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Analysis of the Short Tandem Repeat Loci for *STRX1*, *HPRTB*, *ARA*, *DYS390*, *DYS392* and *DYS393* in Koreans

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Three *STR* loci (*STRX1*[AGAT]$_n$, *HPRTB*[AGAT]$_n$ and *ARA*[AGC]$_n$) on X chromosome and three other STR loci (*DYS390*[CTG(A)T]$_n$, *DYS392*[ATT]$_n$ and *DYS393*[GATA]$_n$) on Y chromosome were analyzed in 154 unrelated healthy Korean subjects. Four loci (*STRX1, HPRTB, DYS390* and *DYS393*) were amplified by quadruplex polymerase chain reaction (PCR) using fluorescent labeled primers (FLP). *ARA* and *DYS392* were amplified separately using single PCR, similarly by using FLP. They were then run in an automated DNA sequencer and were analyzed with Genescan software. We found 7 alleles (308-332 bp) in *STRX1*, 7 alleles (275-299 bp) in *HPRTB*, 16 alleles (252-315 bp) in *ARA*, 6 alleles (203-223 bp) in *DYS390*, 7 alleles (245-263bp) in *DYS392* and 5 alleles (116-132 bp) in *DYS393*. The **13* (34%), **13* (51%), **23* (18%), **23* (50%), **14* (39%) and **13* (40%) alleles were observed to be the highest frequencies of *STRX1, HPRTB, ARA, DYS390, DYS392* and *DYS393*, respectively. The detection of STR loci on sex chromosomes by quadruplex PCR allows simple determination of sex and identification of an individual.

Tandem repetitive DNA sequences are abundantly spread throughout the human genome. Many of these sequences, which are polymorphic due to variation in length of a short tandem repetition (STRs), have been given various names (Jeffreys et al., 1985; Edwards et al., 1991, 1992; Roewer et al., 1992; Hammond et al., 1994). The STRs loci have been used not only in the areas of population genetics, genetic linkage analysis, medical diagnostics, and evolutionary study but also in forensic application such as sex determination and paternity testing (Hearne et al., 1992; Hammond et al., 1994; Hammer and Horai, 1995; Ryu and Kim, 1996; Cavalli-Sforza, 1998; Sasaki and Dahiya, 2000).

Various STRs loci on sex chromosomes as well as autosomes for populations genetic analysis exist (Ciminelli et al., 1995; de Knijff et al., 1997; Kayser et al., 1997a; Pérez-Lezaun et al., 1997). The Y linked STRs loci, in particular, are highly informative and are transmitted from fathers to sons only as a haploid without recombination. They are especially useful in identification of male lineages, estimation of mutation rate and identification of relationships among populations (Kayser et al., 2000; Kim et al., 2000; Carvalho-Silva et al., 2001; Kayser et al., 2001). Many of STR studies in Korean population have also been reported (Kim et al., 1998; Shin et al., 1998; Han et al., 2001).

We analyzed allele distribution of six STRs, the allelic polymorphism of the AGAT repeat of the short arm of X chromosome (*STRX1*), the AGAT repeat within intron 3 of the hypoxanthine phosphoribosyltransferase B gene (*HPRTB*), the AGC repeat within the first exon of the androgen receptor A gene (*ARA*) on X chromosome and the CTG(A)T repeat of *DYS390*, the ATT repeat of *DYS392* and the GATA repeat of *DYS393* on Y chromosome and also showed three Y chromosomal STRs haplotypes in Koreans.

Materials and Methods

DNA extraction

Genomic DNA was extracted from peripheral blood of 154 unrelated healthy Koreans including 94 males with QIAamp Blood Kits (QIAGEN, USA).

PCR amplification for STR typing

The quadruplex PCR for four STR loci, *STRX1, HPRTB, DYS390* and *DYS393*, and two single PCR for *ARA* and *DYS392* were performed, according to methods described previously (Tun et al., 1999). 10-20 ng each of template DNA, 200 μM dNTPs, 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl, pH 8.3, 2 U

