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RESTRICTION FRAGMENT LENGTH POLYMORPHISMS ON THE SHORT ARM OF X CHROMOSOME AMONG THE JAPANESE POPULATION

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Summary Restriction fragment length polymorphisms were studied among the Japanese population using 13 polymorphic DNA probes on the short arm of X chromosome. The calculated molecular sizes of polymorphic bands for the probes 782, pXUT23 and 754 were larger than those in previous reports from North America and Europe, and their frequency distribution was markedly different for the probes C7, pERT87-8 and XJ5.1. The probe pD2 did not show any polymorphism in the present study for the Japanese population. The genetic significance of these differences was discussed.

INTRODUCTION

Restriction fragment length polymorphisms (RFLPs) have been used for carrier detection and prenatal diagnosis of inherited diseases in which the gene defect was identified and the presence of RFLPs was detected around their loci, such as phenylketonuria and ornithine transcarbamylase deficiency (Lidsky *et al.*, 1985; Rozen *et al.*, 1986). Recently linkage analysis of genetic disorders with unidentified biochemical lesions, such as Duchenne and Becker muscular dystrophies and Huntington's disease, has also been performed using RFLPs (Kunkel *et al.*, 1986; Gusella *et al.*, 1983).

More than 400 polymorphic DNA probes were reported by 1985, especially those on X chromosome (Willard *et al.*, 1985), and the number is increasing rapidly. The usefulness of these probes for genetic linkage analysis is determined mainly by their frequency of RFLPs. It seems likely that frequencies of their RFLPs are different between human races. For inhabitants of North America and Europe,

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several data on X chromosome have been reported previously (Bakker *et al.*, 1985; Drayna and White, 1985; Kunkel *et al.*, 1986), but systematic survey of RFLPs has not been performed for the Japanese population up to the present except for a study on 40 DNA probes from various human chromosomes (Suzuki, A. *et al.*, presented at Annual Meeting of the Japan Society of Human Genetics, 1987). Five of them showed no polymorphism, and the probe for 19p13-cen revealed a pattern different from that reported among Caucasians.

In this study, we investigated RFLPs among the Japanese with 13 polymorphic probes on the short arm of X chromosome for the basis of linkage analysis of X-linked disorders in Japan.

MATERIALS AND METHODS

DNA was extracted and purified from leukocytes in heparinized blood by the method described by Blin and Stafford (1976). The genomic DNA thus obtained (5 μg) was then digested with appropriate restriction enzymes, and the DNA fragments were separated by electrophoresis on a 0.7% agarose gel in TAE buffer (0.04 M Tris-acetate containing 1 mM EDTA, pH 8.0), and transferred to a nitrocellulose membrane filter (BA 85, Schleicher & Schuell, Dassel, F.R.G.) by Southern blotting (Southern, 1975). Hybridization was performed with the nick translated DNA probe (final specific activity 1×10^8 cpm/μg) using Amersham Nick Translation Kit (Amersham, Buckinghamshire, England) under the same condition as described by Bakker *et al.* (1985). After hybridization, the filter was washed twice at 65°C in 2×SSC (20×SSC: 3 M NaCl, 0.3 M sodium citrate) containing 0.1% sodium dodecyl sulfate (SDS) for 15 min, and twice at 65°C in 1×SSC containing 0.1% SDS for 15 min. The spots were visualized by autoradiography at −80°C for 1–5 days. The *Hin*dIII digests of lambda phage DNA were used as molecular size markers.

DNA probes were kindly supplied by Dr. P.L. Pearson (782, pXUT23, 99-6, pD2, C7, 754, 754-11 L1.28), Dr. L. Kunkel (pERT87-1, 87-8, 87-15), and Dr. R.G. Worton (XJ1.1, 5.1).

RESULTS AND DISCUSSION

The results of RFLP analysis with 13 DNA probes are summarized in Table 1. Definite differences have been observed between the Japanese and the inhabitants of North America and Europe in previous reports with regard to molecular size and relative frequency of polymorphic bands.

The polymorphic bands for the probes 782, pXUT23 and 754 were larger in molecular size among the Japanese than among the populations reported from North America and Europe (Willard *et al.*, 1985). For example, the restriction fragment length has been reported as 12.0 or 9.0 kb for the probe 754 after digestion with a

Table 1. RFLP for DNA probes on the short arm of X chromosome.

