POPULATION GENETICS OF THE AMPLIFIED FRAGMENT LENGTH POLYMORPHISM AND SHORT TANDEM REPEAT TYPE SYSTEMS IN THE POPULATION OF NORTHERN POLAND (GDANSK AREA)

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Abstract: This paper presents results of the population studies of the VNTR polymorphic systems of human DNA. DNA samples were taken from at least 106 unrelated persons living in the Gdansk area. DNA samples were subjected to the PCR amplification and PCR products separated on polyacrylamide gels. Allelic frequencies of three AMPFLPs systems and six STRs were calculated and homogeneity of alleles distribution between Gdansk and other population samples were compared. The presented data are the core of the planned population-based DNA indentification system for Poland.

Introduction

VNTRs (variable number of tandem repeats) are polymorphic regions of the human DNA consisting among others of minisatellites and microsatellites. Short tandem repeat (STR) microsatellite loci consist of tandemly repeated sequences between 2 and 6 base pair in length, but minisatellite AMPFLP (Amplified Fragment Length Polymorphism) loci contain longer tandemly repeated sequencies. The polymerase chain reaction (PCR) is an in vitro enzymatic process allowing to amplify double stranded DNA using thermal stable DNA polymerase and specific oligonucleotide primers.The working out of the PCR oferred many new diagnostic possibilities, among others in forensic biology, where it is chiefly used to amplify ministatellite (AMPFLP) and microsatellite (STR) VNTR sequences. Our laboratory has applied the PCR technique for biological traces analysis in 1992 and as the first in Poland introduced this technique into laboratory practice. Annually an average of over 10 thousands PCR reactions are carried out, this including analysis of biological traces, population studies, clinical diagnostics of chimerism after bone marrow transplantation, also the investigating the linkage between some human genes and hypertension, as well as cardiac ischaemia and diabetes.

One of the goals of our forensic medicine laboratory is to establish a database consisting of DNA profiles which will make possible the indentification of subjects who were under criminal investigation. The database will consist of approximately 10^6 subjects for whom many different polymorphic systems will be determined. The identification process i.e. the comparison of multiple loci profiles with established database needs powerful computer systems to make this process feasible. Such a DNA databases are already established and effectively used in some Western countries.

Our main research at present, however is concentrated on population studies of new systems, their usefulness and reliability when studying biological traces, as well as the working out of optimum conditions of the separation of their alleles. However, the population studies are needed to assess the frequencies of alleles. The certainty of the investigation process is higher when the population frequency is low. Before polymorphic systems are promoted for use in identification studies, checks must be carried out to ensure that it is in Hardy-Weinberg (H-W) equilibrium. Hardy-Weiberg law states that in large intrabreeding population, the gene and genotype frequencies will remain constant over time. The Hardy-Weiberg law allows genotype frequencies to be determined if allele frequencies are known. For p = frequency of allele A and q = frequency of allele a, the equilibrium genotype frequencies of A/A, A/a and a/a become p^2, 2pq and q^2, respectively in one generation.

The main aim of the work was to establish frequencies of the alleles of the three AMPFLPs and six STRs VNTR systems, as well as comparison of distribution of allele frequencies between Polish and other Caucasian European populations.

Materials and methods

Blood samples were obtained from 106 unrelated donors living in the Gdansk area (North Poland). DNA was extracted using the method described by Lahiri et al. [1]. PCR amplifications of human DNA loci were performed according to previously described methods Wiegand et al. [2] (ACTBP2 and HUMTH01), Möller et al. [3] (HUMFES/FPS and HUMVWA), Wall et al. [4] (HUMCD4), Rand et al. [5] (D1S80, ApoB, D17S5), and Kayser et al [6] (DYS390).

The separation of PCR products was mainly carried out on horizontal polyacrylamide gels, using a discontinuous buffer system as described by Allen et al. [7]. Electrophoregrams were stained with silver using our modification of Bassam method [8] and alleles identified by comparison with allelic ladders.

Statistical calculations.

