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Five highly informative X-chromosomal STRs in Koreans

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Abstract The five X-chromosomal short tandem repeats (STRs) GATA172D05, HPRTB, DXS8377, DXS101 and HumARA were analyzed in 150 males and 150 females from Korea. Markers were amplified in a quadruplex and a monoplex PCR reaction with fluorescently labeled primers. For accurate and reproducible STR typing, sequenced allelic ladders were constructed and a Genotyper macro was programmed. Some differences were found on comparing the allele frequencies of Koreans with those of other populations in DXS8377, DXS101 and HumARA. The forensic efficiency parameters showed that the five X-linked STRs are highly informative for forensic application in Koreans.

Keywords STR · X-chromosome · Korea

Introduction

A large number of autosomal and Y-chromosomal STRs have been studied for forensic purpose and widely applied in human identification and paternity testing [1, 2, 3]. In contrast, X-chromosomal STRs have not made a great contribution to forensic analysis to date. However, X-linked markers can be a more powerful tool than autosomal STRs in deficiency cases, especially when the disputed child is female [4]. Additionally, some special deficiency cases can be solved only through the application of X-linked markers. For example, X-markers are required to exclude paternity where the question is whether two women who were separated as children have the same father.

The aim of this study was to show the polymorphism of five X-chromosomal STR loci (GATA172D05, HPRTB, DXS8377, DXS101 and HumARA) in Koreans, and to evaluate their efficiency in forensic practice. HPRTB [5, 6, 7, 8, 9, 10, 11, 12, 13] and HumARA [5, 7, 11, 12, 13, 14, 15, 16] loci were chosen because they have already been established for forensic applications. On the other hand the GATA172D05 [11, 17], DXS8377 [11, 17, 18, 19] and DXS101 [11, 13, 19, 20, 21] loci were selected as according to recent previous reports they have been suggested to be highly informative for forensic applications.

Materials and methods

Buccal swab samples were obtained from 300 unrelated Koreans (i.e. 150 males and 150 females) as well as 25 families to check regular X-chromosomal inheritance. Genomic DNA was extracted using the QIAamp DNA Mini kit (Qiagen, Hilden, Germany) according to the manufacturer's protocols.

Primer sequences and concentrations for PCR amplification of the five X-chromosomal STRs were as listed in Table 1. A quadruplex PCR for GATA172D05, HPRTB, DXS8377 and DXS101 was carried out in a 10 µl reaction volume containing 1–2 ng DNA, 1.0 µl GeneAmp 10× PCR buffer (Applied Biosystems, Foster City, CA), 200 µM dNTPs and 1.5 U AmpliTaq Gold DNA polymerase (Applied Biosystems, Foster City, CA). A monoplex PCR for HumARA was carried out under the same conditions as the quadruplex PCR except for the use of 0.5 U AmpliTaq Gold DNA polymerase and the HumARA primer set. Thermal cycling was conducted on a PTC-200 DNA engine (MJ Research, Waltham, MA) under the following conditions: 95°C for 11 min, followed by 30 cycles at 94°C for 1 min, 59°C for 1 min, 72°C for 1 min and a final extension at 60°C for 30 min. The PCR products were analyzed by capillary eletrophoresis using an ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Foster City, CA).

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Table 1 Primer sequences and primer concentrations for the five X-linked STR loci

Locus	Sequence (5' to 3')	Label	Primer conc. (μM)
GATA172D05			
Primer 1	5'-TAG TGG TGA TGG TTG CAC AG-3'	FAM	0.07
Primer 2	5'- ATA ATT GAA AGC CCG GAT TC-3'	–	0.07
HPRTB			
Primer 1	5'-TCT CTA TTT CCA TCT CTG TCT CC-3'	FAM	0.08
Primer 2	5'-TCA CCC CTG TCT ATG GTC TCG-3'	–	0.08
DXS8377			
Primer 1	5'-CAC TTC ATG GCT TAC CAC AG-3'	FAM	0.15
Primer 2	5'-GAC CTT TGG AAA GCT AGT GT-3'	–	0.15
DXS101			
Primer 1	5'-ACT CTA AAT CAG TCC AAA TAT CT-3'	HEX	0.25
Primer 2	5'-AAA TCA CTC CAT GGC ACA TGT AT-3'	–	0.25
HumARA			
Primer 1	5'-TCC AGA ATC TGT TCC AGA GCG TGC-3'	HEX	0.50
Primer 2	5'-GCT GTG AAG GTT GCT GTT CCT CAT-3'	–	0.50

