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Use of Polymerase Chain Reaction for Allele Distribution Analysis of Variable-Number Tandem Repeats Among 120 Unrelated Russian Individuals Living in Moscow

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Frequencies of allele occurrence in four variable-number tandem repeat (VNTR) regions (loci D1S80, D17S30, APOB, and IGHJ) were determined in 120 unrelated Russian individuals living in Moscow. A high level of polymorphism was revealed for these hypervariable regions, characterized by the presence of alleles of different length. Distribution of genotypes with respect to these loci was established on the basis of the data obtained. Comparative analysis showed similar allele distribution of the VNTRs studied among Russians and other groups of Caucasians living in Europe and North America.

Key words: polymerase chain reaction; allele polymorphism; hypervariable regions

Genome hypervariable regions (HVR), also known as variable-number tandem repeats (VNTRs) are used as genetic markers in human genome studies. These minisatellite sequences have a high level of length polymorphism and may be used for solving the problems associated with gene mapping and identification of the loci responsible for malignant transformation and development of a number of hereditary diseases. The polymorphic minisatellite sequences find ever-increasing use in establishing parenthood, individual identification, and forensic medicine [1–4].

The copy number in tandem repeats may vary from one to some dozen. A definite set of alleles differing in copy number and, consequently, in length is usually found in the population. A set of repeat sequences of definite length is unique for every individual, therefore allele polymorphism in VNTRs can be efficiently used for individual identification, as shown earlier [5].

Until recently, the typing of hypervariable regions has been based on the use of Southern hybridization [6] or analysis of the repeat length fragment polymorphism (RLFP) upon cleaving the genomic DNA with restrictases [7]. However, these experimental approaches had insufficient resolution in the cases when the VNTRs were part of relatively large DNA fragments, since the alleles were close in length. In these cases it is impossible to differentiate the VNTR alleles differing by 11–70 bp (this interval can include the length of repeated units of known VNTRs). Besides, substantial quantities of DNA, which cannot be extracted from small stains of semen, blood, or from hair roots, are required for successful analysis using the methods mentioned above [8]. The quality of DNA used is also important; degraded or contaminated DNA from old stains proved to be of little use.

Rapid development of DNA amplification methods based on polymerase chain reaction (PCR) radically changed the situation in typing of hypervariable regions and obviated many limitations on the efficiency of methodological approaches mentioned above [9]. Any genome DNA fragment can be amplified using flanking oligonucleotide primers and thermostable DNA polymerase. The DNA amplification technique finds application in VNTR polymorphism analysis. Important results, including individual identification, may be obtained even in the cases when the investigator has the genomic DNA extracted from a single cell [10, 11].

To date, a few hundred of VNTRs have been found in human genome. Some data on VNTRs more frequently used in genome dactyloscopy are presented in Table 1. In this work we carried out the analysis of allele distribution for four VNTRs: pYNZ22 and pMCT118

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TABLE 1. Tandem Repeats with Variable Copy Number Commonly Used in Genome Dactyloscopy Based on PCR

VNTR region	HGM locus	Chromosome localization	Allele number	Repeat length, bp	Amplified fragment length, kb	Heterozygosity index, %	References
Gene *apo*B	APOB	2p23 – p24	12	30	0.54 – 0.87 0.56 – 0.97	75	[13–15]
pYNZ22	D17S30	17p13	10	70	0.17 – 0.87	86	[16]
pMCT118	D1S80	Chromosome 1	21	16	0.39 – 0.72	78	[20]
HVR-Ig_H	IGJH	14q32	6	50	0.5 – 1.7	67	[15]
p33.4	–	Not defined	7	64	0.8 – 1.3	70	[12]
p33.6	D1S111	1q23	8	37	0.5 – 1.0	67	[12]
Gene IL-6	IL6	7p21 – p14	4	30–80	0.60 – 0.76	55	[17]
Collagen gene	COL2A1	12q14.3	6	31–34	0.58 – 0.72	81	[18]
c-Ha-ras	HRAS	11p15.5	18	28	0.86 – 2.65	58	[14]
p602	DXYS17	Xp22/Yp11	10	70	0.52 – 1.24	87	[19]

that are not contiguous to known genes, and tandem repeats located in genes of apolipoprotein B (in 3'-terminal nontranslated region) and immunoglobulin heavy chain (in 5'-terminal segment J) among 120 unrelated Russians living in Moscow.

