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Allele distribution of two X-chromosomal STR loci in a population from Sicily (Southern Italy)

A. Asmundo*, F. Perri, D. Sapienza

Dipartimento di medicina sociale del territorio, Sezione di medicina legale, Università di Messina, Azienda Ospedaliera Universitaria "Policlinico G. Martino", Via Consolare Valeria 98125 Messina, Italy

Abstract. Population genetic data for two X-chromosomal STR loci (DXS7423 and DXS9902) were obtained by analysing a population sample ($n=60$ males and 60 females) and 43 family trios (showing a probability of paternity and maternity >99.9%) from Sicily (Southern Italy) by using PCR and PAGE followed by silver staining. Five and four different alleles of DXS7423 and DXS9902 loci were detected, respectively. The allele frequencies of both ChrX markers were in good agreement with Hardy–Weinberg equilibrium. The analysis of the family trios, based on the investigated meiotic events, showed no mutation. The observed heterozygosity of DXS7423 and DXS9902, together with other forensic parameters were determined, confirming that these markers are useful tools for parentage testing, mainly in deficiency paternity cases when the disputed child is female. © 2006 Published by Elsevier B.V.

Keywords: X-chromosome; Short tandem repeat (STR); Allele distribution; Population data

1. Introduction

The aim of this study was to investigate the distribution of two X-chromosomal STR loci (DXS7423 and DXS9902) in a population sample of 120 unrelated individuals ($n=60$ males and 60 females) and 43 family trios [in which paternity (and maternity) was already proved by probabilities exceeding 99.9%] living in Messina (Sicily, Southern Italy), as well as statistical evaluations, including the possible divergence from Hardy–Weinberg expectations and other parameters of forensic interest. DXS7423 and DXS9902 loci are situated, on opposite site, near the terminal end of the X-chromosome: in the Xq28 band (DXS7423) and in the Xp22.1 band (DXS9902). They are tetranucleotide polymorphism showing "simple" repeat sequences with a fragment length of 175–199 bp and 160–176 bp, respectively [1–4].

* Corresponding author.
E-mail address: alessio.asmundo@unime.it (A. Asmundo).

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2. DNA extraction, PCR amplification and allele typing

DNA was extracted from blood or buccal swabs according to a standard "Chelex®100" method [5]. DXS7423 and DXS9902 alleles were amplified separately (monoplex PCR) on a thermal cycler ("PCR sprint", Hybaid) using primer sequences already reported: DXS7423 [3]: $primer_1$ 5′ GTC TTC CTG TCA TCT CCC AAC 3′; $primer_2$ 5′TAG CTT AGC GCC TGG CAC ATA 3′; DXS9902 [6]: $primer_1$ 5′ TGG AGT CTC TGG GTG AAG AG 3′; $primer_2$ 5′ CAG GAG TAT GGG ATC ACC AG 3′. Each PCR reaction was performed with 2.5 μl extract (5–250 ng DNA), 0.5 μM of each primer, 2.5 μl *Taq* buffer (10× PCR Buffer II, Perkin Elmer), 2 μl $MgCl_2$, 25 mM (Perkin Elmer), 0.5 μl dNTPs mix (10 mM PCR Nucleotide Mix, Promega), 1 U *Taq* polymerase (DyNAzyme II DNA Polymerase, Finnzymes) in a total volume of 25 μl. A total of 30 cycles were carried out as follows: 95 °C for 60 s (denaturation), at 60 °C for 60 s (annealing) and at 72 °C for 60 s (extension). The PCR products were vertically electrophoresed on ultrathin (0.2–0.4 mm) layer polyacrilamide "native" gels in TBE buffer 0.5× and then revealed by silver staining [7]. Starting from already classified [8] standard DNA templates (K562, 9947A and 9948), allelic ladders were calibrated and then alleles were named on the variation of the number of repeats in comparison (side by side) with allelic ladders as recommended by the ISFG [9].

3. Results and discussion

Five and four different alleles were detected for DXS7423 and DXS9902 loci, respectively. PCR with DXS7423 primers resulted in fragments of 179–195 bp, just including alleles with different numbers (13–17) of the "simple" repeat sequence $(TCCA)_{13–17}$. Also DXS9902 locus exhibited clearly distinguishable alleles ranging from a fragment length of 160 bp ("9" allele) to 172 bp ("12" allele) and their designation was referred to the total number of the "simple" repeats: $(GATA)_{9–12}$. The allele frequencies were independently calculated for females and males. Since allele frequencies for both markers were similar in males and females, data from both sexes were combined. Allele frequencies are shown in Table 1 for DXS7423 and in Table 2 for DXS9902. Performing an exact test for both loci according to the GENEPOP software (http://wbiomed.curtin.edu.au/genepop/), no deviations from HWE were found in females. Some statistical parameters (H^{obs}, H^{exp}, PIC, PD, PE) were calculated [10] and are displayed in Tables 1 (for DXS7423) and 2 (for DXS9902). In comparison with other population data, there were no significant differences between allele frequencies. As shown in Tables 1 and 2 both markers were consistently

Table 1
DXS7423 locus

Allele	Length (bp)	Females ($N=60$)	Males ($N=60$)	Pooled alleles
13	179	0.0667	0.0333	0.0500
14	183	0.3583	0.4167	0.3875
15	187	0.4333	0.3500	0.3917
16	191	0.1167	0.1667	0.1417
17	195	0.0250	0.0333	0.0292

HWE (exact test): $p=0.2713$; H^{obs}: 0.66; H^{exp}: 0.66; PIC: 0.61; PD^F: 0.82; PD^M: 0.67; PE: 0.41.

Table 2
DXS9902 locus

Allele	Length (bp)	Females ($N=60$)	Males ($N=60$)	Pooled alleles
9	160	0.0917	0.0500	0.0708
10	164	0.3583	0.3000	0.3292
11	168	0.3250	0.3167	0.3208
12	172	0.2250	0.3333	0.2792

HWE (exact test): $p=0.0031$; H^{obs}: 0.80; H^{exp}: 0.86; PIC: 0.65; PD^F: 0.79; PD^M: 0.70; PE: 0.44.

informative with a probability of discrimination of 0.82 (PD^F) and 0.67 (PD^M) for DXS7423 and of 0.79 (PD^F) and 0.70 (PD^M) for DXS9902 system, respectively. Among the meiosis analyzed, we found no evidence of mutations at the DXS7423 and DXS9902 locus. The distance between loci (offering no problems of linkage), the length of alleles, together with the analytical and statistical results of this study, show that they are polymorphic STRs that can be very useful in forensics. In fact, as other markers on the X-chromosome, they can be useful in analyzing deficient cases of paternity, when the alleged child is a female and a sample of the alleged father is not available, or when the alleged fathers are father and son (not sharing the same X-chromosome). They can also be useful in other paternity testing involving rape and incest cases, or in cases when kinship analysis is requested for identification purposes, especially when is difficult to analyse degraded template material, such as DNA from exhumed samples, or in the analysis of mixed female/male stains, for the assumption that the chance of all male alleles being included in the female component is higher for ChrX than for autosomal markers [11].

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