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YIPING HOU, ASTRID MIELKE, MARTINA MULCAHY-SCHRÖDER, MECHTHILD PRINZ, DANIELA SIVÁKOVÁ, HUBERT WALTER

GENETIC VARIATION AT THE SHORT TANDEM REPEAT LOCI HUMTH01 AND HUMVWA31 WITHIN AND BETWEEN GERMAN AND SLOVAK POPULATIONS

ABSTRACT: The short tandem repeat loci HUMTH01 and HUMVWA31 were typed in several population samples from Germany (Bremen, n = 100; Hannover, n = 100; Köln, n = 122) and Slovakia (Bratislava, n = 107). Within the three German population samples some variation is seen concerning the allele distribution patterns which, however, are within the range of other German population samples so far tested. It is worth mentioning, however, that according to the genetic distance analysis the two samples from Northern Germany (Bremen and Hannover) are more similar as compared with that from Western Germany (Köln). The Slovak sample differs clearly from the three German samples, which is true for the allele distribution pattern of the HUMTH01 locus as well as for that of the HUMVWA31 locus. This suggests different allele distributions of STR loci in Central European populations, which is in accordance with the genetic differences observed concerning the conventional genetic markers of the blood.

KEY WORDS: Short tandem polymorphisms – Germans and Slovaks – Genetic variability

INTRODUCTION

Short tandem repeats (STRs) loci are characterized by repetitive core sequences of only a few base pairs (Edwards *et al.* 1992; Kimpton *et al.* 1993). As for other variable number tandem repeat (VNTR) loci, the observed polymorphism is caused by the variation in the repeat numbers. STRs are present in the human genome as frequently as once every 15kb, providing a rich source of genetic markers. They offer two obvious advantages for the purpose of population genetic analysis. Firstly, since the allele classifications can be based upon the number of tandem repeats relative to a human allele ladder, correct genotyping is permitted. Secondly, the distribution of the allele frequencies is not continuous, so that the conventional formula for the Hardy-Weinberg equilibrium (HWE) can be applied to assess the goodness of fit of the distribution of genotypes for any STR locus. STR markers provide new approaches for population genetic studies, especially in the identification of ethnic groups and in the detection of gene flow from one population to another.

In order to understand the distribution of allele frequencies at STR loci in German and Slovak populations, two STR loci were investigated in three German population samples and in one from Slovakia, using gene amplification by PCR: a tetranucleotide repeat situated within intron 1 of human tyrosine hydroxylase gene (GenBank Accession number D00269 designated HUMTH01) which is located at chromosome 11p15.5 (Edwards *et al.* 1992) and a tetranucleotide repeat situated within intron 40 of Human

von Willebrand Factor gene (GenBank Accession number M25858 designated HUMVWA31) at chromosome 12p13.3–p13.2 (Kimpton *et al.* 1993).

MATERIAL AND METHODS

A total of 429 EDTA-blood specimens were collected from unrelated male and female individuals, which were from four European populations. The three German populations were sampled in Bremen (n = 100), Hannover (n = 100), and Köln (n = 122), the Slovak one was sampled in Bratislava (n = 107).

DNA was extracted from EDTA-blood using the Chelex method (Singer *et al.* 1989). DNA amplification for HUMTH01 as well as for HUMVWA31 was carried out with the primers as described by Edwards *et al.* (1992) and Kimpton *et al.* (1993), respectively *(Table 1)*. Each PCR contained 2–40 ng human genomic DNA, 1x Taq buffer (Promega), 1.5µM $MgCl_2$, 200 µM each nucleotide, 1U Taq polymerase (Promega), and 0.25 µM each primer in a reaction volume of 37.5 ml. A total of 28 cycles were carried out in a Triothermocycler with denaturation for 1 min at 94 °C, annealing for 1 min at 58 °C, and extension for 2 min at 72 °C.

Allele frequencies for each STR locus were estimated directly by gene counting. Two tests were used to verify whether genotype distribution conformed to Hardy-Weinberg equilibrium (HWE) predictions. One is a χ^2 test with one degree of freedom, which was applied to the comparison between the observed and the expected number of heterozygotes (Edwards *et al.* 1992). The expected heterozygosity for each locus was calculated according to the equation $h = 2n(1-\Sigma X_i^2)/(2n-1)$, where h = expected heterozygosity, X_i = allele frequencies and n = sample size. Standard error of this estimate was obtained by using the equation $SE = [h(1-h)/n]^{1/2}$ (Edwards *et al.* 1992). Another is a modified χ^2 test, which contrasts every observed genotype frequency with its respective expectation (Hou *et al.* 1994). Genetic distances between pairs of populations were estimated using Nei's standard distance (Nei 1987). Significance test concerning the estimates of genetic distance was carried out using the equation $\chi^2 = 2nXnY\Sigma(X_i-Y_i)^2/(X_inX+Y_inY)$, where nX and nY are the numbers of individuals in populations X and Y, respectively (Nei 1987). The relationhips among populations were depicted by the tree of the unweighted pair-group clustering method using arithmetic averages (UPGMA), and using programs in the MEGA package (Kumar *et al.* 1994).