MATERIALS AND METHODS

Taq^R DNA polymerase was obtained from NPK Biotekh (Moscow). Labeled [α-P^{32}]dATP was obtained from the Institute of Nuclear Physics, Russian Academy of Sciences (Obninsk). Nylon filters Hybond N were from Amersham.

Oligonucleotide primers were synthesized in the Institute of Applied Enzymology (Vilnius, Lithuania).

Genome DNA extraction from human blood was conducted according to known methods [4, 21].

Polymerase chain reaction was carried out in a Techne PHC-2 Thermal Cycler. The reaction mixture composition was the following: reaction buffer, 0.1–1.0 μg of genomic DNA, 1 μM of primers, 2 un. *Taq*^{R} DNA polymerase. For tandem repeat pYNZ22 amplification, primers TR-1 and TR-2 were used; for pMCT118 amplification, primers TR-5 and TR-6; for hypervariable regions of apolipoprotein B (*apo*B) and immunoglobulin H (HVR-Ig_H), TR-7+TR-8 and TR-13+TR-14, respectively. The reaction buffer was 67 mM Tris-HCl, pH 8.8; 16.6 mM ammonium sulfate, 0.01% Tween-20, 0.15 mg/ml BSA, 0.5 mM nonlabeled triphosphate. To amplify pYNZ22 and HVR-Ig_H, 1.0 mM $MgCl_2$ was used; to amplify pMCT118 and *apo*B, 1.5 and 4.0 mM, respectively. The volume of reaction mixture was 50 or 100 μl.

The program of reaction in the first cycle was the following: denaturation at 94°C, 4 min, then *Taq*^{R} DNA polymerase was added and the reaction was continued *a*) in the case of pYNZ22 and *apo*B, 94°C, 1 min; 57°C, 6 min; 30 cycles; *b*) in the case of pMCT118, 94°C, 1 min; 65°C, 1 min; 70°C, 8 min; 30 cycles; *c*) in the case of HVR-Ig_H, 94°C, 1 min; 68°C, 6 min; 30 cycles.

The amplification products were analyzed by electrophoresis in 6% polyacrylamide or 2% agarose gel, 10–20% volume of reaction mixture was loaded into the wells. Polyacrylamide gel was stained by silver [20]. DNA was transferred to nylon filters as described by Maniatis et al. [22].

The mixture of amplified fragments containing different alleles of VNTRs was labeled with [α-^{32}P]dATP using *Taq*^{R} DNA polymerase. The composition of reaction mixture (volume 20 μl) was the following: reaction buffer for amplification of corresponding VNTR, 0.5 μg of DNA, nonlabeled dTTP, dCTP, and dGTP, 0.05 mM each, 50 μCi of [α-^{32}P]dATP, corresponding primers (10 ng each), 1 un. *Taq*^{R} DNA polymerase. DNA was previously denatured by exposing the reaction mixture at 95°C for 4 min, then the enzyme was added and three cycles of reaction were carried out according to the following program: 94°C, 1 min; 65°C (pMCT118), 57°C (pYNZ22 and *apo*B), or 68°C (HVR-Ig_H), 1 min; 70°C, 1 min. In the final cycle the reaction was conducted at 70°C for 3 min.

Prehybridization was carried out for 2 h at 65C in a mixture of the following composition: 5 × SSPE (SSPE: 180 mM NaCl, 10 NaH_2PO_4, pH 7.4; 1 mM EDTA), 5× Denhardt solution, 0.1% sodium dodecylsulfate (SDS). Hybridization with radioactive probes was conducted overnight at 65°C in the same mixture as prehybridization, with addition of salmon sperm DNA previously denatured by incubation at 100°C for 1 h in the presence of 0.5 N NaOH (up to 50 μg/ml). Nylon filters were washed to remove nonspecifically bound probes with: a) 2 × SSPE, room temperature, 15 min, one time; b) 2 × SSPE, 0.1% SDS, room temperature, 15 min, one time; c) 2 × SSPE, 0.1% SDS, 65°C, 15 min, six times; d) 1 × SSPE, 65°C, 15 min, two times.