Calculations of the Hardy-Weinberg equillibria (HWE) were performed using computer programme GENEPOP (Version 1.2) [9]. To demonstrate homogeneity of

the allele frequencies distribution between different populations, R×C contingency tables were used (computer program kindly provided by G. Carmody, Carleton University, Ottawa, Canada). George Carmody program calculates probablility of obtaining an observed 2-way contingency table even when some cells have very small (i.e.<5) numbers. It calculates this probability by generating 1000 random tables, each having the same marginal totals as the observed table. The program calculates the Chi-square and the G-statistic (Likelihood-ratio test; for the observed table and for all of the 1000 simulated tables. It thus avoids the common problem of having to combine data to make each cell have at least 5 observations.

The power of discrimination (PD) was calculated as $1-\sum(P_i)^2$, where P_i represents the frequency of each phenotype. Discrimination index (DI) was calculated as $DI = q^2 (2-q)$, where q is mean allele frequency and polymorphic information content (PIC) as $PIC = 1-\sum p_i - (\sum p_i^2)^2 + \sum p_i^4$, where p_i is a frequency of every allele and n – number of every allele.

Results

Amplified Fragment Length Polymorphism (AMPFLP) Systems

So far, population studies of three AMPFLP and six STR systems have been completed. The results of population studies are presented in Table I.

D1S80

Population studies embraced a group of 207 unrelated persons. In the population analyzed, 19 alleles and 59 phenotypes were found. Distribution of D1S80 alleles is bi-modal with the most frequent 18 (20,05%) and 24 (36,23%). No deviation from the Hardy-Weinberg equation was observed ($\chi^2 = 17.18$; $0.2<P<0.3$; d.f.=14). Comparison of allele distribution for different populations was carried out using the 2-way R×C contingency table (Table II). For D1S80 locus, comparison of the Polish data with the German [10], Swiss [11], Austrian [12], Danish [13] and Southern Polish [14] populations showed no statistical differences. On the other hand, the general Polish D1S80 distribution was statistically different from the South-Eastern Polish [15], Spanish [16], Hungarian [17] and Russian population[18].

D17S5

In the population sample of 204 individuals from the Gdansk area we found 13 alleles (Table I) and 53 phenotypes of D17S5 system. The most frequent alleles observed in our population are: 4 (26,7%), 3 (19,6%), the most frequent phenotypes being 3,4 and 4,4 with frequencies 10,29% and 8,34% respectively. Alleles with a number of repetitive units higher than 13 were not observed. Distribution of D17S5 alleles in North Polish population fits the H-W equation ($\chi^2 = 15.75$; $0.3<P<0.5$; d.f.=13).

Comparison of allele distribution for D17S5 shows no statistical differences between the Polish and English [19], and Polish and Italian [20] populations. However, there are statistically significant differences between the Polish and Spanish [21] populations and Polish and German [5] populations (Table II). The main problem accompanying the typing of minisatellites is preferential amplification. In the case of D17S5 we observed that this phenomenon depends greatly not only on DNA concentration but also that of magnesium ions (data not shown). In the sample analyzed the heterozygosity observed (H=0.804) was lower than expected (Hexp.=0.853 (0.025). One of the possible explanations of heterozygote deficiency could be preferential amplification.

ApoB

The ApoB system constitutes a third of the minisatellite systems routinely used routinely in our lab. In the analyzed sample of 201 unrelated persons, 21 alleles and allelic variants were observed (Table I). The most frequent are 37 and 35. As it was expected, the most frequent phenotypes are 37,37 and 35,37, with frequencies of 18,4% and 15,4% respectively. No deviation from the H-W equation was observed (χ^2 = 13.99; 0.3<P<0.5; d.f.=12).

A comparison of our ApoB alleles distribution with Italian [22], Austrian [23] and Hungarian [17] population samples showed a significant difference. The highest differences in allele frequencies were observed for small ApoB alleles as 29 (P<0.001) and 31 (P<0.001). No significant difference was observed between our and German population [24].

Short Tandem Repeat (STR) Systems

HUMTH01

Population studies were performed on the population of 203 unrelated persons. Seven TC11 alleles and 19 phenotypes were observed. The most frequent alleles are 9.3 and 6, and phenotype is 6, 9.3 with a frequency of almost 13% (Table I). The system analyzed fits the H-W equilibrium (χ^2 = 11.22; 0.5<P<0.7; d.f.=12).

