

A Large Population Genetic Study of 15 Autosomal Short Tandem Repeat Loci for Establishment of Korean DNA Profile Database

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Genotyping of highly polymorphic short tandem repeat (STR) markers is widely used for the genetic identification of individuals in forensic DNA analyses and in paternity disputes. The National DNA Profile Databank recently established by the DNA Identification Act in Korea contains the computerized STR DNA profiles of individuals convicted of crimes. For the establishment of a large autosomal STR loci population database, 1805 samples were obtained at random from Korean individuals and 15 autosomal STR markers were analyzed using the AmpF/STR Identifier PCR Amplification kit. For the 15 autosomal STR markers, no deviations from the Hardy-Weinberg equilibrium were observed. The most informative locus in our data set was the D2S1338 with a discrimination power of 0.9699. The combined matching probability was 1.521×10^{-17} . This large STR profile dataset including atypical alleles will be important for the establishment of the Korean DNA database and for forensic applications.

INTRODUCTION

Short tandem repeats (STRs), also called microsatellites, are powerful molecular markers for the identification of human individuals due to their hypervariability and wide distribution throughout the genome. STR typing has been facilitated by the ability to amplify several loci simultaneously in a multiplex polymerase chain reaction (PCR). Although di-nucleotide repeat STRs are frequently used in other species study (Lee et al., 2009), STRs with tetra to penta-nucleotide repeats are exclusively applied for human identification. Autosomal STR loci are routinely used for kinship testing and forensic identification (Butler, 2006; Hammond et al., 1994; Tautz, 1989).

Forensic DNA databases have been established in many countries. In the United States, the DNA Identification Act, which was included in the 1994 Crime Bill, authorized the FBI

to establish the Combined DNA Index System (CODIS) for law enforcement purposes. Now, CODIS has been accepted by forensic laboratories all over the world. Owing to the success of criminal DNA profile databases (Martin, 2004), their database has increased rapidly. CODIS in the United States and the National DNA Database (NDNAD) in England each contain more than five million personal profiles.

Recently, the establishment of a new national offender DNA databank was authorized by the DNA Identification Act in Korea, which was initiated in July, 2010. Thousands of DNA profiles will be deposited in the national DNA profile databank each year. Before a discussion on the effectiveness of the database, it is important to consider the standardization of the Korean STR loci in terms of allele frequencies including atypical types or rare allele distribution. When population data are applied to determine forensic statistical values, ethnic group-specific DNA profile databases are essential (Budowle et al., 2001). In particular, while several commercially available human multiplex STR PCR kits are usually fitted to Americans or Europeans, the provided allele frequencies are occasionally not suitable for application for the Korean population. Therefore, there is a need to establish a large population database of Koreans for reliable statistical analyses of the population genetics and forensic applications.

In the course of typing samples, non-standard patterns of STR alleles which are not included in the allelic ladders provided by the manufactures can be encountered and forensic scientists have to be aware of these rare events. Non-standard DNA profiles can result from biological events such as allele dropout, slippage, conversion and copy number variations (Freeman et al., 2006). Thus far, a few autosomal STR data pertaining to variant alleles have been reported in forensic communities (Clayton et al., 2004; Dauber et al., 2009; Egyed et al., 2000).

The aim of this study was to determine allele frequencies and statistical parameters for medicolegal interest of 15 autosomal-

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loci (D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818, and FGA) in subjects with a large population of unrelated Korean individuals. This study also included several atypical variant alleles and triallelic types encountered in the Korean population.

MATERIALS AND METHODS

Sampling

Peripheral blood or buccal swabs were obtained from 1805 unrelated, randomly selected individuals collected from South Korea. All of the samples were collected with their informed consent.