TABLE 2. Nucleotide Sequence of Amplification Primers for Human Genome Fragments Containing Blocks of Tandem Repeats with Variable Copy Number

Code	Sequence 5′ → 3′	Length, bp	Designation	References
TR-1	CGAAGAGTGAAGTGCACAGG	20	pYNZ22	[16]
TR-2	CACAGTCTTTATTCTTCAGCG	21		
TR-5	GAAACTGGCCTCCAAACACTGCCCGCCG	28	pMCT118	[20]
TR-6	GTCTTGTTGGAGATGCACGTGCCCCTTGC	29		
TR-7	ATGGAAACGGAGAAATTATG	20	gene *apo*B	[13]
TR-8	CCTTCTCACTTGGCAAATAC	20		
TR-13	GGGCCCTGTCTCAGCTGGGGA	21	HVR-Ig_H	[14]
TR-14	TGGCCTGGCTGCCCTGAGCAG	21		

The filters were exposed with X-ray film RMV in the presence of intensifying screens EUI-1 overnight at minus 70°C.

RESULTS AND DISCUSSION

To amplify minisatellite repeats situated in four loci of human genome in different chromosomes, we used synthetic oligonucleotide primers (Table 2). Amplification was carried out for four VNTRs in the DNA extracted from blood of 120 unrelated Russians living in Moscow.

We introduced *Taq*^{R}DNA polymerase into the reaction mixture after DNA denaturation at 94°C for 4 min to prevent the nonspecific reaction of second chain synthesis, which results in discrimination of alleles when the synthesis of shorter VNTR alleles in the case of heterozygotous genotype is more probable [23].

The number of PCR cycles is also important. The optimal number is 25–30, provided that the reaction mixture contains no less than 10 ng of genomic DNA [14]. A greater number of cycles gives rise to DNA fragments of abnormal size due to the presence of partly transcribed DNA template molecules at the end of the second chain synthesis stage. These molecules can serve as primers at the subsequent annealing stage and interact with some sites of the hypervariable region by complementation with the tandem repeats. This results in DNA fragments of abnormal length.

The elongation stage is to be continued for a long time (6–10 min), sufficient for complete synthesis of maximal-size alleles. The primer annealing temperature and Mg^{2+} concentration are decisive for reaction selectivity. To amplify VNTRs, we used primer pairs with close values of annealing temperatures, and annealing was conducted at the maximal temperature characteristic for one of the primers of the pair. Besides, in the case of some VNTRs we conducted PCR using a two-stage program, combining the primer annealing stage and the synthesis of the second chain. This experimental approach also facilitates the production of full-length largest alleles.

The optimal Mg^{2+}concentration was determined experimentally, preference was given to the concentration at which no irregular-length DNA fragments were produced while the amount of desired fragments was maximal. The optimal Mg^{2+} concentrations for VNTR amplification differed: pMCT118 amplification was conducted at 1.5 mM Mg^{2+}, pYNZ22 and HVR-Ig_H at 2.0 mM Mg^{2+}, and *apo*B at 4.0 mM Mg^{2+}.

Analysis of DNA from 120 blood samples of unrelated Russians from the Moscow population in D17S30 locus (tandem repeat pYNZ22) revealed 11 alleles of 170–940 bp differing 70 bp (Fig. 1, Table 3). The locus heterozygosity was 78%; this figure is close to the value presented by Horn et al. [16] for US Caucasians (86%).

Alleles No. 3 (FO is 0.171) and No. 4 (0.371) were found to be more representative among pYNZ22 alleles (Table 3). The other alleles had FO within the limits of 0.004–0.092. Among the genotype variants observed, allele combinations 3–4 (0.158) and 4–4 (0.116) were the most frequent (Table 3).

