POPULATION STUDIES OF TWO AMPFLPs AND TWO STRs SYSTEMS IN A NORTH POLISH POPULATION.

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INTRODUCTION

Analysis of variable number of tandem repeats (VNTR) is widely used in forensic testing. PCR amplification of many polymorphic VNTR regions has been described. The most commonly amplified fragment length polymorphisms (AMPFLPs) loci, are D1S80, ApoB, D17S5 and COL2A1. Recently, a number of short tandem repeats (STRs) loci, have been described and applied to the forensic practice.
We describe here the results of population studies of two AMPFLPs and two STR systems investigated in the North Poland, Gdańsk area.
The aims of this investigation were following: 1) to obtain allele frequencies for Polish population, 2) to test whether the allele frequencies conform to Hardy-Weinberg expectations and 3) to compare results with other population samples.

MATERIALS AND METHODS

Blood samples were taken from persons of both sexes living in the North Poland (Gdańsk area). DNA was isolated from blood samples obtained from unrelated persons using non-organic and non-enzymatic method (Lahiri et al. 1992). The analyzed VNTR systems and PCR conditions are shown in Table 1. PCR was carried out in 20μl reaction volumes, using 2-10ng of template DNA, 1 U Taq-DNA-polymerase (Biometra), 200mM of each dNTPs, 1 mM of each primer and 2μl reaction buffer.

Table 1. VNTR loci studied.

Locus	Chromosome location	Chromosomes tested (n)	Primer sequences	Amplification conditions
D1S80	1p36-p35	414	Kasai at al. (1990)	Kloosterman et al. (1993)
D17S5	17p13.3	408	Horn et al. (1989)	Rand et al. (1992)
HUMTH01	11p15.5-p15	406	Edwards et al.(1991)	Wiegand et al. (1993)
HUMVWA	12pter-p12	370	Kimpton et al. (1992)	Möller et al. (1994)

Electrophoretic separation of PCR products was performed by high resolution polyacrylamide gel electrophoresis according to Allen et al. (1989). Gels were silver stained using the modified Bassam (1991) method.

RESULTS AND DISCUSSION

For D1S80 19 different alleles and 59 genotypes were observed. In a sample of 204 persons 13 alleles and 53 genotypes were observed for the D17S5 locus.
For HUMVWA 8 alleles and 27 genotypes were observed and for HUMTH01 7 alleles and

Table 2. Allele frequencies of D1S80 in the Polish population (N=207).

Allele	Frequency	Allele	Frequency	Allele	Frequency	Allele	Frequency
18	0.2005	**23**	0.0386	**28**	0.0507	**33**	0.0024
19	0.0024	**24**	0.3623	**29**	0.0459	**36**	0.0072
20	0.0266	**25**	0.0628	**30**	0.0121	**37**	0.0121
21	0.0072	**26**	0.0290	**31**	0.0604	**41**	0.0024
22	0.0459	**27**	0.0169	**32**	0.0145		

Chi-square = 17.178, df=13, 0.2>P>0.1

Table 3. Allele frequencies of D17S5 in the Polish population (N=204).

Allele	Frequency	Allele	Frequency	Allele	Frequency	Allele	Frequency
1	0.0515	**5**	0.0613	**9**	0.0539	**13**	0.0074
2	0.1324	**6**	0.0490	**10**	0.0539		
3	0.1961	**7**	0.0171	**11**	0.0759		
4	0.2672	**8**	0.0490	**12**	0.0098		

Chi-square = 15.75, 0.3<P<0.5 , df=13

Table 4. Allele frequencies of HUMVWA in the Polish population (N=185).

Allele	Frequency	Allele	Frequency	Allele	Frequency	Allele	Frequency
13	0.0054	**15**	0.0972	**17**	0.2864	**19**	0.0945
14	0.0648	**16**	0.1945	**18**	0.2324	**20**	0.0243

Chi-square = 15.867, df=14, 0.5>P>0.3

Table 5. Allele frequency of HUMTH01 in the Polish population (N=203)

Allele	Frequency	Allele	Frequency	Allele	Frequency	Allele	Frequency
5	0.0024	**7**	0.1256	**9**	0.1847	**10**	0.0123
6	0.2487	**8**	0.1206	**9.3**	0.3054		

Chi-square= 11.224, df=12, 0.5<P<0.7

Table 6. Forensic value of the four analysed systems in the Polish population.

System	H obs.	H exp±SE	DP	DI	PIC	pM
D1S80	0.802	0.812±0.027	0.935	0.071	0.793	0.065
D17S5	0.804	0.853±0.025	0.950	0.069	0.836	0.050
HUMVWA	0.789	0.804±0.029	0.932	0.080	0.775	0.068
HUMTH01	0.782	0.782±0.029	0.916	0.087	0.746	0.084

19 genotypes were observed. Tables 2, 3, 4 and 5 show allele distribution of the analyzed systems. The Chi-square test was carried out to estimate if the population sample conforms to the Hardy-Weinberg (H-W) equilibrium. The Polish population meets H-W expectations for D1S80, D17S5, HUMVWA and HUMTH01. Table 6 shows forensic value of the analysed systems expressed as various statistical parameters. Comparison of allele distributions for different populations was done using the 2-way RXC contingency table.

For D1S80 locus, a comparison of the Polish data with the Danish (Thymann et al. 1993), German (Schnee et al. 1993), and Swiss (Hochmeister et al. 1994) populations showed no statistical differences. On the other hand, the general Polish D1S80 distribution was statistically different from the Spanish (Lareu et al. 1993) and Dutch (Kloostermann et al. 1993) at $P<0.005$. Comparison of allele distribution for D17S5 showed no statistical differences between the Polish and American (Batanian et al. 1990) populations. However there were statistically significant differences between the Polish and Spanish (Gene et al 1995) populations ($P=0.001$), Polish and Italian (Buscemi et al. 1994; $P=0.05$), and Polish and German (Rand et al. 1992) populations ($P=0.031$). For HUMVWA we did not observe statistical differences in alleles distribution between the Polish and German (Möller et al. 1994) and Polish and English (Drozd et al. 1994) populations. Statistically significant differences were observed between the Polish and Finnish ($P<0.05$; Sajantila et al. 1994), and Polish and Spanish ($P<0.001$; Pestoni et al. 1995) populations. We have shown that there is no significant difference in the allele frequencies at the HUMTH01 locus between the Polish and German (Wiegand et al. 1993), Swiss (Hochmeister et al. 1994) and Spanish (Pestoni et al. 1995) populations. However, these distributions in the Polish and Danish populations differed significantly ($P=0.001$).
In conclusions, this study shows that the analyzed loci are highly polymorphic in the Polish population, and could be used as a powerful DNA typing systems.

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