We have shown that there is no significant difference in the allele frequencies at the HUMTH01 locus between the Polish and the German [2], Swiss [25], Spanish [26] and Hungarian [27] populations (Table III). Deviation from population homogeneity was, however, observed when comparing the Polish population sample with French (P=0.038) [28], Danish (P=0.002) [29], Dutch (P=0.033) [30] and Austrian populations (P<0.001) [31]. The greatest discrepancies between the Polish and Austrian populations were observed for alleles 7 (χ^2 = 13.4500; P=0.0000) and 9 (χ^2 = 9.3303; P = 0.0020) and between the Polish and French for allele 10 (χ^2 = 6.3400; P = 0.0150).

HUMVWA

For HUMVWA we observed 8 alleles and 27 phenotypes in the population of 185 persons. The alleles 11, 12 and 21 were not observed in the population sample analyzed (Table I). The most frequent alleles are 16 (19,45%), 17 (28,64%) and 18 (23,24%), the most frequent phenotypes being 16,17 (12,97%) and 17,18 (11,89%). The population sample analyzed meets HW expectations for HUMVWA (χ^2 = 15.8760; 0.3<P<0.5; d.f. = 14). For HUMVWA we did not observe statistical differences in alleles distribution between the Polish and the Dutch [30], Polish and German [3], Polish and two English [32,33] populations, Polish and Austrian [31], as well as Polish and Hungarian [27] populations. Statistically significant differences were, however, observed between the Polish and Finnish (P<0.008) [34] and Polish and Spanish (P<0.001) [35] populations. The greatest difference between the Polish and Spanish populations (using 2×2 contingency tables) was found for allele 14 frequencies (P<0.001).

HUMFES/FPS

In the population of 106 persons from the northern Poland, we observed eight alleles and 20 phenotypes of the HUMFES/FPS system. The alleles 7 and 14 were not observed. The most frequent alleles are 11 (40,57%) and 10a (19,34%) and the most frequently occurring phenotypes are 11,12 (20,75%) and 10a,11 (17,92%). Statistical calculations showed that the analyzed population sample meets the H-W expectation with a high P-value (χ^2 = 0.5317; 0.95<P<0.98; d.f. = 4). Our allele frequencies distribution is similar to those which were observed for German [36], French [28], Spanish [26,37], Dutch [30] and Austrian [38] Caucasian samples (Table III).

HUMCD4

Distribution of HUMCD4 alleles in the Polish population was analyzed in a sample of 129 individuals. We observed alleles 5,6,7,10,11,12 and 13. In the population sample analyzed, we found 15 different phenotypes of 28 possible for this number of alleles. In the Polish population the most frequent alleles are 5 (32,56%), 6 (31,01%) and 10 (30,62%) and the most frequent phenotypes are 5,6 (19,38%), 6,10 (17,83%), and 5,5 and 5,10 present with a frequency of 14,73%. The analyzed population sample is in the H-W equilibrium: χ^2 = 6.8315; 0.2<P<0.3; d.f. = 5. A comparison of our data with German [39] and Austrian [40] population was done using a R×C contingency table. No deviation from population homogeneity was observed in this comparison (Table III).

ACTBP2

The ACTBP2 is one of the most informative systems introduced so far in our laboratory. A total of 25 alleles was observed for ACTBP2 in a population of 176

unrelated persons. The observed frequency of most frequent allele of ACTBP2 is not higher than 9% (Table I) , and the frequency of most common phenotype below 4%. The statistical analysis of the population sample showed clearly that the system is H-W equilibrium ($\chi^2 = 2.45$; $0.95<P<0.98$; d.f. = 8). Possible heterogeneity in ACTBP2 allele frequencies between the north Polish, German [41] and Zagreb [42] population samples was assessed using an R×C contingency table. Deviation from population homogeneity was observed. The north Polish sample, however, was not statistically different from the west Polish [43] sample (Table III).

DYS390

The DYS390 is Y-chromosome specific STR system. Seven alleles (8-14) were observed in a population sample of 128 unrelated males. Table I presents the frequency of observed alleles. In the Polish population the most frequent alleles are 12 (40,6%) and 11 (25,7%). For DYS390 we did not observe statistical differences in alleles distribution between Gdansk and Heidelberg population samples, or significant statistical differences between Gdansk and Leiden, as well as Leicester, Rome and Muenster [44] (Table III).