We have shown the presence of seven alleles of the IGHJ locus of 470–920 bp (Table 3) (the length of repeat unit is 50 bp), and found a novel allele No. 6 of 720 bp not observed early [14]. The heterozygosity index for this hypervariable region is 68%, which is close to the result of Decorte et al. [14] on Caucasians of Belgium (73%).

Alleles No. 3 (FO is 0.387) and No. 5 (0.304) also occurred more frequently among HVR-Ig_H alleles found (Table 3). Alleles No. 3 (0.402) and No. 5 (0.229) were more frequently represented in Caucasians living in Belgium [14]. The genotypes containing allele combinations 3–3 (0.158), 3–5 (0.217), and 5–5 (0.125) were most widely represented in Russians living in Moscow (Table 5).

The length of repeat unit of locus D1S80 (tandem repeat pMCT118) is only 16 bp [24]. The polymorphism level of this locus is high, therefore it is practically impossible to use electrophoresis in agarose gels and subsequent ethidium bromide staining. To improve the resolving capacity, Budowle et al. [24] proposed to use electrophoresis in polyacrylamide gels with subsequent silver staining, and a special mixture of regular mol. wt. markers. This mixture consists of DNA fragments, the sizes of which are multiples of a certain value. For example, Budowle et al. used a mixture of DNA fragments differing in 123- and 1000-bp steps: 123, 246, 369 bp, etc., and 1000, 2000, 3000 bp, etc. [20].

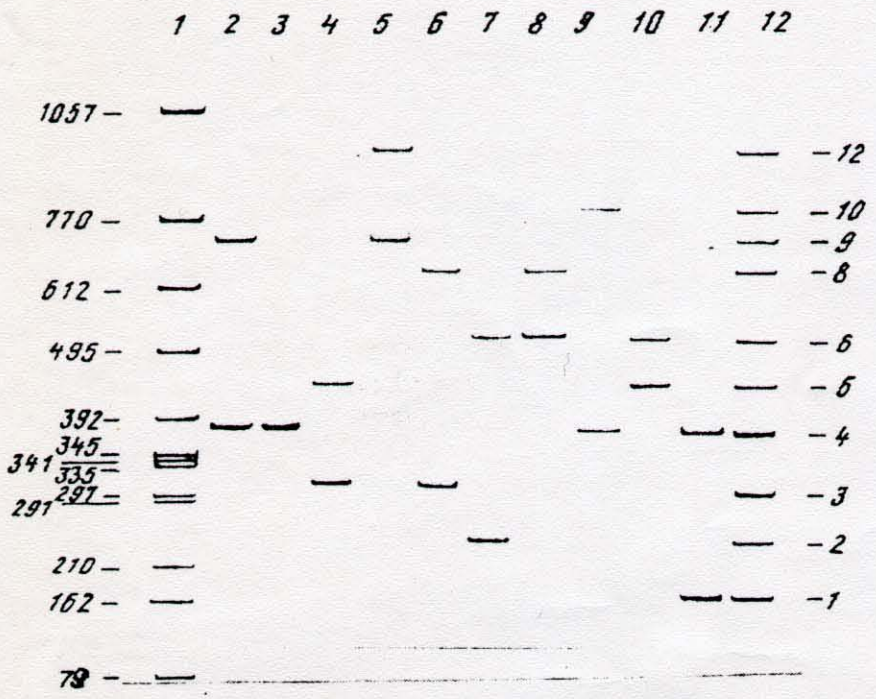

Fig. 1. Electrophoretic separation of tandem repeat pYNZ22 amplification products in 6% polyacrylamide gel (stained with silver): *1*) Phage φX174 DNA fragments cleaved with *Hinc*II (numbers give fragment size in bp); *2–11*) amplification products of tandem repeat pYNZ22 of 10 unrelated genomic DNA samples; *12*) markers containing a mixture of pYNZ22 alleles (ordinal numbers of the alleles are shown on the right).