Table 2 Observed alleles, fragment lengths, and repeat sequence structures of the five X-linked STR markers

Locus	Observed alleles	Fragment length (bp)	Repeat sequence structure	Genotype of standard DNA	
				K562	9947A
GATA172D05	6–12	108–132	---$(TAGA)_{6-12}$---	12, 12	10, 10
HPRTB	11–16	155–175	---$(AGAT)_{11-16}$---	13, 13	14, 14
DXS8377	41–59	216–270	---$(AGA)_x$- (GGA-AGA)$_y$-$(AGA)_2$-GGA- $(AGA)_6$---	52, 52	45, 47
DXS101	21–30	200–227	---$(CTT)_x$- $(ATT)_y$---	23, 24	24, 26
HumARA	12–30	258–312	---$(CAG)_{12-30}$---	25, 25	21, 30

Sequenced allelic ladders were constructed for all markers by combining observed alleles from each locus. Each allele was sequenced on an ABI 310 Genetic Analyzer using a BigDye Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, CA). Allele typing was carried out based on the sequenced allelic ladders and Genotyper software (Applied Biosystems, Foster City, CA). Alleles were assigned according to the recommendations of the International Society of Forensic Genetics (ISFG) Commission [22]. However, HPRTB and HumARA alleles were assigned in a way that they maintained consistency with the nomenclature that has been used in previous studies. The cell line samples K562 and 9947A (Promega, Madison, WI) were used as control DNA for calibrating allelic ladders.

The following statistical analyses were carried out: expected heterozygosity (Het) [23], polymorphic information content (PIC) [24], mean exclusion chance in trio cases (MEC) [16], power of exclusion in motherless cases (PE) [16], power of discrimination (PD) in females and males [16]. To evaluate Hardy-Weinberg equilibrium (HWE) Fisher's exact test using the GDA program (http://lewis.eeb.uconn.edu/lewishome/software.html) was performed.

Results and discussion

Although the initial PCR amplification for five X-linked STRs was designed as a pentaplex reaction, it was found that the simultaneous amplification of DXS101 and HumARA was difficult. Therefore, a quadruplex and a monoplex for HumARA were carried out and the two PCR products were analyzed by an automatic DNA sequencer together. The amplification of all markers was easily done as the above process with 1 ng of DNA sample, and consistent results were observed from repeated typing of DNA samples. Stutter peaks were frequently observed at DXS8377 and HumARA, but they did not make typing of the samples difficult. For accurate and reproducible STR typing, sequenced allelic ladders were constructed and a Genotyper macro was programmed. The investigation of standard DNA, K562 and 9947A revealed that the typing results of the present study were in agreement with reports in the literature for DXS101 [19] but not for DXS8377 [25]. In the case of DXS8377, the alleles of K562 and 9947A were shown to be two repeats more than those of previous reports and this was confirmed by cloning and sequencing of the PCR products (Table 2).

Tables 3, 4, 5, 6, 7 show allele frequencies and the forensic efficiency for the five X-chromosomal STRs in Koreans. The allele distribution of GATA172D05 in a Korean population was similar to that of a German population reported by Edelmann et al. [11]. For HPRTB, the allele distribution in our Korean population was similar to that of Japanese [8] and Chinese in Taiwan [12], while the alleles 9 and 10 common in a German population [10, 11] were not found in Koreans. In the case of DXS8377, alleles 58 and 59 were unique for Koreans while alleles 37–40, which were observed in Germans [11, 19] and the report of Zarrabeitia et al. [18], were not found in a Korean population at all. Also, alleles 55, 56 and 57 of DXS8377 were found in Koreans and Germans [11], but not in the study

Table 3 Allelic frequencies and forensic efficiency of GATA172D05 locus in Koreans

Allele	Female	Male	Cumulated
6	0.083	0.073	0.080
7	0.003	0.007	0.004
8	0.150	0.167	0.156
9	0.087	0.087	0.087
10	0.413	0.380	0.402
11	0.223	0.220	0.222
12	0.040	0.067	0.049
Het	0.743	–	
PIC	0.706	–	
MEC	0.706	–	
PE	0.596	–	
PD	0.898	0.762	

Het Expected heterozygosity.
PIC Polymorphic information content.
MEC Mean exclusion chance in trio cases
PE Power of exclusion in motherless cases
PD Power of discrimination

