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SHORT COMMUNICATION

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Population genetic study from the Zagreb area using 3 STR systems

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Abstract Population genetic studies were carried out on Caucasians from north-west Croatia (Zagreb-area) using the short tandem repeat (STR) systems HumTHO1, HumVWA and HumACTBP2. After electrophoresis in PAG, 6 alleles could be identified for HumTHO1 in a sample size of 100 unrelated individuals and 7 alleles were found for VWA. For ACTBP2, 25 alleles have been identified. No significant deviations from Hardy-Weinberg equilibrium could be observed.

Key words Short tandem repeats · HumTHO1 · HumVWA · HumACTBP2-Population studies · Zagreb area

Zusammenfassung Populationsstichproben nordwestkroatischer Kaukasier (Zagreb-Region) wurden mit den 3 Short tandem repeat (STR) Systemen HumTHO1, HumVWA und HumACTBP2 untersucht. Nach elektrophoretischer Auftrennung der Fragmente in PAG konnten 6 Allele für HumTHO1 in einer Bevölkerungsstichprobe von 100 nicht verwandten Personen und 7 Allele für HumVWA differenziert werden. Für ACTBP2 ließen sich 25 Allele unterscheiden. Eine signifikante Abweichung vom Hardy-Weinberg-Gleichgewicht wurde nicht festgestellt.

Schlüsselwörter Short tandem repeats · HumTHO1 · HumVWA · HumACTBP2 · Populationsstudien · Zagreb-Region

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Introduction

Among the different categories of variable number of tandem repeat (VNTR) polymorphisms, short tandem repeat (STR) polymorphisms seem to offer various advantages: (1) the fragments/alleles can be clearly assigned to one locus, (2) the alleles can be assigned by side-to-side comparisons with mixtures of alleles thus avoiding fragment size measurements, (3) the allele distribution is discontinuous, (4) due to the benefits of the PCR there is extreme sensitivity, (5) as the fragments are extremely small PCR products can also be obtained in degraded DNA samples.

The loci selected for this survey have been recently introduced (HumTHO1 – Edwards et al. 1992, HumVWA – Kimpton et al. 1992, HumACTBP2 (SE33) –Polymeropoulos et al. 1992) and various systematic studies have already been performed on population genetics, applicability, sensitivity and structure (Kimpton et al. 1993; Möller and Brinkmann 1994; Möller et al. 1994). However, data on genotype frequencies in different populations are still rare. Since this is a basic prerequisite also for the forensic application we present our preliminary data on 3 STR systems in a population sample from the Zagreb area.

Materials and methods

Blood samples from healthy unrelated Caucasians living in the Zagreb area were extracted as described previously (Brinkmann et al. 1991). The 3 loci were amplified using primer sequences as published (HumTHO1 – Gill et al. 1992; Hum VWA – Kimpton et al. 1992; HumACTBP2 – Polymeropoulos et al. 1992). The reaction assay and the amplification conditions were also carried out as described (Wiegand et al. 1993; Möller et al. 1994).

Electrophoresis was performed on polyacrylamide gels, separation distance 18 cm, silver staining according to Budowle et al. (1991) using the published conditions (Wiegand et al. 1993; Möller et al. 1994).

The VWA and THO1 allelic ladders used were based on a number of repeats (Brinkmann and Wiegand 1994). For SE33 the same ladder and allele designation was applied as previously described (Wiegand et al. 1993). The sequenced structure of a selected number of fragments is indicated (Table 1).

Table 1 Frequency values for HumACTBP2 (nomenclature according to Wiegand et al. 1993), HumTHO1 and HumVWA in a Croatian population. The ACTBP2 alleles which are indicated with an asterisk have been sequenced (Möller and Brinkmann 1994); the new nomenclature according to the number of repeats is written in brackets.

Allele	HumACTBP2	HumTHO1	HUMVWA
6		0.300	
7		0.090	
8		0.100	
9		0.175	
9.3		0.325	
11A	0.005		
11	0.010	0.010	
12	0.060		
13* (15)	0.040		
14* (16)	0.045		0.130
15* (17)	0.070		0.115
16* (18)	0.080		0.165
17* (19)	0.055		0.335
18* (20)	0.025		0.200
19* (21)	0.050		0.035
20	0.045		0.020
21	0.030		
22	0.055		
23* (22)	0.025		
24	0.015		
25	0.050		
26* (25)	0.025		
27	0.050		
28	0.055		
29* (27)	0.040		
30* (28)	0.085		
31	0.020		
32* (29)	0.035		
33* (30)	0.010		
34	0.020		

Table 2 Comparison of the observed number of alleles and heterozygosity for HumTHO1, HumVWA and HumACTBP2 in the Croatian, German, UK and American population studies.

HumTHO1		
	alleles	heterozy. (n = ind.)
CRO (This study)	6	0.82 (100)
FRG (Wiegand et al. 1993)	6	0.73 (110)
USA (Edwards et al. 1992)	6	0.76 (186)
HumVWA		
	alleles	heterozy. (n = ind.)
CRO (This study)	7	0.75 (100)
FRG (Möller et al. 1994)	9	0.81 (321)
UK (Kimpton et al. 1992)	7	0.73 (100)
HumACTBP2		
	alleles	heterozy. (n = ind.)
CRO (This study)	25	0.96 (100)
FRG (Wiegand et al. 1993)	26	0.93 (180)
USA (Polymeropoulos et al. 1992)	21	0.93 (39)