Valuation parameters of DNA polymorphic systems.

Table IV shows the forensic value of the analyzed systems expressed as various statistical parameters.The presented frequencies of alleles are generally the first findings in the Polish population. These data are often used for forensic application. It is of great importance that the collected data will permanently increase in terms of subjects, as well as new polymorphic systems. In the nearest future we are planning to establish the system which allows us to communicate with other centers in order to create a general database for Poland. Such system will also be effective in an international data exchange.

Summary

Summing up, it can be stated that the obtained parameters characterising the systems studied and our experience in applying them in examining biological traces for identification purposes permit us to recommend them as very helpful in forensic-medical practice.

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Table I. *Allele frequencies of nine VNTR systems in the Polish population*

Alleles	*D1S80 n=207*	*ApoB n=201*	*D17S5 n=204*	*ACTBP2 n=176*	*HUMTH01 n=203*	*HUMVWA n=185*	*HUMFES n=106*	*DYS390 n=128*	*HUMCD4 n=129*
1			0,0515						
2			0,1324						
3			0,1961						
4			0,2672						
5			0,0613		0.0025				0.3256
6			0,0490		0.2488				0.3101
7			0,0171		0.1256				0.0116
8			0,0490		0.1207		0.0189		---
9			0,0539		0.1847		0.0094		—
9.3					0.3054				—
10			0,0759		0.0123		0.0708		0.3062
10a							0.1934		—
11			0,0098				0.4057		0.0194
11a							0.0142		
12			0,0294	0.0170			0.2217		0.0194
13			0,0074	0.0057		0.0054	0.0660		0.0078
14				0.0426		0.0648			
15				0.0540		0.0972			
16				0.0625		0.1945			
17				0.0739		0.2864			
18	0.2005			0.0540		0.2324			
19	0.0024			0.0852		0.0945			
20	0.0266			0.0739		0.0243			
21	0.0072			0.0369				0.0156	
22	0.0459			0.0483				0.1172	
23	0.0386			0.0227				0.1484	
24	0.3623			0.0341				0.2578	
25	0.0628			0.0227				0.4063	
26	0.0290			0.0370				0.0469	
27	0.0169			0.0653				0.0078	
28	0.0507			0.0653					
<29		0.0075							
29	0.0459	0.0572		0.0454					
30	0.0121	---		0.0682					
31	0.0604	0.0319		0.0426					
32	0.0145	—		0.0227					
33	0.0024	0.0348		0.0085					
34	—	—		0.0057					
35	—	0.1866		0.0028					
36	0.0072	—		0.0028					
37	0.0121	0.4527							
39	—	0.0224							
41	0.0024	0.0299							
43		0.0050							
45		0.0249							
47		0.0547							
49		0.0796							
51		0.0100							
53		0.0025							

Table II. *Pairwise comparison of allele frequencies for three AMPFLPs between Caucasian population samples.*

Legend:

A – Austria,CH – Switzerland, D – Germany, DK – Denmark, E – Spain, F – France,GB – Great Britain, H – Hungary, I – Italy, NL – Nederland, PL – Poland, SF – Finnland, N – North, S – South, W – West, E – East, SE – South-East, NW – North-West.

Compared populations	x^2	*P*	*G-statistic*	*P*
D1S80				
PL × CH [11]	18.9490	0.4580	19.7167	0.5650
PL × D [10]	24.9661	0.2345	29.5823	0.1692
PL × A [12]	27.8501	0.0783	31.1569	0.0720
PL × DK [13]	24.2263	0.2890	27.4135	0.2910
PL × Russians [18]	31.7921	0.0242	35.3524	0.0246
N PL × S PL [14]	32.1064	0.0504	35.3978	0.0656
N PL × SE PL [15]	38.0481	0.0106	33.8087	0.0358
PL × H [17]	41.2778	0.0120	47.9612	0.0140
PL × E [16]	42.5180	0.0031	48.9742	0.0025
D17S5				
PL × GB [19]	10.3812	0.6800	10.8478	0.6920
PL × I [20]	20.9571	0.0530	21.2366	0.0602
PL × D [5]	24.6248	0.0260	24.5652	0.0380
PL × E [21]	29.8415	0.0040	30.5608	0.0060
APOB				
PL × D [24]	20.4931	0.0710	19.5042	0.1202
PL × I [22]	50.9674	0.0000	52.4002	0.0000
PLx H [17]	72.3707	0.0000	84.5764	0.0000
PL × A [23]	58.2928	0.0000	70.6315	0.0000