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AmpliTaq Gold™(Perkin Elmer, Applied Biosystems, Foster City, CA, USA) and 3 pmol of primers for *STRX1*, 3 pmol for *HPRTB*, 15 pmol for *DYS390* and 5 pmol *DYS393* were mixed in a total 25 μl reaction mixture for quadruplex PCR. Also, most of the reaction buffer concentrations of each single PCR for *ARA* and *DYS392* were the same as those of the quadruplex PCR except for primer and Taq polymerase. The concentration of primer and Taq polymerase were 20 pmol and 1.25 U for *ARA* and 15 pmol and 2 U for *DYS392*. Each primers for the STR loci were labeled with different fluorescence dye with reverse primer (Nippon Flourmills Co. Ltd, Japan) and the primer sequences for each STR loci were followed as Edwards et al. (1992) for X STR loci and Kayser et al. (1997b) for Y STR loci. The quadruplex PCR were performed with initial denaturation cycle of 94℃ for 10 min, and then 30 cycles of 94℃ for 20 sec, 55℃ for 20 sec, 72℃ for 20 sec with final cycle of 72℃ for 2 min, by using Perkin Elmer 2400 Thermal Cycler (Norwalk, CT, USA). Each single PCR for *ARA* and *DYS392* was performed with the initial denaturation cycle of 94℃ for 9 min, and then 30 cycles of 94℃ for 1 min, 60℃ for 1 min, 72℃ for 1.5 min with final cycle of 72℃ for 5 min for *ARA* and 94℃ for 10 min, 29 cycles of 94℃ for 1 min, 55℃ for 1 min, 72℃ for 2 min for *DYS392*, by using ASTEC PC-700 (Fukuoka, Japan).

Electrophoresis and detection

The amplified products were checked by electrophoresis on 1% agarose (Seakem) gels and stained with ethidium bromide. Then 0.5 μl of each amplified PCR products were mixed with 0.5 μl for GS 500 TAMRA, a yellow labeled internal size standard (PE, Applied Biosystems, Foster City, CA, USA), 2.5 μl formamide and 1 μl loading buffer. After denaturation at 90℃ for 3 min, the mixture was applied to 373A automated DNA Sequencer (PE, Applied Biosystems, Foster City, CA, USA) using 6% sequencing gel (polyacrylamide: bisacrylamide = 19:1, 8 M urea). Electrophoresis data were collected and automatically analyzed by 672 GeneScan software (PE, Applied Biosystems, Foster City, CA, USA) which employs Southern local method for calculation of fragment size utilizing the known internal size standard. The allelic ladder of all loci was loaded in lanes flanking the samples for convenient allele determination. Designation and nomenclature of each alleles were according to the recommendations of the International Society of Forensic Haemogenetics (Bär et al., 1997).

Statistical analysis

Distribution of allele frequencies for each STR loci were calculated from observed genotypes. The values of the heterozygosity (Het), polymorphism information content (PIC), power of discrimination (PD) and mean exclusion chance (MEC) for *STRX1*, *HPRTB* and *ARA* were calculated by 'DNA VIEW' program and the Hardy-Weinberg Equilibrium in these three X STR loci were assessed for the genotype distribution. The Y-linked marker diversity (D) was calculated by using the formular $D=1-\sum(fi)^2$, where fi represents the frequency of the i-th allele.

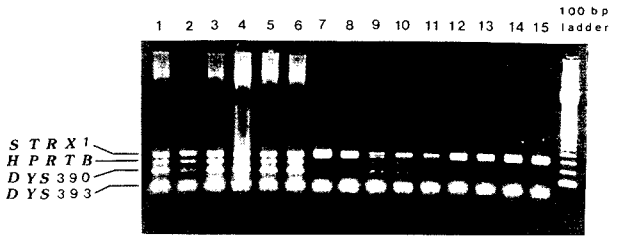

Fig. 1. Detection on 1% agarose gel of amplification products after quadruplex PCR for *STRX1*, *HPRTB*, *DYS390* and *DYS393*. Lane 1-6, 9-11: male samples, Lane 7, 8, 12-15: female samples

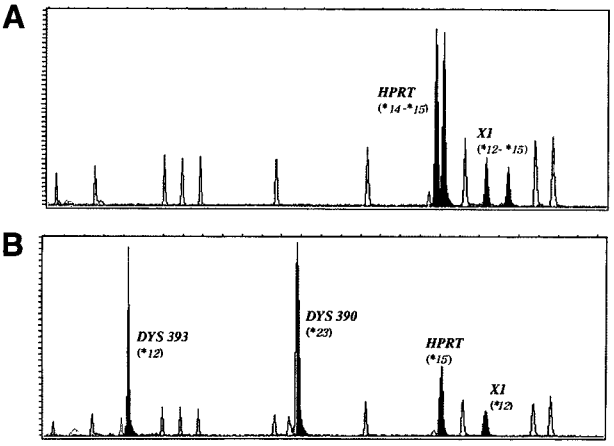

Fig. 2. These are the results of automatically analyzing by 672 GeneScan software after electrophoresis using an automated DNA sequencer 373A. A, Electropherogram of a female sample with *'14/15* in *HPRTB* and allele *'12/15* in *STRX1*. B, Electropherogram of a male sample with *'12* in *DYS393*, allele *'23* in *DYS390*, allele *'15* in *HPRTB* and allele *'12* in *STRX1*.