DNA extraction, PCR amplification and genotyping

DNA was isolated from the samples using a QIAamp DNA Micro kit (Qiagen, Germany). The 15 autosomal STR loci were simultaneously amplified by the multiplex PCR method using an AmpFSTR Identifier PCR Amplification kit (Applied Biosystems, USA). DNA amplification was carried out in a Mastercycler gradient (Eppendorf AG, Germany). The PCR products were subjected to capillary electrophoresis on a 3130XL Genetic Analyzer (Applied Biosystems). Data collection was performed with Data Collection v3.0 software (Applied Biosystems)

and the results were analyzed by GeneMapper ID v3.2 software (Applied Biosystems). Quality control was ensured with laboratory internal control standards and kit controls.

Determination of microvariant allele structure

To prepare the PCR products for D7S820 and D21S11, locus-specific primers were designed from genomic DNA. The cloning of these PCR products was performed using pT7 Blue T vector and DH5 α *E. coli* cells for transformation of the recombinant plasmids. The sequencing reaction was performed with a BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems). Electrophoresis was performed on a 3130XL Genetic Analyzer and the results were analyzed using ABI PRISM data collection software.

Statistical analyses

The Hardy-Weinberg equilibrium (HWE) exact test (dememorization value of 10,000 iterations per batch) and interpopulation comparison were assessed using Arlequin 3.5 software (Excoffier and Lischer, 2010). Genotype distributions were considered to be significantly deviated from the HWE at $P < 0.05$. Bonferroni correction was applied on loci showing a departure from the HWE (Weir, 1990). Power of discrimination (PD) and matching probability (MP) were calculated using Fisher's method (Fisher, 1951).

Table 1. Allele frequencies and forensic parameters for 15 STR loci in Korean population (n = 1805)

Allele	D3S1358	D8S1179	D7S820	CSF1PO	TH01	D13S317	D16S539	TPOX	D5S818
5	-	-	0.0003	-	0.0003	-	-	-	-
6	-	-	-	-	0.1590	-	-	-	0.0003
7	-	-	0.0036	0.0008	0.2540	0.0019	-	-	0.0100
8	-	-	0.1391	0.0008	0.0452	0.2662	0.0025	0.4892	0.0044
8.1	-	-	0.0003	-	-	-	-	-	-
9	0.0003	0.0017	0.0529	0.0482	0.4801	0.1371	0.2947	0.1208	0.0870
9.1	-	-	0.0014	-	-	-	-	-	-
9.2	-	-	-	-	-	-	-	-	-
9.3	-	-	-	-	0.0490	-	-	-	-
10	-	0.1116	0.1709	0.2432	0.0114	0.1438	0.1493	0.0283	0.1886
10.1	-	-	0.0019	-	-	-	-	-	0.0003
10.3	-	-	0.0003	-	-	-	-	-	-
11	-	0.0928	0.3651	0.2399	0.0011	0.2343	0.2407	0.3296	0.3263
12	0.0066	0.1427	0.2269	0.3770	-	0.1693	0.2058	0.0307	0.2357
13	0.0008	0.2266	0.0346	0.0806	-	0.0377	0.0895	0.0008	0.1349
14	0.0438	0.1953	0.0028	0.0091	-	0.0072	0.0175	0.0006	0.0114
15	0.3839	0.1510	-	0.0003	-	0.0019	-	-	0.0011
16	0.3058	0.0668	-	-	-	0.0003	-	-	-
17	0.1920	0.0097	-	-	-	0.0003	-	-	-
18	0.0626	0.0014	-	-	-	-	-	-	-
19	0.0036	-	-	-	-	-	-	-	-
20	0.0006	-	-	-	-	-	-	-	-
H _{obs}	0.7224	0.8327	0.7457	0.7368	0.6715	0.8083	0.7623	0.6277	0.7679
H _{exp}	0.7165	0.8420	0.7629	0.7325	0.6754	0.8048	0.7824	0.6359	0.7766
PD	0.8693	0.9549	0.9097	0.8818	0.8474	0.9332	0.9201	0.8043	0.9169
MP	0.1307	0.0451	0.0903	0.1182	0.1526	0.0668	0.0799	0.1957	0.0831
HWE	0.0206	0.4446	0.6428	0.6040	0.3716	0.2478	0.3698	0.5478	0.4990

(Continued)

