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Polymorphism of 8 X-Chromosomal STR loci in Turkish population

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Abstract

In forensic sciences, X-chromosomal short tandem repeats (X-STRs), a complementary marker to the other genetic markers (autosomal, Y-chromosomal or mitochondrial), can be used in complex kinship analysis. Especially when a biological sample is not available from the putative father and samples from paternal relatives could be used instead. In this study eight X-chromosomal STR loci (DXS7132, DXS7423, DXS8378, DXS10074, DXS10101, DXS10134, DXS10135, HPRTB and Amelogenin) were investigated in population samples of 129 unrelated healthy Turkish volunteer [male (n=56), female (n=73)]. X-STRs were amplified in a multiplex PCR reaction with Mentype® Argus X-8 Kit. The separation and detection of PCR products were performed by capillary electrophoresis on a 310 Genetic Analyzer (Applied Biosystems). Statistical analysis was performed using POPgene v.1.32. and Arlequin v3.1 software's. Data was compared with Turkish populations living in Europe (Germany and Denmark) and other populations (Japan, Ghana, Finland, Portugal and Germany). The high genetic distances were observed for the geographically distant populations while no significant differences found between in studied and tested Turkish populations. Results showed that eight STR loci on the X chromosome in Turkish population were found as highly polymorphic and useful for forensic applications.

Keywords: X-STR, kinship analysis, Turkish population

Introduction

In forensic genetics STR markers are widely used for identification of biological evidences and kinship analysis [1]. However, in some complex kinship cases, analysis of the standard STR markers may be insufficient due to missing individuals in paternities or kinship. X chromosome markers are having a very special inheritance pattern in which women inherit one X chromosome from the mother and the second from the father and men transfer only one X chromosome from the mother as the father passes on a Y chromosome instead [2]. Therefore, markers such as X-STRs may be useful indirect examinations of father-daughter, mother-son relationships and also in more distant relationships testing. X chromosome markers are particularly informative for determining paternity and kinship relationships that involve at least one female [i.e. mother–daughter, mother–son, half-sisters and father–daughter duos). X chromosome markers have advantages in deficient paternity cases, when females have the same father, they also share the same paternal X chromosome [3].

Within paternity investigations, a child's genotype would be compared to his or her mother's and the alleged father(s) under investigation. If there is no match between the questioned sample and the known, then the samples may be thought to have originated from different sources. The term "exclusion" is used for such fail DNA profile matching conditions. If a match or 'inclusion' results, then a comparison of the DNA profile is questioned from a database, which is a collection of genetic content obtained from unrelated individuals of a particular population groups with specific marker(s) [2,3]. Thus, it is necessary to establish such population data for forensic purposes in Turkey.

The aim of this study was determining Turkish population data for eight X-STR loci (DXS7132, DXS7423, DXS8378, DXS10074, DXS10101, DXS10134, DXS10135 and HPRTB), and compared it with other published population data (Turkish populations -living in Germany and Denmark-, Japan, Germany, Ghana, Portugal and Finland).

Material and methods

Blood samples were collected from 129 unrelated volunteers of Turkish population (56 males and 73 females) with their informed consents. The study was approved by Ethical Committee of Cerrahpaşa Medicine Faculty, Istanbul University (Number: 27050). The DNA was extracted using two different methods Chelex [4] or the QIAmp DNA Minikit [5], quantitated by using Invitrogen Qubit Fluorometer. DNA was amplified using Mentype® Argus X–8 PCR Amplification Kit (DXS7132, DXS7423, DXS8378, DXS10074, DXS10101, DXS10134, DXS10135, HPRTB and Amelogenin) that allows a single-tube co-amplification and detection of eight loci [6]. The separation and detection of PCR

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products were performed by capillary electrophoresis on the ABI PRISM® 310 Genetic Analyzer (Applied Biosystems, USA), using the ROX 550 internal lane standard (Applied Biosystems, USA). The alleles were typed using the GeneScan® software (Applied Biosystems, USA). Allele frequencies were calculated with software POPGENE Version 1.32 [7]. Polymorphism Information Content (PIC), Expected Heterozygosity (Het), Power of Identity (PI), Mean Exclusion Chance (MEC), Power of Discrimination in males (PDm) and in females (PDf), expected probability of exclusion (PE) values were calculated via the ChrX database (http://www.chrx-str.org/) that has been developed by forensic chromosome X research group (ChrX The Forensic Research Group) [8]. Arlequin v3.1 software tool was used to test for significant deviations from Hardy–Weinberg equilibrium and p-Value of exact test of population differentiation.