Probe	Restriction enzyme	Polymorphic band	Japanese			Previous reports [a]	
			Size(kb)	Frequency(n)		Size(kb)	Frequency
782	*Eco*RI	D	15.0	0.37	(118)	14.0	0.60
		d	9.4	0.63		7.0	0.40
pXUT23	*Bgl* II	H	19.0	0.85	(27)	17.5	0.84
		h	14.0	0.15		12.5	0.16
99-6	*Pst*I	F	22.0	0.78	(58)	22.0	0.71
		f	13.0	0.22		13.0	0.29
pD2	*Pvu*II	E	6.4	1.00	(32)	6.6	0.71
		e				6.0	0.29
C7	*Eco*RV	J	8.0	0.97	(32)	8.0	0.15
		j	7.5	0.03		7.5	0.85
pERT 87-15	*Xmn*I	L	2.8	0.47	(120)	2.8	0.32
		l	1.6, 1.2	0.53		1.6, 1.2	0.68
pERT 87-8	*Bst*XI	K	4.4	0.32	(112)	4.4	0.60
		k	2.2	0.68		2.2	0.40
pERT 87-1	*Xmn*I	P	8.7	0.44	(129)	8.7	0.66
		p	7.5	0.56		7.5	0.34
XJ1.1	*Taq*I	Q	3.8	0.35	(71)	3.8	0.28
		q	3.1	0.65		3.1	0.72
XJ5.1	*Sph*I	R	24.0	0.72	(32)	24.0	0.21
		r	17.0	0.28		17.0	0.79
754	*Pst*I	M	22.0	0.03	(36)	12.0	0.62
		m	12.0	0.97		9.0	0.38
754-11	*Eco*RI	C	4.2	0.64	(113)	4.2	0.16
		c	2.4	0.36		2.4	0.84
L1.28	*Taq*I	A	12.0	0.55	(32)	12.0	0.68
		a	9.0	0.45		9.0	0.32

[a] Data from North America and Europe: Kunkel *et al.* (1986) for probes pERT87-15, 87-8 and 87-1, Thompson *et al.* (1986) for probe XJ1.1, Worton (1987, personal communication) for probe XJ5.1, and Willard *et al.* (1985) for other probes.

restriction enzyme *Pst*I (Willard *et al.*, 1985), whereas our study demonstrated a larger band calculated as 22.0 kb instead of 9.0 kb among the Japanese (Fig. 1). The polymorphism was not observed for the probe pD2; only the 6.4 kb band was found in our present study for the Japanese population in contrast to two bands reported previously (6.6 and 6.0 kb). The fragment size and frequency of these two probes, 754 and pD2, should further be evaluated for larger numbers of individuals in the future. For other probes, no significant differences were found in the fragment sizes between the two populations. New polymorphisms were not found with the other restriction enzymes we tried in this study.

Some differences were observed also in the incidence of each polymorphic band. The relative frequency was significantly different from previous reports for the probes

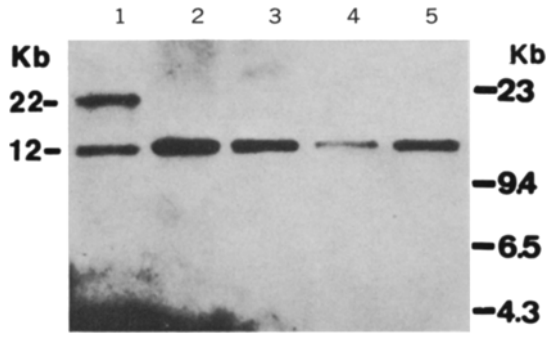

Fig. 1. Southern blot hybridization patterns of *Pst*I digest with the probe 754 showing two polymorphic bands (22 and 12 kb) in normal Japanese individuals. The *Hin*dIII digests of lambda phage DNA were detected by fluorescence, and their molecular sizes (23, 9.4, 6.5 and 4.3 kb) are indicated on the right. Lane 4, male; other lanes, females.

C7 (Willard *et al.*, 1985), pERT87-8 (Kunkel *et al.*, 1986) and XJ5.1 (Worton, personal communication, 1987). Akita *et al.* (1987) also reported similar results for the frequency of polymorphic bands with the pERT87 probes among the Japanese. There are differences in molecular size and relative frequency of polymorphic bands at the apolipoprotein loci among different ethnic groups (Paul *et al.*, 1987).

Among the probes in this study, the frequency of heterozygosity among the Japanese females was high enough to use for linkage analysis with 782, 99-6, pERT87-15, pERT87-8, pERT87-1, XJ1.1, XJ5.1, 754-11, and L1.28. These probes are mapped on loci from Xp222-223 (782) to Xp113 (L1.28) on the short arm of X chromosome (Bakker *et al.*, 1985). The loci of some inherited diseases have been identified in this region; adrenal hyperplasia (White *et al.*, 1984), glycerol kinase deficiency (Wieringa *et al.*, 1985), Duchenne and Becker muscular dystrophies (Burghes *et al.*, 1987; Monaco *et al.*, 1986), chronic granulomatous disease (Royer-Pokora *et al.*, 1986) and ornithine transcarbamylase deficiency (Rozen *et al.*, 1986). We have also performed carrier detection of Duchenne and Becker muscular dystrophies by linkage analysis using RFLPs among the Japanese families, and confirmed the usefulness of the probes described above (Shimmoto *et al.*, 1987; Shimmoto *et al.*, 1988). Our linkage studies will be extended also to the diseases mentioned above in the near future.

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