Allele	D19S433	vWA	D18S51	Allele	D2S1338	FGA	Allele	D21S11
9	-	-	-	16	0.0075		26	0.0006
9.2	0.0014	-	-	17	0.0895	0.0036	27	0.0006
10	0.0003	-	0.0017	18	0.1343	0.0183	28	0.0404
11	0.0028	-	0.0055	19	0.1659	0.0584	28.1	0.0003
11.2	0.0017	-	-	20	0.1155	0.0573	28.2	0.0064
12	0.0330	0.0003	0.0474	20.2		0.0003	29	0.2271
12.2	0.0033	-	-	21	0.0213	0.1188	29.2	0.0028
13	0.3258	0.0014	0.2330	21.2		0.0014	30	0.3582
13.2	0.0343	-	-	22	0.0449	0.1765	30.2	0.0061
14	0.2729	0.2161	0.2108	22.2		0.0044	30.3	0.0028
14.2	0.0961	-	-	23	0.1767	0.2452	31	0.0903
15	0.0499	0.0307	0.1604	23.2		0.0030	31.2	0.0698
15.2	0.1385	-	-	24	0.1457	0.1693	31.3	0.0003
16	0.0058	0.1770	0.1097	24.2		0.0036	32	0.0274
16.2	0.0285	-	-	25	0.0740	0.0972	32.2	0.1144
17	0.0003	0.2795	0.0693	25.2		0.0008	33	0.0050
17.2	0.0050	-	-	26	0.0177	0.0321	33.2	0.0435
18	-	0.2069	0.0479	27	0.0053	0.0072	34	0.0022
18.2	0.0006	-	-	28	0.0014	0.0022	34.2	0.0017
19	-	0.0742	0.0463	29	0.0003	0.0003	35	0.0003
20	-	0.0125	0.0274					
21	-	0.0014	0.0211					
22	-	-	0.0130					
23	-	-	0.0044					
24	-	-	0.0019					
25	-	-	0.0003					
H _{obs}	0.7789	0.7961	0.8504		0.8659	0.8488		0.7817
H _{exp}	0.7855	0.7946	0.8509		0.8727	0.8486		0.7898
PD	0.9249	0.9265	0.9612		0.9699	0.9592		0.9304
MP	0.0751	0.0735	0.0388		0.0301	0.0408		0.0696
HWE	0.6323	0.7899	0.8097		0.2510	0.5754		0.3060

H_{obs}, observed heterozygosity; H_{exp}, expected heterozygosity; PD, power of discrimination; MP, matching probability; HWE, Hardy-Weinberg equilibrium (exact test based on 10,000 shufflings).

RESULTS AND DISCUSSION

Population genetic data of 15 autosomal STRs

This study was performed to set a standard large Korean population database for the important autosomal STR markers containing 13 CODIS loci ($n = 1805$), which might be useful for establishment of the National DNA Profile Databank and applications like forensics and population genetics. In particular, the database included rare variant alleles and atypical genotypes. Allele frequencies and several statistical values for the 15 STR profiles are shown in Table 1 (The genotypes for each sample are available in Supplementary Table S1). This analysis revealed a total of 182 alleles with a mean allele number of 12.13 per locus. D21S11 revealed the highest allele number ($n = 20$), whereas D16S539 and TPOX revealed the lowest number ($n = 7$). All the examined loci showed no deviation from the HWE with the exception of D3S1358. The forensic community recommends Bonferroni correction to correct for departures from the HWE expectations due to multiple comparisons of each autosomal STR loci (Weir, 1990). After employing Bonferroni

correction, the departure observed at D3S1358 was not significant ($0.05/15 = 0.0033$). The expected heterozygosities ranged from 0.6359 (TPOX) to 0.8727 (D2S1338) with a mean value of 0.7781, while the observed heterozygosities ranged from 0.6277 (TPOX) to 0.8659 (D2S1338) with a mean value of 0.7731. The power of discrimination (PD) index ranged from 0.8043 at TPOX to 0.9699 at D2S1338 (mean = 0.9140). In a comparison of the allele frequencies of previous Korean population studies, no significant differences were found (Han et al., 2000; 2002; Kim et al., 2003).

