

Full Length Research Paper

X-Chromosomal short tandem repeat loci in the Turkish population

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In this study, we aimed to demonstrate the importance and utility of polymorphic short tandem repeat (STR) found on the human X chromosome and to provide the first allelic frequency data of X-STR (X chromosomal) loci in the Turkish population. Blood samples were taken from unrelated individuals (135 males and 129 females) from different regions of the country. Primers were designed according to gene bank data (www.gdb.org) and synthesised by the Köln Blutgruppen Institute. The primers amplified the following loci on the X chromosome: DXS8377, DXS101, DXS6789, STRX-1 and HUMHPRTB. Our data showed that two loci, DXS8377 and DX101, had the highest number of alleles (18) and the polymorphism information content (PIC) values of these loci were 0.9 and 0.87, respectively and were higher than those of other loci. In the other loci examined, 11 (STRX1), 10 (DXS6789) and 9 (HPRTB) alleles were detected and the PIC values of these loci were 0.78, 0.68 and 0.70, respectively. The highest (0.899) and lowest (0.674) rate of heterozygosity was found in the DXS8377 and HPRTB loci, respectively. In all loci, the power of discrimination for female (PD^F) values were higher than the power of discrimination for male (PD^M) values. The locus with the highest PD^F value was DXS8377 (0.9841). The mean exclusion change (MEC) value of this locus also proved to be the highest for both father-mother-child and father-child. The locus with the lowest MEC value was DXS6789. To use calculations of probabilities of genetic results for forensic purposes, allelic frequencies in individuals in the community should be determined. Our study provides the first data from the Turkish population.

Key words: X chromosomal (X-STR), forensic genetics, forensic sciences, DNA, kinship testing, paternity.

INTRODUCTION

Short tandem repeat (STR) analysis is the most effective method to identify individuals for forensic purposes. Initially, only autosomal STRs were used, but later, gonosomal STRs have been introduced for use in criminal identification and paternity cases (Asicioglu et al., 2002, 2003; Liang et al., 2005). One of the gonosomal STRs, Y chromosomal STR (Y-STR), has proved to be particularly effective in paternity cases, if the

child is male and the putative father cannot be determined. Y-STR is also useful in rape cases in which the DNA profile is mixed (Henke et al., 2001). After YSTR, studies have focused on X-STR (X chromosomal STR). At the beginning, only a small number of X-STR loci have been analysed with different populations (Lv et al., 2004; Hering et al., 2004; Pepinski et al., 2005), but at recent years, a growing number of X-STR loci have been reported for certain ethnic groups. (Asamura et al., 2006; Aler et al., 2007; Wu et al., 2009; Hwa et al., 2010; Mukerjee et al., 2010; Poetsch et al., 2010). X-STR multiplex polymerase chain reaction (PCR) systems, including a commercially available X-chromosome amplification kit (MENTYPE ARGUS X-8, BIOTYPE, Dresden, Germany), have also been used at the majority of these studies (Becker et al., 2008; Zalan et al., 2008; Hedman et al., 2009; Tie et al., 2010). X-STR multiplexes

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Abbreviations: STR, Short tandem repeat; PCR, polymerase chain reaction; PIC, polymorphism information content; PD^F , power of discrimination for females; PD^M , power of discrimination for males; MEC, mean exclusion chance.

utilizing reduced-size amplicons for identity and kinship testing have also been an effective system for analyzing degraded DNA (Asamura et al., 2006; Diegoli and Coble 2010). X-STR polymorphisms can be analysed in all cases of forensic identification as an adjunct to autosomal STRs and this technique can be helpful, especially for resolving cases in which the biological parents are unknown. As in the case with all other STR analyses, allelic frequencies of X-STRs in the population in which the individual belongs should be identified for statistical analysis. Here, we demonstrate the importance and utility of polymorphic STR loci on the human X chromosome and provide the first allelic frequency data of X-STR loci of the Turkish population.

MATERIALS AND METHODS

DNA was isolated from healthy unrelated males (135) and females (129) who applied to the Biology Department of the Council of Forensic Medicine, Ministry of Justice, to resolve paternity conflicts. The subjects were carefully chosen from all geographical regions of the country to serve as representative samples of the entire population. DNA was isolated using the Chelex method. Chelex 100 (BIO-RAD Laboratories, Hercules, CA), a chelating resin, scavenges metal contaminants to a high degree of purity. The resin is composed of styrene divinylbenzene copolymers containing paired iminodiacetate ions that act as chelating groups in binding polyvalent metal ions. Using this method, DNA is recovered from whole blood samples of donors, consisting of an initial wash step and then boiling in a 5% suspension of Chelex 100 (Lincoln and Thomson, 1998). Amplification was performed using fluorescently labelled primers for the DXS8377, DXS101, DXS6789, STRX-1 and HUMHPRTB loci as described by Henke et al. (2001). A PCR touchdown protocol was used and the cycling conditions were as follows: Denaturation at 95°C for 8 min, 7 cycles of denaturing at 94°C for 60 s, annealing at temperatures decreasing from 61 to 58°C (0.5°C decrease every cycle) for 60 s, extension at 72°C for 60 s and 23 repeats of the last cycle. The final extension step was 60°C for 15 h. A capillary electrophoresis ABI Prism 310 genetic analyser was used for typing. The internal lane standard (ILS) 400 size standard was used and consisted of a total of 16 DNA fragments with sizes ranging from 60 to 400 bp. The DNA fragment sizes increased by 20 from 60 to 200 bp and by 25 from 200 to 400 bp.