TABLE 3. Allele FO for Four VNTRs Among 120 Unrelated Representatives of Russian Population

Allele number	Length, bp	Frequency of occurrence	Allele number	Length, bp	Frequency of occurrence
pYNZ22					
1	170	7/0.029	7	590	3/0.012
2	240	22/0.092	8	670	8/0.033
3	310	35/0.147	9	730	19/0.079
4	380	94/0.387	10	800	19/0.079
5	450	16/0.067	12	940	1/0.004
6	520	16/0.067			
HVR-IgH					
1	470	29/0.121	6	720	11/0.046
2	520	17/0.071	9	870	15/0.062
3	570	93/0.387	10	920	2/0.008
5	670	73/0.304			
pMCT118					
1(18)	430	72/0.300	8(25)	542	12/0.050
2(19)	446	5/0.021	9(26)	568	7/0.029
3(20)	462	6/0.025	10(27)	584	5/0.021
4(21)	478	10/0.042	11(28)	600	10/0.042
5(22)	494	8/0.033	12(29)	616	8/0.033
6(23)	510	9/0.037	13(30)	632	1/0.004
7(24)	526	72/0.300	14(31)	648	15/0.062
apoB					
1(29)	541	7/0.029	7(41)	721	21/0.088
2(31)	571	8/0.033	8(43)	751	1/0.004
3(33)	601	7/0.029	9(45)	781	8/0.033
4(35)	631	49/0.204	10(47)	811	12/0.050
5(37)	661	86/0.358	11(49)	841	11/0.046
6(39)	691	18/0.075	12(51)	871	12/0.050

Footnote. Numeration of locus D1S80 alleles (tandem repeat pMCT118) is suggested by Budowle et al. [24], and APOB locus (in parentheses) by Boerwinkle et al. [13].

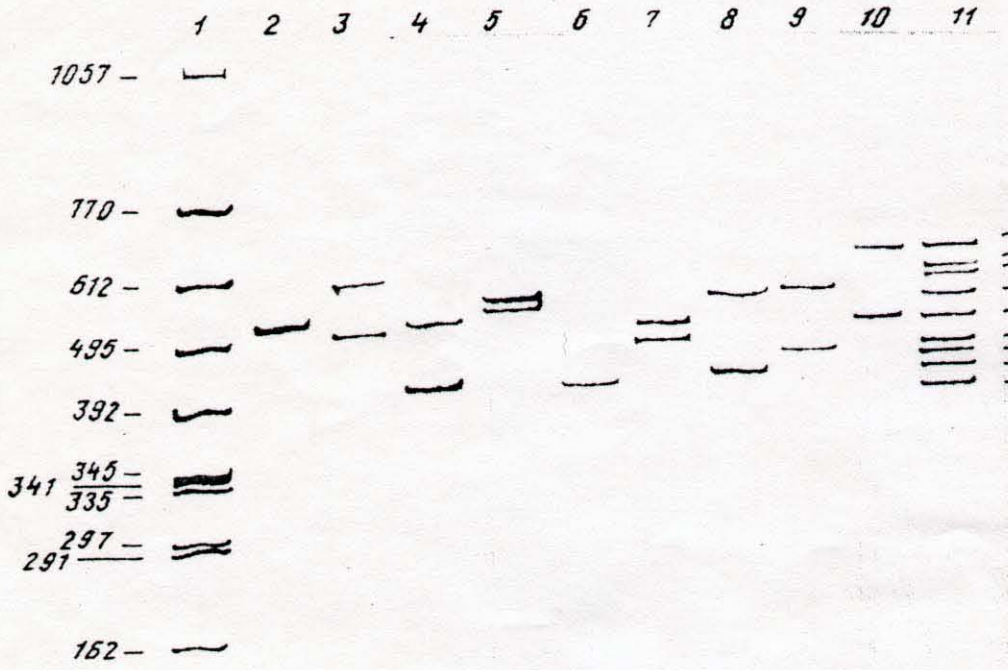

Fig. 2. Electrophoretic separation of tandem repeat pMCT118 amplification products in 6% polyacrylamide gel (stained with silver): *1*) Phage ϕX174 DNA fragments cleaved with *Hinc*II (numbers give fragment size in bp); *2–10*) amplification product of tandem repeat pMCT118 of 9 unrelated individual genomic DNA samples; *11*) markers containing a mixture of pMCT118 alleles (ordinal numbers of the alleles are shown on the right).