RESULTS AND DISCUSSION

Figures 1 and *2* display the typing results for the two loci under study: HUMTH01 and HUMVWA31, respectively. The genotype distributions in the four population samples are shown in *Tables 2* and *3*, while the

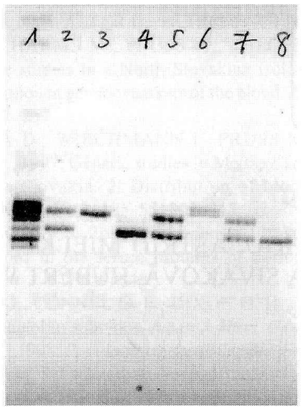

FIGURE 1. Typing of HUMTH01 STR. Lane 1: allele ladder. Lanes 2, 5 and 7: 7–9. Lane 3: 9–9. Lanes 4 and 8: 7–7. Lane 6: 9.3–10.

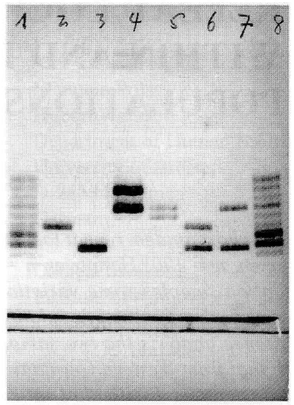

FIGURE 2. Typing of HUMVWA31 STR. Lanes 1 and 8 allele ladder. Lane 2: 16–16. Lane 3: 14–14. Lane 4: 18–20. Lane 5: 17–18. Lane 6: 14–16. Lane 7: 14–18.

allele frequencies at two STR loci HUMTH01 and HUMVWA31 are depicted in *Tables 4* and *5*. A total of 7 alleles in 20 genotypes were observed at the HUMTH01 locus in the three German samples, while in the Slovak sample only 6 alleles in 17 genotypes were found. Similarly, a total of 9 alleles in 28 genotypes were observed at the HUMVWA31 locus in the three German samples, while in the Slovak population only 7 alleles in 21 genotypes were seen. No evidence of deviation from Hardy-Weinberg equilibrium was observed in these four population samples using both χ^2 tests.

In these four European population samples, generally high frequencies of alleles 6 and 9.3 at HUMTH01 locus are to be seen, while at the HUMVWA31 locus allele 17 is the most frequent one *(Tables 4* and *5)*. This is in good accordance with the results of other studies on European populations, which were summarized recently by

TABLE 1. PCR primers used.

Locus	Primers	Reference
HUMTH01	5' GTG ATT CCC ATT GGC CTG TTC CTC 5' GTG GGC TGA AAA GCT CCC GAT TAT	(Edwards et al. 1992)
HUMVWA31	5' CCC TAG TGG ATG ATA AGA ATA ATC 5' GGA CAG ATG ATA AAT ACA TAG GAT GGA TGG	(Kimpton et al. 1993)

TABLE 2. Distribution of HUMTH01 genotypes in German and Slovak populations.

	Germans			Slovaks
	Bremen	**Hannover**	**Köln**	**Bratislava**
5–6	–	–	1	–
5–7	1	–	–	–
6–6	6	4	9	9
6–7	5	7	8	4
6–8	4	5	4	8
6–9	5	6	9	11
6–9.3	11	15	13	13
6–10	3	2	1	1
7–7	4	3	5	2
7–8	5	6	6	8
7–9	8	4	5	6
7–9.3	15	8	13	12
7–10	1	1	–	–
8–8	1	1	1	1
8–9	4	2	4	5
8–9.3	4	5	10	1
8–10	–	–	–	1
9–9	1	–	6	2
9–9.3	8	19	16	10
9.3–9.3	13	12	11	7
9.3–10	1	–	–	–
Total	100	100	122	101
Obs. Heterozygosity	0.7500	0.8000	0.7377	0.7921
Exp. Heterozygosity	0.7931	0.7717	0.7858	0.7931
SE	±0.0405	±0.0420	±0.0371	±0.0403

Table 3. Distribution of HUMVWA31 genotypes in German and Slovak populations.

