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FOR THE RECORD

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Unlinked Tetrameric Microsatellites on the X Chromosome: Frequency Data in Males from Cantabria (Northern Spain)

POPULATION: Cantabria, northern Spain ($n = 141$)

KEYWORDS: forensic science, DNA typing, microsatellites, short tandem repeat, X chromosome, population genetics

We studied unrelated male donors ($n = 141$) living in Cantabria, a region in northern Spain. DNA was extracted from peripheral blood by using the Qiagen blood kit (Qiagen, Hilden, Germany). Loci of interests in the X-chromosome (DXS9895, GATA172D05 and DXS9898) were amplified in a single PCR with primers of published sequences (1–3). PCR conditions consisted of an initial denaturation step at 94° for 10 min, followed by eight cycles with denaturation at 94° for 1 min, annealing at decreasing temperature between 62° and 59° (1° decrease every two cycles) and extension at 72° for 1 min. Then 24 cycles at 94° 1 min, 58° 1 min, 72° 1 min; followed by a final extension at 72° for 30 min. PCR products were analyzed by capillary electrophoresis (ABI310, Applied Biosystems), with sequenced controls. Allele designation was as reported previously (1,2).

Allele and haplotype-like frequencies were estimated by counting. The presence of a disequillibrium linkage was tested with GDA software (4). Unbiased haplotype diversity was estimated according to Nei (5).

Allelic and haplotypic frequencies are shown in Tables 1 and 2. Seventy nine different haplotypes were found. Haplotype diversity was 0.9913, and the resulting matching probability was 0.0087.

As male subjects transfer the same X chromosome to all their daughters, the analysis of markers on the X chromosome may be very useful in some identification and kinship cases, particularly when DNA from the alleged father is not available. A number of microsatellites of forensic interest have been reported previously by several authors, including our own group (6–8). In the present study we chose to study a set of distant tetrameric microsatellites (which are less prone than trimeric ones to develop stutter bands after amplification). A protocol was developed to amplify them in a single PCR.

The loci were located wide apart on the chromosome and no evidence of linkage was found ($P > 0.45$). Therefore, alleles from the loci here studied could be considered as independent, as least in case of large, unstructured populations. Nevertheless, it is usually safer to use the more conservative "haplotypic" analysis when computing combined probabilities of loci situated on the same chromosome.

The complete dataset is available upon request from the corresponding author.

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TABLE 1—*Allelic frequencies*.

	DXS9895		GATA172D05		DXS9898	
Alleles	Frequency	SE	Frequency	SE	Frequency	SE
6			0.177	0.032		
7			0.007	0.007		
8			0.177	0.032		
8.3					0.277	0.038
9			0.071	0.022		
10			0.248	0.036	0.021	0.012
11			0.213	0.034	0.227	0.035
12	0.007	0.007	0.106	0.026	0.248	0.036
13	0.298	0.039			0.156	0.031
14	0.121	0.027			0.071	0.022
15	0.390	0.041				
16	0.156	0.031				
17	0.028	0.014				
PDf	0.874		0.939		0.916	
PDm	0.719		0.814		0.780	
PE	0.672		0.787		0.745	

PD: power of discrimination in female (PDf) or male (PDm) cases.
PE: power of exclusion in trio cases.

TABLE 2—*Haplotype-like groups determined by loci DXS9895, GATA172D05 and DXS9898*.

Haplotype	*n*	Haplotype	*n*
12-11-10	1	15-8.3-10	5
13-8.3-6	1	15-8.3-11	3
13-8.3-6	3	15-8.3-12	2
13-8.3-10	3	15-10-12	1
13-8.3-11	1	15-11-6	1
13-10-11	2	15-11-8	3
13-11-8	2	15-11-9	2
13-11-10	1	15-11-10	2
13-11-11	4	15-11-11	5
13-11-12	1	15-12-6	1
13-12-6	4	15-12-8	1
13-12-8	3	15-12-8	2
13-12-9	2	15-12-9	2
13-12-10	2	15-12-11	3
13-12-11	1	15-12-12	2
13-12-12	2	15-13-6	2
13-13-6	1	15-13-8	3
13-13-8	1	15-13-10	3
13-13-10	1	15-13-11	2
13-13-11	1	15-13-12	1
13-13-12	1	15-14-6	2
13-14-8	1	15-14-10	2
13-14-10	1	16-8.3-6	1
13-14-11	2	16-8.3-8	2
13-14-11	1	16-8.3-10	2
14-8.3-6	2	16-8.3-12	2
14-8.3-8	1	16-11-6	2
14-8.3-9	1	16-11-9	1
14-8.3-10	1	16-11-10	1
14-8.3-11	2	16-11-12	2
14-11-7	1	16-12-9	1
14-11-10	1	16-12-10	1
14-12-6	2	16-12-10	1
14-12-8	1	16-12-11	1
14-12-9	1	16-13-6	1
14-12-10	1	16-13-10	2
14-12-11	1	16-13-12	1
14-13-10	1	16-14-8	1
14-13-11	1	17-8.3-6	1
15-8.3-6	2	17-8.3-10	1
15-8.3-8	3	17-11-10	2