Results and Discussion

The DNA amplification by quadruplex PCR for two X STR loci, *STRX1* and *HPRTB*, and two Y STR loci, *DYS390* and *DYS393*, was successfully carried out and we consequently determined the sex as well as X and Y STR genotypes accordingly. A DNA sample derived from a male who carries both X and Y chromosomes yielded four fragments in quadruplex PCR, whereas the DNA derived from a female who carries two X chromosomes yielded only two fragments of X STR in quadruplex PCR (Fig. 1). The electropherograms of the female and male samples are presented in Fig. 2.

The genotypic frequencies for *STRX1*, *HPRTB* and *ARA* STR loci in females are presented in Table 1. We were able to detect 7 alleles from 308 bp (**11*) to 332 bp (**17*) in *STRX1*, 7 alleles from 275 bp (**10*) to 299

Table 1. Genotype frequencies of *STRX1*, *HPRTB* and *ARA* in Korean females

STRX1		*HPRTB*		*ARA*	
Genotypes	%	Genotypes	%	Genotypes	%
*11/*13	1.7	*10/*12	1.7	*10/*27	2.6
*11/*14	1.7	*11/*12	1.7	*18/*25	2.6
*12/*12	3.3	*11/*13	5.0	*19/*23	5.3
*12/*13	1.7	*11/*14	1.7	*20/*22	2.6
*12/*14	4.9	*11/*15	1.7	*20/*23	2.6
*12/*15	6.7	*12/*12	1.7	*20/*24	2.6
*12/*16	1.7	*12/*13	33.3	*21/*22	2.6
*13/*13	15.0	*12/*14	6.7	*21/*24	2.6
*13/*14	23.3	*12/*15	3.3	*21/*30	2.6
*13/*15	18.3	*13/*13	21.5	*22/*23	10.6
*13/*16	3.3	*13/*14	13.3	*22/*24	2.6
*14/*14	6.7	*13/*15	6.7	*22/*25	2.6
*14/*15	10.0	*14/*15	1.7	*22/*27	5.3
*15/*15	1.7			*22/*28	2.6
				*23/*24	10.6
				*23/*25	8.0
				*23/*26	5.3
				*23/*27	5.3
				*24/*26	2.6
				*24/*28	5.3
				*25/*25	5.3
				*26/*29	2.6
				*27/*28	2.6
				*30/*30	2.6

bp(**16*) in *HPRTB*, 16 alleles from 252 bp (**10*) to 315 bp (**31*) in *ARA*, 6 allele from 203 bp (**21*) to 223 bp (**26*) in *DYS390*, 7 alleles from 245 bp (**10*) to 263 bp (**16*) in *DYS392* and 5 alleles from 116 bp (**11*) to 132 bp (**15*) in *DYS393* in Koreans. The allele frequencies of the six loci in all sampled individuals and the values of forensic efficiency for *STRX1*, *HPRTB* and *ARA* are presented in Table 2. The values of heterozygosity (Het), polymorphism information content (PIC), power of discrimination (PD) and mean exclusion chance (MEC) for the three X STR loci indicated the highest values for *ARA* compared with those for *STRX1* and *HPRTB* and especially, the PD of *ARA* indicated a highly informative value with 97%. These

Table 2. Allele frequencies of *STRX1*, *HPRTB*, *ARA*, *DYS390*, *DYS392* and *DYS393* in Koreans

	STRX1 (214)	*HPRTB* (214)	*ARA* (163)	*DYS390* (94)	*DYS392* (71)	*DYS393* (94)
Alleles						
*10		0.005	0.006		0.014	
*11	0.009	0.042			0.070	0.011
*12	0.108	0.252			0.141	0.394
*13	0.341	0.514			0.352	0.404
*14	0.294	0.126			0.394	0.085
*15	0.215	0.056			0.014	0.106
*16	0.028	0.005			0.014	
*17	0.005		0.006			
*18			0.006			
*19			0.037			
*20			0.049			
*21			0.049	0.011		
*22			0.152	0.063		
*23			0.178	0.500		
*24			0.123	0.309		
*25			0.123	0.106		
*26			0.104	0.011		
*27			0.073			
*28			0.037			
*29			0.025			
*30			0.025			
*31			0.006			
Heterozygosity	0.726	0.658	0.876			
PIC[1]	0.681	0.613	0.864			
PD[2]	0.880	0.838	0.973			
MEC[3]	0.489	0.422	0.755			