Table III. *Pairwise comparison of allele frequencies for six STRs between Caucasian population samples.*

Compared populations	x^2	*P*	*G-statistic*	*P*
HHUMCD4				
PL × D [39]	13.108	0.092	13.851	0.113
PL × A [40]	9.830	0.252	12.209	0.208
HUMFES/ FPS				
PL × A [38]	8.380	0.285	8.864	0.318
PL × F [28]	7.583	0.268	7.923	0.306
PL × N Spain [37]	3.939	0.584	4.606	0.544
PL × NW Spain [26]	3.609	0.612	3.615	0.622
PL × D [36]	8.074	0.331	9.242	0.338
PL × NL [30]	8.3155	0.121	7.984	0.172

HUMTH01				
PL × D [2]	7.034	0.314	7.452	0.317
PL × H [27]	4.245	0.640	4.253	0.662
PL × CH [25]	5.954	0.438	5.723	0.512
PL × E [26]	10.642	0.079	12.582	0.056
PL × F [28]	12.834	0.038	14.802	0.027
PL × NL [30]	11.583	0.033	11.691	0.044
PL × DK [29]	21.694	0.002	22.626	0.003
PL × A [31]	22.345	0.0000	22.998	0.0000
HUMVWA				
PL × NL [30]	6.470	0.590	8.018	0.510
PL × D [3]	8.138	0.418	8.718	0.413
PL × GB [33]	7.574	0.362	8.242	0.349
PL × GB [32]	10.440	0.147	10.682	0.166
PL × A [31]	13.423	0.094	14.194	0.108
PL × H [27]	13.964	0.063	15.265	0.057
PL × SF [34]	18.984	0.006	19.552	0.011
PL × E [35]	26.619	0.0000	30.171	0.0000
ACTBP2				
N PL × W PL [43]	27.101	0.298	27.279	0.371
PL × D [41]	39.922	0.038	39.677	0.043
PL × Zagrebpopulation [42]	19.540	0.018	19.090	0.021
DYS390				
Gdansk × Heidelberg [44]	10.243	0.098	11.4166	0.093
Gdansk × Leiden [44]	37.378	0.000	41.553	0.000
Gdansk × Leicester [44]	54.086	0.000	52.514	0.000
Gdansk × Rome [44]	48.346	0.000	54.916	0.000
Gdansk × Muenster [44]	23.087	0.000	24.406	0.000

Table IV. *Forensic value of the nine analyzed systems in the Polish population.*

System	*PD*[a]	*H*[b]	*DI*[c]	*PIC*[d]	*Most frequent phenotype*	*(%)*
ACTBP2	0.995	0.955	0.004	0.942	17,30	(3,40)
D17S5	0.950	0.804	0.069	0.836	3,4	(10.29)
D1S80	0.935	0.802	0.071	0.793	18,24	(17.87)
HUMVWA	0.932	0.789	0.080	0.775	16,17	(12.97)
ApoB	0.927	0.771	0.093	0.773	37,37	(18.40)
HUMTHO1	0.916	0.778	0.087	0.746	6,9.3	(12.81)
HUMFES/FPS	0.883	0.736	0.121	0.702	11,12	(20.75)
HUMCD4	0.862	0.636	0.217	0.644	6,10	(17.83)
DYS390	0.730	0.730	0.126	0.689	12	(40.6)

[a] PD indicates power of discrimination which is calculated using the formula $PD=1-(\sum Pi)^2$, were Pi is the frequency of each genotype; [b] H observed heterozygosity ; [c] DI — discrimination index [45]; [d] PIC –- indicates polymorphic information content [46].

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