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Genetic Study in a Hungarian and a Japanese Population at the Short Tandem Repeat Locus HUMVWFA31

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Abstract: Genetic study was carried out for short tandem repeat (STR) locus HUMVWFA31 in a population sample of Hungarian-Caucasian individuals from Baranya County of Hungary (H) and in a Japanese population sample from Shiga Prefecture, Japan (J). Allele frequency and genotype distributions were determined. In the case of this locus 8 different alleles and 21 genotypes out of the possible 36 were observed in both populations. Using two tests for assessment of agreement with Hardy-Weinberg Equilibrium (HWE) no deviations could be found. Population comparisons were carried out using previously published databases of different racial groups. Using the conventional chi-square analyses we found statistically significant differences between Hungarian and Japanese population and between populations belonging to different racial groups.

Key words: Short tandem repeats, HUMVWFA31, Baranya County of Hungary, Shiga Prefecture of Japan, Population study

Introduction

Short tandem repeat loci (STR) form subgroups of the variable number of tandem repeats (VNTRs) and are characterized by tandemly repeated 2-7 base-pair (bp) length core sequences. Their total length, consisting of the flanking regions, is generally under 350 bp, thus, STRs can be successfully typed in cases of highly degraded forensic biological evidence when other conventional marker systems give no or not sufficient results. Bearing some laboratory precautions in mind application of multiplex systems (triplex, quadruplex) or multiple (sequencial) loading procedures can all be easily performed. According to their allele structures, especially whether they have any alterations in the regular allele series because of mutations, we could subdivide the STRs into three main groups[3].

Before their use in any application in human identification and paternity cases, detailed databases should be compiled for the relevant populations where the STRs are intended to be used. Therefore, the aim of this study was to carry out population genetic studies using a Hungarian-Caucasian (H) and a Japanese-Mongoloid (J) population sample representing

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two main racial groups.

The multiplex (triplex) PCR was carried out for STR loci HUMVWFA31, HUMFESFPS and HUMF13A01, strictly following instructions of the Promega manufacturer. Allele frequency and genotype distributions were determined. Population comparisons were carried out using previously published databases for different racial groups. The present study summarizes the statistical data concerning only the locus HUMVWFA31. The results regarding to the other two systems will be published elsewhere. Characteristics of the locus analysed are shown in Table 1.

Materials and methods

Whole blood samples were obtained in EDTA vacutainer tubes by venipuncture from 82 unrelated Japanese individuals, collected at the Shiga University of Medical Science. For the Hungarian survey, 82 dried blood samples of unrelated healthy individuals living in Baranya County in the south-west region of Hungary were collected. These were preserved on filter papers or on sterile cotton fabric. DNA extractions-using CHELEX® 100 method-and further steps of the Multiplex PCR analyses were performed by strictly following the instructions as specified in the *Promega Technical Manual for Gene Print™ STR Systems* (# TMD004).

All of the amplification reactions were carried out under "Hot-Start" PCR conditions. The mixture of the 10xSTR Primers, 10xSTR buffer [consists of 500 mM KCl, 100 mM Tris-HCl (pH9.0 25°C), 15 mM $MgCl_2$, 1% Triton® x-100, 2 mM each dNTP] and templates were heated at 95°C for 3minutes before adding the Taq+polymerase. The reaction conditions were as follows: in 25μl reaction volume, 2.5μl 10x STR buffer, 2.5μl 10x primer pairs, 0.15U Promega Taq Polymerase, and 2.5μl Chelex extracted DNA was amplified. K562 DNA was used as +control.

The PCR cycling parameters were set up according to the # TMD004 instructions (Protocol №2, briefly: 96°C 2 min; 10 cycles of 94°C 1 min, 60°C 1 min, 70°C 1.5 min and 20 cycles of 90°C 1 min, 60°C 1 min, 70°C 1.5 min). The PCR Thermal Cycler 900 of Takara Co. (Japan) was used. The success of amplifications were prechecked by vertical 6%T, 3%C polyacrylamide gel (PAG) electrophoresis followed by ethidium bromide staining. The final allele separations were achieved by vertical PAG electrophoresis through a 0.35-mm thick 6%T, 5%C (19:1; acrylamide:bisacrylamide) denaturing (7 M urea) gel. The electrophoresis buffer was 1x Tris-borate-EDTA. PCR products (2.5μl) were diluted with equal amount of 2x STR loading buffer. A 3.8μl of diluted mixture was loaded onto the vertical PAG electrophoresis system (Atto Co. Japan) and PAG electrophoresis was carried out at a constant 35W (approx. 1850 V, 20 mA) after a 50-minute pre run period. The running was stopped when xylene-cyanol migrated approximately 4cm from the anode (31cm running distance).