Table 4 Allelic frequencies and forensic efficiency of HPRTB locus in Koreans

Allele	Female	Male	Cumulated
11	0.030	0.047	0.036
12	0.323	0.293	0.313
13	0.420	0.407	0.416
14	0.193	0.180	0.189
15	0.027	0.053	0.036
16	0.007	0.020	0.011
Het	0.682	–	
PIC	0.621	–	
MEC	0.621	–	
PE	0.520	–	
PD	0.839	0.711	

of Zarrabeitia et al. [18]. In DXS101, the Korean population showed a distinctive allele distribution since the common alleles 15, 18, 19 and 20 in Germans [11], Austrians [19] and the paper of Zarrabeitia et al. [13] were not observed in Koreans. On the other hand, the allele distribution of HumARA in the Korean population is similar to that of Japanese [15]. However, the alleles below allele 12 in Germans [11] and the alleles above allele 30 in Chinese in Taiwan [12] were not found in Koreans at all.

All markers are located on the q arm of chromosome X [4], and yet in our study, no deviation of linkage equilibrium was found among loci ($p>0.05$). Also, we did not find significant deviations from Hardy-Weinberg equilibrium for each individual marker ($p>0.05$). In the statistical analysis and forensic efficiency data, DXS8377 showed the highest heterozygosity (Het), polymorphic information content (PIC), mean exclusion chance (MEC), power of exclusion (PE) and power of discrimination (PD), followed by HumARA, DXS101, GATA172D05 and HPRTB in that sequence (Tables 3, 4, 5, 6, 7). All statistical parameters for forensic efficiency were found to be lower in

Table 5 Allelic frequencies and forensic efficiency of DXS8377 locus in Koreans

Allele	Female	Male	Cumulated
41	0.003	–	0.002
42	0.007	0.013	0.009
43	0.043	0.013	0.033
44	0.030	0.027	0.029
45	0.083	0.100	0.089
46	0.110	0.113	0.111
47	0.150	0.087	0.129
48	0.087	0.133	0.102
49	0.120	0.167	0.136
50	0.130	0.073	0.111
51	0.077	0.080	0.078
52	0.057	0.080	0.064
53	0.047	0.027	0.040
54	0.023	0.033	0.027
55	0.007	0.040	0.018
56	0.013	0.007	0.011
57	0.007	–	0.004
58	0.003	0.007	0.004
59	0.003	–	0.002
Het	0.908	–	
PIC	0.897	–	
MEC	0.897	–	
PE	0.814	–	
PD	0.983	0.901	

Table 6 Allelic frequencies and forensic efficiency of DXS101 locus in Koreans

Allele	Female	Male	Cumulated
21	0.017	–	0.011
22	0.037	0.040	0.038
23	0.113	0.100	0.109
24	0.310	0.213	0.278
25	0.183	0.273	0.213
26	0.167	0.207	0.180
27	0.110	0.120	0.113
28	0.047	0.027	0.040
29	0.007	0.007	0.007
30	0.010	0.013	0.011
Het	0.816	–	
PIC	0.790	–	
MEC	0.790	–	
PE	0.662	–	
PD	0.942	0.810	

Koreans than in Germans [11], demonstrating that Koreans are a more homogeneous population.

Although the number of meioses analyzed was not sufficient for evaluating the mutation rates of these systems, we found 1 mutation out of 25 proven families (36 meioses) in DXS8377. The father had allele 51, the mother had alleles 47, 50 and the daughter was homozygous for allele 50, suggesting a loss of 1 repeat unit from the paternal allele. The other X-chromosomal STRs exhibited typical X-linked inheritance.

Table 7 Allelic frequencies and forensic efficiency of HumARA locus in Koreans

Allele	Female	Male	Cumulated
12	0.010	0.007	0.009
13	–	0.007	0.002
14	–	–	–
15	0.007	0.013	0.009
16	0.013	0.013	0.013
17	0.017	0.027	0.020
18	0.027	0.033	0.029
19	0.033	0.067	0.044
20	0.067	0.093	0.076
21	0.153	0.107	0.138
22	0.133	0.207	0.158
23	0.153	0.113	0.140
24	0.157	0.093	0.136
25	0.090	0.073	0.084
26	0.037	0.047	0.040
27	0.053	0.027	0.044
28	0.037	0.033	0.036
29	0.007	0.020	0.011
30	0.007	0.020	0.011
Het	0.893	–	
PIC	0.880	–	
MEC	0.880	–	
PE	0.812	–	
PD	0.978	0.899	

In conclusion, the 5 X-chromosomal STRs are highly informative for forensic applications, and DXS101, DXS8377 and HumARA are especially useful in the investigation of kinship analysis and deficiency cases.

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