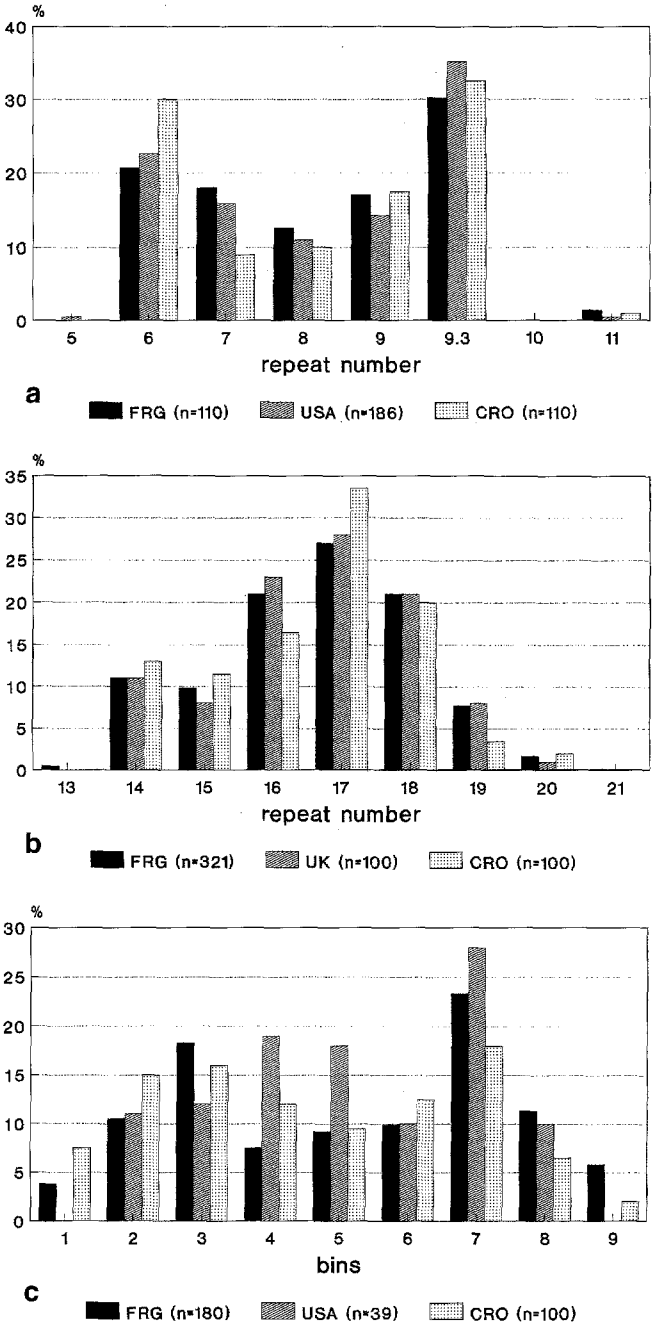

Fig. 1 a–c Comparison of the allele frequencies of different population studies. FRG = white caucasians from Münster area; USA = white caucasians; UK = white caucasians; CRO = white caucasians from Zagreb area (for references see table 2). a) HumTHO1 b) HumVWA c) HumACTBP2; the alleles for ACTBP2 were bined in groups of 3 alleles

The population genetic comparisons were carried out using a test for heterogeneity (R x C contingency table; G. Carmody, Ottawa, Canada).

Results and discussion

The results of this survey (Table 1) are in good accordance with other Caucasian populations with minor dif-

Table 3 Mean exclusion chance and discrimination index (DI) for the 3 STRs.

STR	mean excl. chance	DI
HumTHO1	0.60	0.88
HumVWA	0.63	0.93
HumACTBP2	0.89	0.99

Table 4 Chi-square test for Hardy-Weinberg-calculations (allele-group-model according to Rand et al. 1992).

alleles (groups)	HumTHO1 5 allele-model	HumVWA 5 allele-model	HumACTBP2 4 allele-model
I	allele 6	allele 14–15	allele 11 A–17
II	allele 7	allele 16	allele 18–25
III	allele 8	allele 17	allele 26–30
IV	allele 9	allele 18	allele 31–34
V	allele 9.3, 11	allele 19–20	
Chi-square	12.57	11.2	2.18
P	0.5–0.6	0.6–0.7	0.97–0.99
df	14	14	9

ferences in the number of observed alleles and heterozygosity rates (Table 2; Fig. 1 a–c). Some alleles were not found in this study: THO1 alleles 5 and 10, VWA alleles 13 and 21, SE33 alleles 35 and 36 (Brinkmann and Wiegand 1994).

We have observed a variant allele 11a in SE33 with a frequency of 0.5%. The efficiency data are shown in Table 3. The single discrimination indices will always > 88% and the combined mean probability of exclusion reaches 95%. Hardy-Weinberg calculations applying the cluster approach (Rand et al. 1992) show no significant deviation from equilibrium (Table 4) for all 3 systems.

A comparison of the frequency profiles for HumTHO1 (Fig. 1 a) shows good agreement with other Caucasian populations (Edwards et al. 1992; Wiegand et al. 1993) using an R x C contingency table (Chi-squared = 8.24, $P = 0.80$; G-statistic = 8.64, $P = 0.83$). Good homogeneity was also found for HumVWA (Fig. 1 b) (Chi-squared = 4.89, $P = 0.97$; G-statistic = 5.10, $P = 0.96$). Some minor differences were found for HumACTBP2 (Fig. 1 c) compared with the data from Wiegand et al. (1993) and Polymeropoulos et al. (1992). Allele frequencies for bins 4 and 5 in the Croatian sample and the German sample (Wiegand et al. 1993) show differences from the US sample (Polymeropoulos et al. 1992) which resulted in a low *P*-value ($P < 0.05$) while the Croatian and German population samples can be considered as homogenous (Chi-square = 6.94, $P = 0.56$; G-statistic = 7.08, $P = 0.56$). Also allele 11 a was not found in the 2 other studies. Some of the differences observed could be due to the sample sizes.

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