Tabel 1. Characteristics of the STR locus analysed

STR SYSTEM	GENE/CHROMOSOME LOCALIZATION	PRODUCT LENGTH	ALLELES IN THE LADDER LANE	REFERENCES
HUMVWFA31	Von Willebrand factor (12-p 12pter)	139-167	13,14,15,16,17,18,19,20	10) 22)

10) Kimpton CP *et al.* (1992) 22) Urquhart A *et al.* (1994)

The allelic ladders and the bands of unknown individuals were detected by silver staining method using the DNA Silver Staining Kit (Promega Co., USA). Allele designations were determined by comparisons of the sample fragments with those of the allelic ladders supplied in the kit. The allele designations were made according to recommendations of the DNA commission of the International Society of Forensic Haemogenetics[7]. The genotype of the K562 DNA positive control sample was 16;16 for HUMVWFA31.

The allele frequency distributions were calculated from the observed genotypes of individuals. The Hardy-Weinberg equilibrium was tested using the conventional chi-square method according to Rand *et al.*[20] by forming allele classes. Moreover, the unbiased estimates of expected heterozygote frequencies and the standard deviations were computed according to Nei *et al.*[16] using the formula $H = (1-\Sigma x_{ij}^2) 2n/2n-1$ where x_{ij} = allele frequency value of i th allele of j locus, and n = number of individuals sampled. The power of discrimination (PD) was calculated from equation $PD = 1-\Sigma y_{kj}2$ where y_{kj} = the frequency value of the observed genotypes k at locus j[8]. The PIC (polymorphic information content) was calculated according to Botstein *et al.*[2] and the exclusion chance (EC) as Ohno *et al.*[18]. Population comparisons were performed using 2-way RxC contingency tables and chi-squares and P values were calculated according to Alonso *et al.*[1]. During the calculations for testing the homogeneity of different populations, alleles were eliminated if their observed frequencies were equal to 0 in any of the populations. The degree of freedom was equal to number of alleles-1.

Results and Discussion

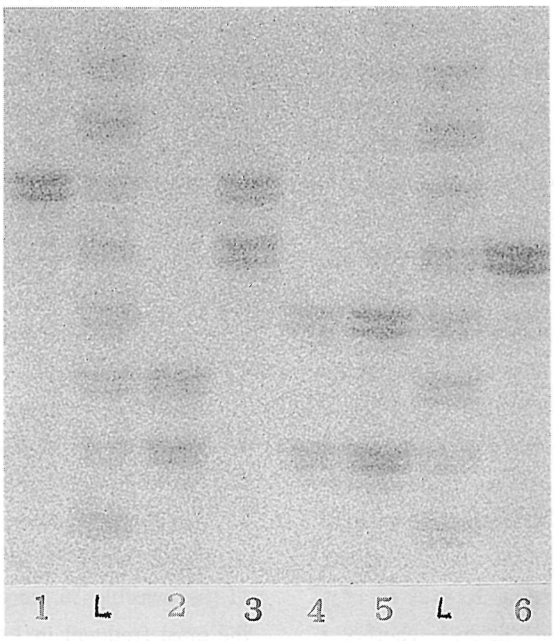
The allele frequency distributions for H and J are summarized in Table 2, and the observed number of different genotypes are shown in Table 3. In both populations, 8 different alleles (allele 13-20) and in H 21; in J, also, 21 genotypes out of the possible 36 were observed. Allele 17 was the most frequent in J (freq =0.268) compared to H in which allele 18 (f =0.286) was the most frequent.

This is the second time, that such high score at allele 18 can be observed in Hungarians from Baranya County of Hungary[11]. Similar allele distributions, as we found among the Hungarians,

Table 2. The observed allele frequency distributions for STR locus HUMVWFA31 in two racial groups (HUN-Hungarian; JPN-Japanese)

	HUMVWFA31	
Allele	HUN	JPN
13	0.006	0.006
14	0.116	0.226
15	0.110	0.024
16	0.171	0.195
17	0.231	0.268
18	0.286	0.189
19	0.067	0.071
20	0.012	0.012

Lanes marked with *L* are the ladder lanes supplied by the kit (from bottom to top allele 13-20). Doublets are the consequence of the different sequence structure of the amplified DNA strands.