Results

The distribution of observed allele frequencies for the 8 loci in Argus X-8 kit, calculated for Turkish population. The allele frequency of the male and female individuals also computed separately. Table 1 shows the observed allele frequencies and forensic statistical parameters for the eight markers investigated in our data set. We detected 19 alleles for DXS10135 locus, 15 alleles for DXS10101, 14 alleles for DXS10134, DXS10074, 9 alleles for HPRTB, 7 alleles for DXS7423, DXS8378 and DXS7132. Observed genotype frequencies in female individual confirmed Hardy–Weinberg Equilibrium after Bonferroni correction, except for locus 1and 2 for which the p-values were lower than 0.00625. DXS10135 and DXS10101 were the most polymorphic markers with a heterozygosity of 0.92 and 0.90 respectively, whereas DXS8378 showed the lowest degree of heterozygosity (0.71). Among all markers, DXS10135, DXS10101 and DXS10074 showed the highest values of all forensic parameters investigated: Polymorphism Information Content, Mean Exclusion Chance, PDF and PDM; whereas, markers of DXS8378 and DXS7423 presented lowest values for the forensic parameters investigated (Table 1). The allele frequencies of Turkish population compared with other populations: Turkish populations living in Germany [9], Turkish populations living in Denmark [10], German [11], Portugal [12], Finland [13], Japan [14] and Ghana [15] populations by applying the Fisher exact test differentiation were shown in Table 2. After Bonferroni's correction ($p < 0.00625$), population differentiation tests showed that the population of Turkey had statistically significant differences with Japan [14] in 7 out of 8 loci (DXS7132, DXS7423, DXS8378, DXS10074, DXS10101, DXS10134 and HPRTB), with Ghana [15] in 7 out of 8 loci (DXS7423, DXS8378, DXS10074, DXS10101, DXS10134, DXS10135 and HPRTB), with Finland [13] in 6 out of 8 loci (DXS7132, DXS7423, DXS8378, DXS10074, DXS10134 and HPRTB) and with Germany [11] in 5 out of 8 loci (DXS7132, DXS7423, DXS8378, DXS10101, DXS10135). No significant differences were observed between the Turkey and Portugal [12] populations. Moreover, there were no significant differences between Turkish population in this study and Turkish population those living in Denmark [10]. We observed only on loci (HPRTB) difference in comparison with Turkish population in this study and Turkish population those living in Germany [11].

Discussion

This study indicates that a high genetic diversity was determined in Turkish population for the 8 studied X-STRs. In general, the X-STRs (DXS7132, DXS7423, DXS8378, DXS10074, DXS10101, DXS10134, DXS10135, HPRTB) used in this work proved to be highly discriminative due to high heterozygosity among all the locus and therefore useful for forensic purposes. We found that DXS10135 and DXS10101 has highest heterozygosity in Turkish population and these are the most informative X-STR loci. The study from Ruivo et al. (2008), Poulsen et al. (2016) and Sufian et al (2017) also reported DXS10135 and DXS10101 locus as the most polymorphic X-chromosomal marker in all populations [9,12,16].

Overall values of the power of discrimination were high, supporting the potential of this octaplex in identification studies. The high values obtained for the combined mean exclusion chance also confirm the effectiveness of this system in kinship testing, in paternity tests including female offspring when complete trios or only alleged father/daughter duos are available, in paternity cases implicating a father and his son as putative fathers and in maternity investigations of male descendants.

This study shows that the polymorphism information content (PIC) of the eight X-STR loci ranged from 0.66626 (DXS8378) to 0.91440 (DXS10135) and power of discrimination ranged from 0.86905 (DXS8378) to 0.98789 (DXS10135) in females, 0.71729 (DXS8378) to 0.92013 (DXS10135) in males. The finding of this study provide similarities with other studies those calculated male and female forensic parameters [11,13].

