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SHORT COMMUNICATION

DNA polymorphisms identified by six X-chromosome probes, linked to genetic diseases, in a Spanish population

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ABSTRACT

Using six different X-chromosome probes, the frequency of restriction fragment length polymorphism (RFLP) with restriction endonucleases was estimated in unrelated people of a Spanish population from Valencia. The allele frequencies were similar to those of other European populations, in particular to the French and Turkish. A high degree of polymorphism was found for all markers, the rare allele frequency ranging between 0.484 and 0.357, and a high level of heterozygosity of the markers *L1.28*, *754*, *OTC* and *p58.1* was found in this population, confirming their utility for diagnosis.

INTRODUCTION

An important application of genetic markers, such as restriction fragment length polymorphism (RFLP), is the characterization of diseases and the determination of the genetic or environmental basis of disorders which cluster in families, by linkage analysis (White *et al.*, 1985). Even when the disease is caused by a characterized gene, molecular diagnosis can use intragenic polymorphic markers instead of direct detection of the mutation.

MATERIAL AND METHODS

Population

Blood specimens from 185 healthy non-related individuals (96 females and 89 males) were obtained through the Hematology Department of the General Hospital in Valencia (Spain).

DNA extraction and analysis

Total genomic DNA was extracted from peripheral blood (Kunkel *et al.*, 1982). Ten µg of DNA was digested to completion with the appropriate restriction endonuclease, size fractioned in 0.8% agarose gels, and transferred to nylon membranes (Southern, 1975).

Probes

Six X-chromosome-specific cloned DNA probes (see Fain *et al.*, 1995) were used to detect polymorphism.

L1.28 (Bakker *et al.*, 1983) linked to Duchenne muscular dystrophy (DMD) (Wilcox *et al.*, 1985), retinitis pigmentosa (RP2), Norris disease, congenital stationary night blindness (CSNB) and Wiscott-Aldrich syndrome (WAS) (Cremin *et al.*, 1993).

p58.1 (Peacocke and Siminovitch, 1987) linked to RP2 and WAS (Cremin *et al.*, 1993).

754 (Hofker *et al.*, 1986) linked to DMD, McLeods' syndrome, glycerol kinase deficiency and retinitis pigmentosa (RP3) (Musarella *et al.*, 1988).

OTC (Francke *et al.*, 1985) linked to DMD and RP3 (Musarella *et al.*, 1988; Dahl *et al.*, 1991).

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cX55.7 (Hofker *et al.*, 1986) linked to X-fragile (FRAX), hemophilia B and agammaglobulinemia (Lovering *et al.*, 1993).

xJ2.3 (Thompson *et al.*, 1986) linked to DMD (Towbin *et al.*, 1993) and RP3 (Musarella *et al.*, 1988).

Each one of these probes detects two allelic fragments. Details are given in Table I. For hybridization, the probes were labeled non-radioactively with d-UTP digoxigenin.

Table I - RFLP loci and probes used to detect polymorphism in a Spanish population.

Locus	Probe	Location	Enzyme	Alleles	Frequencies
DXS7	*L1.28*	Xp11	Taq I	12, 9	0.68, 0.32
DXS14	*p58.1*	Xp11-cent.	Msp I	4, 2.5	0.65, 0.35
DXS84	*754*	Xp21.1	Pst I	11, 9	0.62, 0.38
OTC	*OTC*	Xp21.1	Msp I	6.6, 5.8	0.64, 0.36
DXS101	*cX55.7*	Xq22	Msp I	6.6, 6.2	0.59, 0.41
DXS206	*xJ2.3*	Xp21.1	Taq I	7.8, 6.4	0.30, 0.70

Hybridization and detection

The prehybridization, hybridization and washing of the membranes were performed according to standard methods (Sambrock *et al.*, 1982). Detection was made using an antibody-conjugate (anti-digoxigenin alkaline phosphatase conjugate), by two different non-radiactive methods: color reaction with X-phosphate and NBT and chemiluminescent reaction with Lumigen PPD and exposure to X-rays.

RESULTS AND DISCUSSION

The six DNA probes revealed a polymorphic pattern of hybridizing restriction fragments in the population. Each RFLP showed two alleles of different fragment length, in agreement with the information from the original reports. No new alleles were found. The allele frequencies are shown in Table II.