For the loci located on the same chromosomes (e.g., TPOX and D2S1338, CSF1PO and D5S818), a pairwise analysis of the linkage disequilibrium revealed no significant disequilibrium (data not shown). Therefore these 15 STR markers can be treated as independent for calculation of the matching probability.

It was suggested that the allele frequencies are considerably different between ethnic groups. The genotyping results were compared with several ethnic groups to determine how much genetically different Koreans are from other populations (Sup-

Table 2. Exact repeat motif structures of rare variant alleles identified from D7S820 and D21S11

Locus	Allele	Rpt ^a	Repeat structure ^b	<i>n</i>	Frequency
D7S820	8.1	59	TAACA(GATA) ₈ GACAGATTGATAG(T) ₉	1	2.77×10^{-4}
	9.1	63	TAACA(GATA) ₉ GACAGATTGATAG(T) ₉	4	1.11×10^{-3}
		63	TAAC_ (GATA) ₉ GACAGATTGATAG(<u>I</u>) ₁₀	1	2.77×10^{-4}
	10.1	67	TAACA(GATA) ₁₀ GACAGATTGATAG(T) ₉	6	1.66×10^{-3}
		67	TAAC_ (GATA) ₁₀ GACAGATTGATAG(<u>I</u>) ₁₀	1	2.77×10^{-4}
	10.3	69	TAAC_ (GATA) ₁₁ GACAGATTGATAG(<u>I</u>) ₈	1	2.77×10^{-4}
D21S11	28.1	124	(TCTA) ₆ [**] ^c (TCTA) ₃ ΔTCTA	1	2.77×10^{-4}
	30.3	134	(TCTA) ₆ [**] ^c (TCTA) ₅ TCA(TCTA) ₆	9	2.49×10^{-3}
		134	(TCTA) ₆ [**] ^c (TCTA) ₃ (TCTG)(TCTA)TCA(TCTA) ₆	1	2.77×10^{-4}
	31.3	138	(TCTA) ₆ [**] ^c (TCTA) ₁₀ TCA(TCTA) ₂	1	2.77×10^{-4}

^aRpt, Repeat region size^bNucleotides with insertion and deletion are underlined.^c[**]: (TCTG)₅(TCTA)₃TA(TCTA)₃TCA(TCTA)₂TCCATA (constant region)**Table 3.** Triallelic types identified from D21S11 and TPOX loci

Locus	Allele	Observed number	Type ^a
D21S11	29, 30, 32.2	1	E
	29, 32.2, 33.2	1	UE
TPOX	8, 11, 12	2	E

^aType, the peaks in the three-peak pattern are of even (E) or uneven (UE).

plementary Table S2). Allele frequencies of most loci in the Korean population were significantly different from those in European or American populations ($p < 0.05$). For example, by comparisons of a Korean population, Greek (Sánchez-Diz et al., 2008) and Brazilian populations differed at 12 loci (Fridman et al., 2008), while US Caucasian and African-American populations differed at 11 STR loci (Budowle et al., 1999). Further, two Chinese populations revealed that a relatively small number of loci were different from Koreans: a Shanghai population differed at 6 loci (Li et al., 2009) and a Yunnan Han China population at 4 loci (Nie et al., 2008). Although, a Southwestern American Hispanic population showed significant differences at only 5 loci (Budowle et al., 1999), the R_{st} values indicated that two Chinese populations are more close to Korean population than American Hispanic population (R_{st} value: Shanghai, 0.00301; Yunnan-Han, 0.00147; US Hispanic, 0.02589).