According to the obtained results, Turkish those living in Europe and Portugal populations show more genetic similarities with the Turkish population in this study than with the other compared populations. The amount of information about polymorphic

X-STR loci in the globally differentiated populations (Germany, Finland, Ghana, and Japan) are sufficient which could be explained by population migration, historical events, and geographic barriers etc. Therefore, the results are generally in accordance with the biogeography distribution of the previously studied populations and their historical relationships with the Turkish population. Noteworthy, Guo et al. (2017) compared Northern Han Chinese population with 35 different populations from Asia, Europe, Africa and America regions. Further, in accordance with our results, the study has been thoroughly explained differences and similarities between all available populations [17].

Conclusion

Overall, our work has demonstrated the possibility of simultaneous genotyping of 8 highly polymorphic X-STRs in a single multiplex PCR, which can be used both in paternity investigations and in more complex forensic cases. This report of haplotype frequencies would serve as a reference database for individual identification in Turkey.

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Table 1. Allele frequencies and forensic parameters of 8 X-STR loci

Allele	DXS8378			HPRTB			DXS7423			DXS7132			DXS10134			DXS10074			DXS10101			DXS10135		
	Male	Female	Total	Male	Female	Total	Male	Female	Total	Male	Female	Total	Male	Female	Total	Male	Female	Total	Male	Female	Total	Male	Female	Total
4																0.0000	0.0000	0.0000						
7																0.0364	0.0139	0.0201						
8	0.0000	0.0274	0.0198													0.1636	0.0972	0.1156						
9	0.0357	0.0205	0.0248	0.0000	0.0141	0.0102										0.0182	0.0139	0.0151						
10	0.4107	0.3356	0.3564	0.0000	0.0000	0.0000										0.0000	0.0069	0.0050						
11	0.3214	0.3151	0.3168	0.2037	0.2324	0.2245				0.0364	0.0145	0.0207				0.0000	0.0000	0.0000						
11.2				0.0000	0.0211	0.0153										0.0000	0.0000	0.0000						
12	0.1964	0.2397	0.2277	0.2963	0.2465	0.2602	0.0000	0.0069	0.0050	0.0909	0.0942	0.0933				0.0000	0.0000	0.0000						
13	0.0179	0.0616	0.0495	0.3704	0.3521	0.3571	0.0364	0.0972	0.0804	0.2545	0.2971	0.2850				0.0364	0.0000	0.0101						
14	0.0000	0.0000	0.0000	0.0741	0.0986	0.0918	0.2727	0.3611	0.3367	0.3455	0.2971	0.3109				0.0182	0.0486	0.0402						
15	0.0179	0.0000	0.0050	0.0556	0.0211	0.0306	0.4727	0.3056	0.3518	0.2364	0.2391	0.2383				0.0364	0.0833	0.0704						
16				0.0000	0.0070	0.0051	0.1636	0.1389	0.1457	0.0182	0.0435	0.0363				0.2182	0.2361	0.2312				0.0182	0.0000	0.0051
16.2																0.0182	0.0347	0.0302						
17				0.0000	0.0070	0.0051	0.0545	0.0833	0.0754	0.0182	0.0145	0.0155				0.1273	0.1875	0.1709				0.0000	0.0071	0.0051
18							0.0000	0.0069	0.0050							0.1455	0.1458	0.1457				0.0364	0.0357	0.0359
19																0.1455	0.0833	0.1005				0.0727	0.1000	0.0923
20																0.0182	0.0486	0.0402				0.0364	0.1143	0.0923
21																0.0182	0.0000	0.0050				0.1818	0.0714	0.1026
22																						0.0909	0.1071	0.1026
23																						0.0545	0.1143	0.0974
24																						0.1273	0.0857	0.0974
24.2																			0.0000	0.0000	0.0000			
25																						0.0727	0.0929	0.0872
26																						0.0727	0.0429	0.0513
26.2																			0.0000	0.0000	0.0000			
27																			0.0000	0.0145	0.0106	0.0364	0.0571	0.0513
27.2																			0.0192	0.0290	0.0263			
28																			0.0192	0.0290	0.0263	0.0545	0.0786	0.0718
28.2																			0.0769	0.1304	0.1158			
29																			0.0192	0.0580	0.0474	0.0545	0.0500	0.0513
29.2																			0.1731	0.0870	0.1105			
30																			0.0769	0.1159	0.1053	0.0182	0.0214	0.0205
30.2																			0.0962	0.1667	0.1474			
31													0.0000	0.0317	0.0221				0.0962	0.0797	0.0842	0.0182	0.0143	0.0154
31.2																			0.0962	0.0870	0.0895			
32													0.0182	0.0476	0.0387				0.0577	0.0580	0.0579	0.0182	0.0000	0.0051
32.2																			0.1154	0.0290	0.0526			
33													0.0364	0.0238	0.0276				0.1346	0.0797	0.0947	0.0364	0.0000	0.0102
33.2																			0.0000	0.0072	0.0052			
34													0.2000	0.1270	0.1492				0.0192	0.0290	0.0263	0.0000	0.0000	0.0000
35													0.2000	0.2143	0.2099							0.0000	0.0000	0.0000
36													0.2545	0.2063	0.2210							0.0000	0.0071	0.0051