The polymorphism information content (PIC) of a marker can range from 0 (always uninformative) to 1 (always informative) but the maximum possible PIC value for an RFLP marker with two alleles is 0.5. The value of PIC in the six markers studied in this work was over 0.35 confirming a high polymorphism for all them in our population.

The rare allele frequency ranged between 0.484 and 0.357 for the six markers, so dimorphism is established for all them. Nevertheless, the low heterozygosity degree found for marker *cX55.7* discourage its use as marker in our population while the high heterozygosity of *754*, *L1.28*, *OTC* and even *p58.1* confirms their utility for the diagnosis of diseases.

Table II - Distribution of six marker phenotypes and allele frequencies in a Spanish population.

Sex	Marker	Samples	Number of chromosomes	Phenotype			Allele frequencies		Degree of heterozygosity
				A_1	A_1A_2	A_2	A_1	A_2	
Females	*754*	96	192	31	36	29	0.510	0.490	
Males	*754*	89	89	47		47	0.528	0.472	
Total	*754*	185	281				0.516	0.484	0.375
Females	*L1.28*	96	192	52	30	14	0.698	0.302	
Males	*L1.28*	88	88	46		42	0.523	0.477	
Total	*L1.28*	184	280				0.645	0.357	0.313
Females	*OTC*	84	168	26	26	32	0.464	0.536	
Males	*OTC*	78	78	38		40	0.487	0.513	
Total	*OTC*	162	246				0.471	0.529	0.310
Females	*xJ2.3*	90	180	16	22	52	0.300	0.700	
Males	*xJ2.3*	72	72	36		36	0.500	0.500	
Total	*xJ2.3*	162	252				0.357	0.643	0.244
Females	*cX55.7*	86	172	46	10	30	0.593	0.407	
Males	*cX55.7*	78	78	42		36	0.538	0.462	
Total	*cX55.7*	164	250				0.576	0.424	0.116
Females	*p58.1*	88	176	42	26	20	0.625	0.375	
Males	*p58.1*	78	78	42		36	0.538	0.462	
Total	*p58.1*	166	254				0.598	0.402	0.295

The frequencies of RFLPs detected by these probes differ in different human populations. For marker *L1.28*, the frequency of the rarer allele (9 kb) is higher in our population than in other European ones but close to those of the Dutch (Davies *et al.*, 1983), British (Brown *et al.*, 1985) and Turkish (Schürmann *et al.*, 1987). For the A_2 allele, an interesting clinal increase from West (European frequency 23-29%) to East (Australian aborigenes frequency 64%) has been proposed (Wadhwa *et al.*, 1989) but must be confirmed by further genetic analysis. For marker *754*, the frequency of the rarer allele (9 kb) is very high in our population, the same as in the French (Dandieu *et al.*, 1985) and Turkish (Schürmann *et al.*, 1987) ones.

None of the six probes were in equilibrium, which disagrees with the study of Papiha *et al.* (1988) for *L1.28* probe, in which in all three female population samples, good agreement with those expected under Hardy-Weinberg equilibrium was observed.

Significant differences (*t*-test, $P < 0.01$) were found between the gene frequencies of both sexes in the markers *L1.28* (higher frequency of A_1 in females) and *xJ2.3* (higher frequency of A_2 in females), possibly due to the lack of equilibrium in the populations. However, Schürmann *et al.* (1987) did not find significant differences between sexes in allelic frequencies of markers *L1.28* and *754*.

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RESUMO

Usando seis diferentes probes de cromosomo X, estimou-se a freqüência de polimorfismo de comprimento de fragmento de restrição (RFLP) com endonucleases de restrição em pessoas não parentes, em uma população espanhola de Valência. As freqüências de alelos foram semelhantes às de outras populações européias, em particular francesas e turcas. Um alto grau de polimorfismo foi encontrado para todos os marcadores, sendo que a freqüência do alelo raro variou de 0,484 a 0,357 e um alto nível de heterozigose dos marcadores *L1.28, 754, OTC* e *p58.1* foi encontrado nesta população, confirmando sua utilidade para a diagnose.

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