed above must be quite useful for the linkage analysis in Japan. We would like to perform carrier detection and prenatal diagnosis of ALD by using linkage analysis with these probes. Previous studies have shown a tight linkage between ALD and St14-1 (Aubourg *et al.*, 1987) and also between coagulation factor VIII and St14-1 (Gitschier *et al.*, 1985). Therefore, these probes should also be helpful to identify the defective ALD gene.

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