	Germans			Slovaks
Genotype	**Bremen**	**Hannover**	**Köln**	**Bratislava**
13–14	–	–	1	–
13–18	–	1	–	–
14–14	2	3	1	–
14–15	2	3	5	8
14–16	2	3	2	1
14–17	1	7	4	10
14–18	6	3	2	8
14–19	2	–	–	2
14–20	–	–	–	1
14–21	–	1	–	–
15–15	–	1	–	1
15–16	4	3	4	6
15–17	5	5	7	8
15–18	4	3	2	6
15–19	2	1	5	1
15–20	–	1	1	–
16–16	5	2	5	6
16–17	10	10	20	11
16–18	8	6	10	10
16–19	2	2	5	2
16–20	–	–	1	–
17–17	12	10	7	4
17–18	10	10	15	10
17–19	7	10	9	7
17–20	1	–	1	–
18–18	6	7	6	3
18–19	5	6	8	–
18–20	1	2	1	1
19–19	3	–	–	1
Total	100	100	122	107
Obs. Heterozygosity	0.7200	0.7700	0.8443	0.8598
Exp. Heterozygosity	0.8057	0.8071	0.8071	0.8200
SE	±0.0396	±0.0395	±0.0357	±0.0371

Huckenbeck *et al.* (1996a, 1996b, 1997). Comparing the German and Slovak allele distribution patterns it is seen that they are somewhat different. The Slovak sample displays a higher frequency of allele 6 and a lower frequency of allele 9.3 at the HUMTH01 locus *(Table 4)*. At the HUMVWA31 locus the alleles 14 and 15 are more frequent in the Slovak sample than in the three German samples, while the allele 17 displays a relatively lower frequency in the Slovak population *(Table 5)*. This suggests that among Central European populations the allele distributions of these two STR loci might be different from those of Western Europe. Such interpopulational genetic differences cannot surprise, however, because similar differences between Central and Western European populations are also known concerning the conventional genetic markers of the blood (Walter 1998).

Figure 3 displays an UPGMA tree based on both STR loci. It reveals that 1) the samples from Bremen and Hannover show the lowest genetic distance; 2) both these

TABLE 4. Distribution of allele frequencies for HUMTH01 in German and Slovak populations.

	Germans			Slovaks
Allele	**Bremen**	**Hannover**	**Köln**	**Bratislava**
5	0.005	–	0.004	–
6	0.200	0.215	0.221	0.272
7	0.215	0.160	0.172	0.168
8	0.095	0.100	0.107	0.124
9	0.135	0.155	0.189	0.178
9.3	0.310	0.355	0.303	0.248
10	0.035	0.015	0.004	0.010

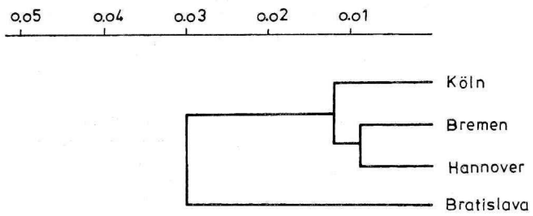

FIGURE 3. Genetic distances between German and Slovak population samples, based on STR loci HUMTH01 and HUMVWA31.

samples cluster with the Köln sample, and 3) the Slovak sample shows a clear genetic distance from all the three German samples. The small genetic distance between Bremen and Hannover can be explained with the fact that both are neighbouring towns in Northern Germany, having genetically similar populations, whereas Köln is located in Western Germany, having a population which differs genetically more or less from that of Northern Germany. This could be shown e.g. by Scheil and Strunz (1995, 1996), who analyzed the distribution of AB0 allele and Rhesus haplotype frequencies in the area of Köln. The reason for such genetic differences among the German population samples can be seen in their different historical backgrounds (Walter 1998). The considerable genetic distance of the Slovak sample from the three German ones is in line with the observations dealing with regional differences concerning the distribution of the conventional genetic markers of the blood, which can be explained by the different history of Central and Western European populations (Walter 1998).

It would be of considerable interest to analyze these two STR loci on other population samples from Central and Eastern Europe, too, in order to see whether the HUMTH01 and HUMVWA31 allele distribution patterns in these parts of Europe are really different from those of Western, Northern and Southern Europe (Huckenbeck *et al.* 1996a, 1996b, 1997). If this could be demonstrated this would be another indication to the genetic differentiation processes which took place in the course of the history of European populations.

TABLE 5. Distribution of allele frequencies for HUMVWF31 in German and Slovak populations.

	Germans			Slovaks
Allele	**Bremen**	**Hannover**	**Köln**	**Bratislava**
13	–	0.005	0.004	–
14	0.085	0.115	0.068	0.140
15	0.085	0.090	0.096	0.145
16	0.180	0.140	0.213	0.196
17	0.290	0.310	0.287	0.252
18	0.230	0.225	0.205	0.192
19	0.120	0.095	0.111	0.066
20	0.010	0.015	0.016	0.009
21	–	0.005	–	–

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Yiping Hou
Institute of Forensic Medicine
West China University of Medical Sciences
610044 Chengdu, Sichuan, PR China

Astrid Mielke
Martina Mulcahy-Schröder
Hubert Walter
Department of Human Biology
University of Bremen
D-28334 Bremen
Germany

Mechthild Prinz
Institute of Forensic Medicine
University of Köln
D-50823 Köln
Germany

Daniela Siváková
Institute of Anthropology
Comenius University
SK-84215 Bratislava
Slovakia
E-mail: sivakova@fns.uniba.sk