Data analysis

Heterozygosity was calculated based on the results from females (PowerStats V12 program). Other relevant data, such as polymorphism information content (PIC) (Salaru, 1993), power of discrimination for females (PD^F), power of discrimination for males (PD^M) (Jeffreys et al., 1985), mean exclusion chance (MEC) for "father, mother and daughter" (Gill et al., 1985) and MEC for "father and daughter" values were estimated using a previously published equation (Kishi et al. 1996; Jeffreys et al. 1985).

RESULTS AND DISCUSSION

Allelic frequencies of the loci investigated and their

statistical evaluation are presented in Tables 1 and 2, respectively. The two loci with the highest number of alleles were DXS8377 and DX101 and the highest PIC values (0.9 and 0.87, respectively) were detected at these loci. We found that, STRX 1 had 11 alleles, DXS6789 had 10 alleles and HPRTB had 9 alleles and the PIC values of these loci were found to be 0.78, 0.68 and 0.70, respectively. DXS8377 had the highest degree of heterozygosity at 0.899 and HPRTB had the lowest degree of heterozygosity at 0.674. In all loci, the PD^F value was higher than the PD^M value. The DXS8377 locus had the highest PD^F value at 0.9841. The locus with the lowest PD^F value was DXS6789.

Higher PIC, heterozygosity, PD and MEC values indicate the superiority of a locus in terms of its value to forensic investigation. Among the loci we analysed, the DXS8377 and DXS101 loci could be effectively utilised in forensic genetic investigations. The higher values obtained for these loci relative to the other loci are secondary in importance to the higher number of alleles and the more homogenous distribution of allelic frequencies in DXS8377 and DXS101 when compared with the other loci. Data from the same loci in different populations are in agreement with our results; the DXS8377 and DXS101 loci have been reported to be more informative than the other three loci analysed (Edelmann and Szibor, 2001; Chen et al., 2002; Zarrabeitia et al., 2002; Wiegand et al., 2003; Shin et al., 2004; Gomez et al., 2007; Martins et al., 2010).

Among the other three loci, STRX1 had higher values than HPRTB and DXS6789. However, the presence of only one base pair difference between alleles of this locus can complicate interpretations. The DXS6789 locus had a higher number of alleles and greater heterozygosity than the HPRTB locus, but with relatively lower statistical values. This result may be due to the fact that, up to 70% of the population has alleles 20 and 21.

Data obtained by Bini et al. (2005) from samples taken from 556 Italians were identical to our results with respect to allelic variation and the frequencies of alleles at the DXS101 and HPRTB loci (Bini et al., 2005).

For locus STRX1 (DXS981), the reverse was true. Because of the paucity of alleles and their heterogeneous distribution in the Han population, the PD^F (0.837), PD^M (0.691) and PIC (0.63) values were much lower than those found in the Turkish population (Zhang et al., 2004). In a study of 401 Koreans, approximately identical and even somewhat higher values were found (PD^F : 0.940; PD^M : 0.831; PIC: 0.806) at the STRX1 (DXS981) locus (Shin et al., 2005).

These results demonstrate that, a locus with insufficient informative value in one population can be beneficial in a different population. Allelic frequencies at each locus in question in every community should be determined so that, they can be utilised to calculate the probability of genetic results for forensic purposes. In this respect, our study provides the first data in the Turkish population.

Table 1. Contd.

Allele number	n	f	n	f	n	f	n	f	n	f	n	f	n	f	n	f	n	f	n	f	n	f	n	f	n
44	13	0.0963	17	0.0659	30	0.0763																			
45	17	0.1259	32	0.1240	49	0.1247																			
46	20	0.1481	34	0.1318	54	0.1374																			
47	15	0.1111	33	0.1279	48	0.1221																			
48	13	0.0963	27	0.1047	40	0.1018																			
49	10	0.0741	16	0.0620	26	0.0662																			
50	9	0.0667	29	0.1124	38	0.0967																			
51	7	0.0519	8	0.0310	15	0.0382																			
52	6	0.0444	8	0.0310	14	0.0356																			
53	2	0.0148	2	0.0078	4	0.0102																			
54	1	0.0074	5	0.0194	6	0.0153																			
55																									
56	0	0.0000	1	0.0039	1	0.0025																			

Table 2. Statistical parameters of DXS8377, DXS101, DXS6789, STRX-1 and HUMHPRTB loci for Turkish population.

Parameter	DXS 6789	STR X1	DXS 8377	DXS 101	HPRTB
PIC	0.6800	0.7800	0.9000	0.8700	0.7000
Het.	0.6900	0.6980	0.8990	0.8530	0.6740
PD female	0.8847	0.9371	0.9841	0.9758	0.8896
PD male	0.7140	0.8085	0.9082	0.8537	0.7145
MEC trios	0.6838	0.7842	0.9020	0.8639	0.6974
MEC F/D	0.5435	0.6627	0.8280	0.7721	0.5582

PIC: Polymorphism information content; Het: observed heterozygosity; PD: power of discrimination; MEC: mean exclusive change.

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