[1] PIC : Polymorphism information content, [2] PD : Power of discrimination, [3] MEC : Mean of exclusion chance
Allelic designations refer to the number of repeats of the core sequence motif indicated in the locus column. Numbers in the pharensis indicate the number of chromosomes samples

Table 3. Distribution of Y chromosome haplotypes (*DYS390-DYS392-DYS393*) in Koreans

Frequency of Haplotype							
DYS390-DYS392-DYS393			%	*DYS390-DYS392-DYS393*			%
*23	*14	*13	11.3	*23	*11	*15	1.4
*23	*13	*13	9.9	*23	*12	*15	1.4
*24	*13	*12	9.9	*23	*13	*12	1.4
*24	*14	*12	9.9	*23	*13	*14	1.4
*23	*12	*12	5.7	*23	*14	*15	1.4
*23	*14	*12	5.7	*23	*16	*13	1.4
*25	*13	*12	4.2	*24	*12	*13	1.4
*22	*13	*13	2.8	*24	*13	*11	1.4
*23	*11	*14	2.8	*24	*13	*13	1.4
*23	*12	*13	2.8	*24	*13	*14	1.4
*23	*14	*14	2.8	*25	*12	*12	1.4
*24	*11	*15	2.8	*25	*12	*13	1.4
*24	*14	*13	2.8	*25	*13	*15	1.4
*24	*14	*15	2.8	*25	*14	*12	1.4
*22	*15	*13	1.4	*26	*13	*13	1.4
*23	*10	*12	1.4				

four values of *ARA* were similar to those of Japanese (Kishida and Tamaki, 1997). However, we have observed very low MEC values for *STRX1* and *HPRTB* and the reason for this may be due to the small sample size. The χ^2 test for *STRX1, HPRTB* and *ARA* in female samples revealed no significant deviation from the Hardy-Weinberg Equilibrium. The haplotype frequencies of three Y STR loci, *DYS390-DYS392-DYS393*, are presented in Table 3. Gene diversities of the three Y STR loci in this study were observed to be 0.63 in *DYS390*, 0.70 in *DYS392* and 0.66 in *DYS393*. These values were similar to the results of Kim et al. (2001). The allelic frequencies and gene diversities of *DYS390*, *DYS392* and *DYS393* in Koreans were similar to those of Chinese in Taiwan, but were different from Italians (Table 4).

In this paper, we have reported genetic informativeness for *STRX1, HPRTB, ARA, DYS390, DYS392* and *DYS393* in the Korean population and presented that simultaneous amplification by multiplex PCR of the STR loci on the X and Y chromosomes allows rapid and easy determination of sex and individual identification.

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Table 4. Allele frequencies and gene diversities of *DYS390, DYS392* and *DYS393* in other populations

Loci	Alleles	Population							
		Korean[1]		Korean[2]		Chinese (in Taiwan, 582)[3]		Italian[4]	
		Allele frequency	Gene diversity	Allele frequency	Gene diversity	Allele frequency	Gene diversity	Allele frequency	Gene diversity
DYS390	*20		0.63		0.60	0.01	0.70		0.55
	*21	0.01				0.01		0.01	
	*22	0.06		0.10		0.06		0.06	
	*23	0.50		0.56		0.39		0.19	
	*24	0.31		0.27		0.33		0.63	
	*25	0.11		0.06		0.17		0.11	
	*26	0.01		0.01		0.02			
	*27					0.01			
DYS392	*9		0.70			0.01	0.64		0.52
	*10	0.01				0.01		0.02	
	*11	0.08				0.03		0.19	
	*12	0.14				0.07		0.07	
	*13	0.35				0.41		0.66	
	*14	0.39				0.42		0.04	
	*15	0.01				0.04		0.02	
	*16	0.01				0.01			
	*17					0.01			
DYS393	*11	0.01	0.66	0.02	0.65	0.01	0.62		0.43
	*12	0.39		0.41		0.52		0.16	
	*13	0.40		0.41		0.30		0.73	
	*14	0.09		0.09		0.14		0.10	
	*15	0.11		0.04		0.03		0.01	
	*16			0.01					
	*17			0.01					

[1] Present study; [2] Kim et al, 2001; [3] Wu and Hu, 2001; [4] Possi et al. 1998.

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