Characterization of rare variant alleles

Several rare variant alleles (intermediates) in 1805 individuals were present at three STR loci, D21S11, D7S820 and D5S818 (Table 2). At D21S11, 3 variant alleles were identified: 28.1 ($n = 1$), 30.3 ($n = 10$) and 31.3 ($n = 1$). D7S820 revealed 4 variant alleles: 8.1 ($n = 1$), 9.1 ($n = 5$), 10.1 ($n = 7$) and 10.3 ($n = 1$). D5S818 showed the variant allele of 10.1 from one sample. Sequences of variant alleles from D7S820 revealed an insertion of A nucleotide just before the repeat region's GATA motif or a modification (deletion or insertion) in the same block of a "T" stretch. Particularly, the variant alleles 9.1 and 10.1 displayed two subtypes sequence variations: TAACA(GATA)₉GACAGATTGATAG(T)₉ or TAAC-(GATA)₉GACAGATTGATAG(I)₁₀ for 9.1 and TAACA(GATA)₁₀GACAGATTGATAG(T)₉ or TAAC-(GATA)₁₀GACAGATTGATAG(I)₁₀ for 10.1. The allele 30.3 for D21S11 also revealed two sequence variants,

(TCTA)₆(TCTG)₅(TCTA)₃TA(TCTA)₃TCA(TCTA)₂TCCATA(TCTA)₅TCA(TCTA)₆ and (TCTA)₆(TCTG)₅(TCTA)₃TA(TCTA)₃TCA(TCTA)₂TCCATA(TCTA)₃(TCTG)(TCTA)TCA(TCTA)₆. These structures are in concordance with the data reported by Tsuji et al. (2006). In addition, a new structural variant was found in allele 30.3, which has a TCTG unit in variable region 3, thus far described as a TCTA repeat block (GenBank Accession No. AP000433).

This study also revealed four cases of triallelic types in locus D21S11 and in TPOX (Table 3). D21S11 revealed two triple allele types, "29-30-32.2" and "29-32.2-33.2", each in one sample. TPOX revealed a triple allele "8-11-12" in 2 samples. These unusual genotypes were confirmed by repeated analysis using a different multiplex PCR system (Powerplex 16 system, Promega, USA). The triallelic patterns are usually classified into two types based on the relative intensities of their component alleles: type 1 with an uneven height of the peaks and type 2 with an even height of the peaks (Clayton et al., 2004). In this study, three variants were type 2 (even) and one variant was type 1 (uneven). Previous data also suggested that D21S11 and TPOX loci have relatively high tendencies to show triallelic patterns at the Short Tandem Repeat DNA Internet database (<http://www.cstl.nist.gov/biotech/strbase/>).

In forensic casework, rare types can greatly increase the power of discrimination. However, particular care should be taken in kinship matching and forensic cases, since incorrect designation of any of deviations from allelic ladders could lead to a false conclusion. The atypical alleles distribute ethnic group-specifically, therefore, it is necessary to increase the number of useful references on non-standard allele patterns. Although several reports have described several rare alleles (Cho et al., 2006; Han et al., 2002; Kim et al., 2003; Park et al., 2005), this study may be the largest dataset to present that presents all the rare alleles or atypical types shown in the Korean population.

Powerful discrimination ability of STR profile database

All of the 15 STR loci analyzed here were found to be highly polymorphic in our Korean population, with a mean observed heterozygosity and power of discrimination of 0.773 and 0.914, respectively. Particularly, the combination of these loci revealed powerful discrimination ability with the combined matching probability of 1.521×10^{-17} . Therefore, this STR profile may serve as a reference database for kinship testing and forensic applications in Korea. This database also can be a good preparation

for the preliminary construction of a DNA database of the Korean population. The STR genotype data here were contributed to the online calculator PopAffiliator (<http://cracs.fc.up.pt/popaffiliator>) for individual affiliation to a major population group.

Note: Supplementary information is available on the Molecules and Cells website (www.molcells.org).

ACKNOWLEDGMENTS

This research was supported by a grant from National Forensic Service (NFS), and by Mid-Career Researcher Program through a National Research Foundation of Korea (NRF) grant funded by the Ministry of Education, Science and Technology (R01-2008-000-20604-0) in Korea. We also thank Hwan Young Lee for her helpful comments and reviewing of this manuscript.

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