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# Population genetic comparisons of three X-chromosomal STRs (DXS7132, DXS7133 and GATA172D05) in North and South Italy

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**Abstract.** Population genetic data of three tetranucleotide X-chromosomal STRs, DXS7132, DXS7133 and GATA172D05, were obtained by analyzing 295 unrelated healthy individuals living in North and South Italy (160 females and 135 males), and 40 family trios with female children. PCR primers for the loci DXS7132 and DXS7133 were redesigned in order to reduce the length of the amplification products compared with conventional design so that improved typing success rate when highly degraded DNA is used as a template. The comparison of the allele frequencies of these three ChrX markers gave similar distributions for North and South Italy although minor variations were found for some alleles. Additionally, some differences were found when comparing the allele frequencies of the male and female samples independently. Based on the investigated meiotic events, mutations were not found. 

*Keywords:* Forensic DNA typing; Paternity testing; Short tandem repeat; X chromosome

## 1. Introduction

While many autosomal STRs and several Y chromosome markers have been evaluated and widely applied to forensic individual identification and paternity testing, X-linked STRs are scantily studied in forensic sciences. To our knowledge, only 21 X-linked STR markers have been described for forensic applications so far [1].

The analysis of X-chromosomal STRs could help to solve deficiency paternity cases when the disputed child is female or the alleged father cannot be typed. Therefore, as males are hemizygous for the X chromosome, the mean exclusion chance (MEC) of X-chromosomal markers tends to be higher than that of diploid autosomal STR loci with comparable polymorphism information content (PIC) values.

The aim of this work was the study, in two different populations from North and South Italy, of three tetranucleotide STR loci (DXS7132, DXS7133 and GATA172D05) located

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at the X chromosome in the range from Xq 11 to Xq 24 to validate their potential utility in paternity testing and other forensic cases.

## 2. Material and methods

DNA was extracted from blood samples of 295 unrelated healthy individuals living in North and South Italy (160 females and 135 males) using GenomicPrep Blood DNA Isolation Kit (APB, Milan, Italy), and 40 family trios including female children were checked for regular X-chromosomal inheritance.

PCR amplification was performed in singleplex using the primers previously published for GATA172D05 and newly designed forward primer sequence for DXS7132 and reverse primer sequence for DXS7133. The newly designed primers were:

| | |
|---|---|
| DXS7132 | 5′ -CCCTCTCATCTATCTGACTG-3′ |
| | 5′ -GCCAAACTCTATTAGTCAAC-3′ |
| DXS7133 | 5′ -GCTTCCTTAGATGGCATTCA-3′ |
| | 5′ -GCCTGTCGTTCATGCTTA-3′ |

Primer forward for each locus was Cy5-labelled.

Amplifications were carried out in 25 μl of reaction volume containing 2–5 ng DNA, 0.4 μM of each primer, 200 μM of each dNTP, 1.5 mM $MgCl_2$, 1.5 U AmpliTaq DNA polymerase and 2.5 μl AmpliTaq buffer II (ABI, Foster City, CA). The amplification conditions were the same for the three ChrX markers and consisted of initial denaturation at 94 °C for 5 min, followed by 28 cycles at 94 °C for 60 s, 58 °C for 45 s, 72 °C for 60 s and final extension step at 72 °C for 10 min, in a GeneAmp PCR System 9700 Thermal Cycler (Applied Biosystems, Foster City, CA).

The amplified products were separated on a denaturing polyacrylamide gel by a Automated Laser Fluorescent DNA sequencer (ALF-Express, APB, Uppsala, Sweden), and allele typing was carried out based on sequenced alleles.

Heterozygosity (HET), polymorphism information content (PIC), power of discrimination (PD) and mean exclusion chance in trios involving daughters (MEC(I)) and in father/daughter duos lacking maternal genotype information (MEC(II)) were determined as proposed by Desmarais [2].

## 3. Results and discussion

The allele frequencies for each ChrX marker were calculated separately for females and males in both Italian samples and their comparisons did not show any significant statistical difference (Table 1), although rare allelic variants were found.

The allele distribution met the Hardy–Weinberg equilibrium for all three markers studied ($p>0.05$). Furthermore, both populations showed a similar PIC and corresponding values for MEC and HET. Based on the investigated meiotic events, no mutations were detected.

In paternity analysis and especially in deficiency cases, it should be borne in mind that DXS7132, DXS7133 and GATA172D05 are in the same "linkage group" [1]. The new primers were designed to shift closer to the repeat region in order to reduce the length of the amplicons compared with the conventional design.

Table 1
Allele frequencies and forensic efficiency of DXS7132, DXS7133, GATA172D05 markers

| Allele | DXS7132 | | | | DXS7133 | | | | GATA172D05 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Female | | Male | | Female | | Male | | Female | | Male | |
| | North | South | North | South | North | South | North | South | North | South | North | South |
| 5 | | | | | | | | | – | 0.007 | – | – |
| 6 | | | | | | | | | 0.218 | 0.213 | 0.180 | 0.114 |
| 7 | | | | | | | | | – | – | – | – |
| 8 | | | | | | | | | 0.167 | 0.167 | 0.180 | 0.205 |
| 9 | | | | | 0.446 | 0.467 | 0.517 | 0.591 | 0.034 | 0.053 | 0.045 | – |
| 10 | – | – | – | 0.023 | 0.163 | 0.147 | 0.090 | 0.091 | 0.316 | 0.333 | 0.270 | 0.455 |
| 11 | 0.006 | 0.020 | – | 0.023 | 0.331 | 0.333 | 0.315 | 0.295 | 0.178 | 0.160 | 0.213 | 0.159 |
| 12 | 0.128 | 0.213 | 0.124 | 0.182 | 0.042 | 0.033 | 0.056 | – | 0.086 | 0.067 | 0.090 | 0.068 |
| 13 | 0.291 | 0.347 | 0.315 | 0.341 | 0.012 | 0.007 | 0.022 | – | | | | |
| 14 | 0.343 | 0.253 | 0.315 | 0.341 | 0.006 | 0.013 | – | 0.023 | | | | |
| 15 | 0.192 | 0.120 | 0.180 | 0.091 | | | | | | | | |
| 16 | 0.041 | 0.047 | 0.045 | – | | | | | | | | |
| 17 | – | | 0.022 | – | | | | | | | | |
| HET | 0.692 | 0.712 | | | 0.596 | 0.578 | | | 0.741 | 0.746 | | |
| PIC | 0.712 | 0.725 | | | 0.646 | 0.634 | | | 0.774 | 0.773 | | |
| PD | 0.845 | 0.857 | 0.752 | 0.726 | 0.721 | 0.692 | 0.622 | 0.555 | 0.893 | 0.890 | 0.807 | 0.709 |
| MEC(I) | 0.574 | 0.537 | | | 0.414 | 0.342 | | | 0.656 | 0.626 | | |
| MEC(II) | 0.700 | 0.713 | | | 0.602 | 0.585 | | | 0.752 | 0.751 | | |

Furthermore, due to their small size, generally less than 140 bp, DXS7132, DXS7133 and GATA172D05 could be very useful in several specific forensic caseworks when highly degraded DNA samples are involved (e.g., DNA recovered from exhumed skeletons and formalin-fixed, paraffin-embedded tissues).

Like other ChrX markers, these three X-chromosomal STRs are highly informative markers for kinship testing and particularly interesting in deficiency paternity cases.

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