Lane	genotypes HUMVWFA31
1	18,18
2	14,15
3	17,18
4	14,16
5	14,16
6	17,17

Figure 1. STR DNA patterns for HUMVWFA31

was published for Caucasians by Sajantila *et al.*[21] concerning Kirillov-Russians (a subgroup of Vologda-Russians whereas the allele 18 f = 0.2247). Pestoni *et al.*[19] found same values for allele 17 and 18 in Galician population (NW Spain) with f =0.2247. In a recent study of Brinkmann *et al.*[4] such allele distribution was found among Papuans.

Figure 2 indicates the unimodal distribution of HUMVWFA31 alleles for H, which is similar to those histograms of Caucasian populations, listed in Table 4 (histograms not shown). In contrast, the allele frequency distribution of J follows a bimodal type with its two peaks at allele 14 (f =0.226) and 17, which is the same as that published by Meyer *et al.*[13] concerning Asians (Chinese and Japanese populations).

The two tests for assessment of agreement with HWE expectations (see Table 3) revealed no deviations in either population. The calculated values of PD for H and J were 0.898 and 0.922, respectively, an the PIC values were 0.776 and

Table 3. Genotypes for HUMVWFA31 system and data of statistical analyses in two racial groups (Hungarian = HUN; Japanese = JPN; n = number of alleles analysed)

Allele	HUMVWFA31 (HUN n =164)								HUMVWFA31 (JPN n =164)							
	13	14	15	16	17	18	19	20	13	14	15	16	17	18	19	20
13	-								-							
14	-	-							-	4						
15	-	8	1						-	1	-					
16	1	4	1	6					1	7	1	5				
17	-	4	1	2	4				-	11	-	8	5			
18	-	3	6	6	20	5			-	9	1	3	9	2		
19	-	-	-	1	3	2	2		-	1	1	2	5	4	-	
20	-	-	-	1	-	-	1	-	-	-	-	-	1	1	-	-

Observed number of alleles	:	8	8
Heterozygosity (obs.)	:	0.781	0.805
(exp.±SD):		0.809±0.07	0.801±0.07
Power of Discrimination	:	0.898	0.922
Results of χ^2 tests	:	χ^2=16.513; df =9	χ^2=4.02; df =9
		0.20>P>0.10	0.95>P>0.90
Polymorphic information content (PIC)	:	0.776	0.774
EC	:	0.631	0.6

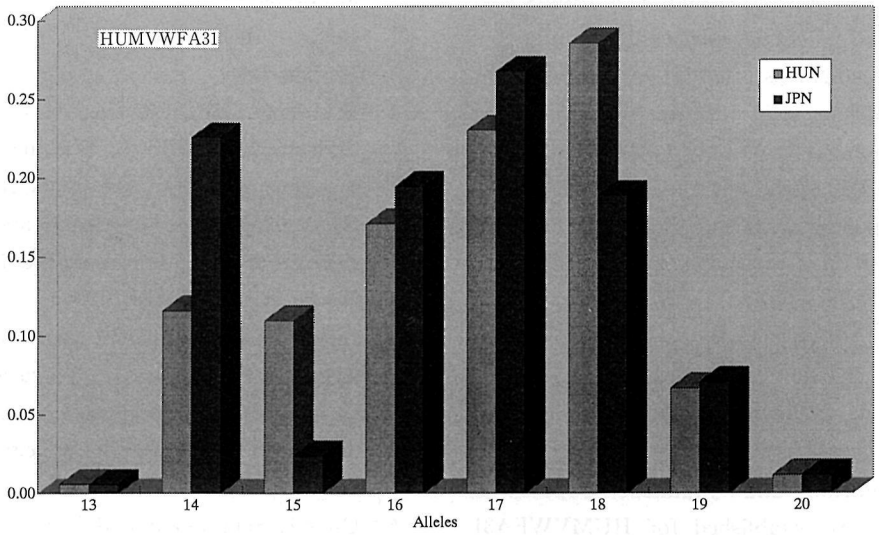


Figure 2. Histograms of allele frequency distributions for HUMVWFA31 in Hungarian and Japanese populations

0.774, respectively. These are as high as the values which were published in other databases for relevant populations for HUMVWFA31, previously.