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37					0.1091	0.2063	0.1768	
38					0.0909	0.0397	0.0552	
38.3					0.0364	0.0159	0.0221	
39					0.0000	0.0079	0.0055	
39.3					0.0000	0.0238	0.0166	
40.3					0.0364	0.0079	0.0166	
41.3					0.0000	0.0159	0.0110	
42.3					0.0000	0.0000	0.0000	
43.3					0.0182	0.0079	0.0110	
44.3					0.0000	0.0000	0.0000	
PIC	0.666262	0.702861	0.684935	0.713678	0.828718	0.848473	0.899397	0.914400
Het	0.717287	0.744622	0.729442	0.754638	0.846475	0.862820	0.906917	0.920126
PE	0.455508	0.500609	0.475263	0.517734	0.687930	0.720282	0.809572	0.836692
PI	0.141356	0.127689	0.135279	0.122681	0.076762	0.068590	0.046542	0.039937
PDf	0.869049	0.893021	0.882291	0.898838	0.958672	0.966835	0.983815	0.987894
PDm	0.717287	0.744622	0.729442	0.754638	0.846475	0.862820	0.906917	0.920126
MEC Krüger	0.468376	0.515827	0.495862	0.528044	0.678108	0.727206	0.811089	0.837283
MEC Kishida	0.666262	0.702743	0.684935	0.713678	0.809941	0.848250	0.899397	0.914293
MEC Desmarais	0.666262	0.702861	0.684935	0.713678	0.828718	0.848473	0.899397	0.914400
MEC Desmarais Duo	0.523589	0.564519	0.544636	0.576881	0.722121	0.749155	0.823771	0.847331
HWE	0.00609	0.00283	0.17396	0.75130	0.02790	0.39206	0.23881	0.16126

Table 2. Population differentiation tests between Turkey and other populations ($p < 0.05$)

Populations	DXS7132	DXS7423	DXS8378	HPRTB	DXS10074	DXS10134	DXS10101	DXS10135
Japan	0.00370*	0.00000*	0.00000*	0.00000*	0.00000*	0.00000*	0.00000*	0.80375
Ghana	0.76010	0.00000*	0.00455*	0.00065*	0.00000*	0.00000*	0.00000*	0.00000*
Germany	0.67340	0.05605	0.05970	0.00105*	0.00485*	0.00000*	0.05845	0.56930
Portugal	0.74630	0.18475	0.36665	0.25835	0.53885	0.08020	0.19400	0.09115
Finland	0.00000*	0.00000*	0.00000*	0.00005*	0.00075*	0.00000*	0.00935	0.24915
Turkish population in Germany	0.59900	0.27090	0.79040	0.00000*	**NA**	**NA**	**NA**	**NA**
Turkish population in Denmark	0.92320	0.88785	0.26145	0.90645	0.30470	0.00120	0.38635	0.49655

* Significant differentiation test P-values, after Bonferroni correction ($p<0.00625$)
Na = No data available

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