The interpopulation comparisons in Table 4a and 4b demonstrate statistically significant differences between the main racial groups. Thus, H and J also differ in their allele distributions for HUMVWFA31 ($P<0.0005$). Within the Caucasoid race itself, statistically significant differences have also been found between Hungarians and Finns[21]. The other three Hungarian databases[6,9,11] for HUMVWFA31 also represent statistically significant differences between Hungarian and Finnish population (data not shown). (Similar difference was published by Alonso *et al.*[1] comparing the Finnish and Basque databases.) This observation is notable because of several, very convincing historical and linguistic evidences about the possible close location of Finnish and a Hungarian ancient home between the River Volga and the Ural Mountain in East Europe. Both of nations have own unique languages in Europe, that origin from the same, Finno-Ugrian linguistic system. Our results suggest the relative genetic isolation of Finnish population as was demonstrated by Nevanlinna[17] for conventional genetic markers and/or the continous genetic flow in Hungarian population.

Within the Mongoloid race, comparisons to another Japanese database[15] and to a Chinese[23] for HUMVWFA31 locus revealed no statistically significant differences.

Conclusion

Hungarian and Japanese population databases were established for HUMVWFA31 STR system. Rapid typing with high resolution was obtained after vertical denaturing PAG electrophoresis followed by silver staining. No deviations from Hardy-Weinberg predictions could be found. Using conventional chi-square analyses we found statistically significant differences between populations of different racial groups. In contrast, within a single race results show such only case between Hungarian and Finnish population.

Our allele frequency distributions for HUMVWFA31 can be used to estimate DNA profile in Hungarian and Japanese populations.

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Table 4a. χ^2comparisons of different populations with the Hungarian data from this study
(n = number of individuals in the referenced works; the asterisks indicate the statistically significant differences)

HUMVWFA31					
Population		n	χ^2	*P*	df
[1]	Hungary I.	244	6.64	0.4>P>0.3	6
[2]	Hungary II.	99	1.23	0.995>P>0.990	7
[3]	Hungary III.	105	12.47	0.10>P>0.05	6
[4]	Finland	175	22.47	0.0005>P*	7
[4]	Kirillov-Russian	48	7.17	0.4>P>0.3	6
[5]	Germany	321	5.98	0.6>P>0.5	7
[6]	Croatia	100	9.13	0.2>P>0.1	6
[7]	Spain/Basque	100	4.85	0.5>P>0.4	5
[8]	Spain	158	6.3	0.4>P>0.3	6
[9]	Italy	211	7.12	0.4>P>0.3	6
[10]	Japan	82	19.9	0.01>P>0.005*	7
[4]	US Blacks	101	14.58	0.05>P*	7

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Table 4b. χ^2comparisons of different populations with the Japanese data from this study
(n = number of individuals in the referenced works; the asterisks indicate the statistically significant differences)

HUMVWFA31					
Population		n	χ^2	*P*	df
[1]	Hungary I	82	19.9	0.01>P>0.005×	7
[2]	Hungary II	244	23.05	0.001>P×	6
[3]	Germany	321	21.9	0.005>P×	6
[4]	Italy	211	28.24	0.0005>P×	6
[5]	Blacks	101	45	0.0005>P×	7
[6]	Chengdu (SW China)	121	4.33	0.8>P>0.7	7
[7]	Japan (Gifu)	362	2.94	0.9>P>0.8	7

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ハンガリーと日本人集団における HUMVWFA31座位の遺伝子頻度

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要　旨

ハンガリー南西部居住者と滋賀県居住者の血液・血痕を試料として，HUMVWFA31のSTR座の同時検出を試みた．HUMVWFA31座位につき検討した結果を報告する．この座位では，8つの対立遺伝子と，それにより想定される36の遺伝子型のうち21の遺伝子型が双方の住民にみられた．これらの結果は，ハーデイ・ワインベルグ平衡成立に関して2種類の検定を行ったが否定されなかった．また，このHUMVWFA31領域に見られた遺伝子頻度分布は今回検討の2つの住民集団間や既報の他の民族との間で，比較すると明らかに有意な差が認められた．