We used a set of pMCT118 alleles containing alleles 1, 3, 4, 5, 7, 9, 11, 12, 14 as markers. The amplification mixtures containing these alleles were combined, the solution obtained was diluted 1,000,000 times in bidistilled water, and PCR was performed in conditions for pMCT118 allele amplification.

According to the old nomenclature, the smallest allele of 430 bp had a number of 18, because it contained 18 tandem repeats of 16 bp. The next allele had 19 repeats and had a number of 19 and so on. Budowle et al. suggested a novel nomenclature, assigning No. 1 to allele No. 18, No. 2 to allele No. 19, and so on [24]. We decided to use both ways of numeration; the numbers of pMCT118 alleles according to the old nomenclature are given in parentheses (Tables 3 and 6).

We observed 14 pMCT118 alleles of 430–648 bp (Fig. 2, Table 3). Budowle et al. discovered that Finns had, besides these 14 alleles, an additional allele No. 20 (37); US Caucasians had 13 alleles, but alleles No. 10 (27) and No. 20 (37) were absent [20]. The heterozygosity degree of locus D1S80 is 72%. which is close to the value for Finns (77%) [20].

Like Finns and North-American Caucasians, Russians have alleles No. 1 (18) and No. 7 (24) more frequently. The values of FO for this alleles were close: 0.300 and 0.300 (Russians) (Table 3); 0.307 and 0.311 (Finns) [20]; 0.293 and 0.335 (US Caucasians) [20], respectively. The combinations of alleles 1–1 (FO is 0.092), 1–7 (0.208), and 7–7 (0.117) had the most wide distribution among the studied genotypes of Russians from Moscow (Table 6). Similar results were obtained when the distribution of alleles among the Finnish population was analyzed, the values of FO in more most common variants of genotypes were close to our data: 0.086, 0.207, and 0.107, respectively [20].

McClure and Scharf [25], analyzing the distribution of pMCT118 alleles in US Caucasians and in Spaniards by the PCR technique, observed in some probes DNA fragments with sizes considerably exceeding the maximal length characteristic of pMCT118 alleles. The investigators designated these fragments as No. 51 and No. 58 according to the old nomenclature, considering that these fragments are novel high-mol.-wt. pMCT118 alleles not observed earlier. Other investigators analyzing amplified DNA fragments also observed these fragments in some samples; however, they did not classify them with pMCT118 alleles [24, 26]. We also sometimes obtained similar fragments in amplification, but they did not hybridize with ^{32}P-labeled probes of alleles No. 1 (18) and No. 7 (24) of pMCT118 (results not shown), and cannot be considered as alleles of this locus.

We revealed 12 alleles of locus APOB with lengths of 541–871 bp (Table 3). Boervinkle et al. [13] observed the same number of alleles in Caucasians of France [13]; only 11 alleles were discovered in Finns: allele No. 43 was absent [20].

The hypervariable region of the *apo*B gene consists of repeats of 15 bp, however the allele lengths of this locus differ each from other by 30, 60, 90 bp, etc. i.e., in 2, 4, 6 repeated units and so on [13]. On this basis, Boervinkle et al. designated the smallest amplified *apo*B allele that contained 29 repeated units as No. 29; the next allele that contained two additional repeats was designated as No. 31, etc. [13]. We suggest a new classification of alleles *apo*B analogous to that introduced by Budowle et al. for pMCT118 [24]. According

to our nomenclature, the smallest amplified allele No. 29 (in the Boervinkle et al. nomenclature [13]) is No. 1, allele No. 31 is No.2 and so on. This nomenclature is more convenient than the old one, primarily for ordering the numeration for those *apo*B alleles that can be amplified by the PCR technique.

The value of heterozygosity index for APOB locus is 77% in Russians, it slightly differs from that in Finns (79%) [20].

Alleles No. 4 (35) and No. 5 (37) are the most widely represented in locus APOB (FO are 0.208 and 0.375, respectively, Table 3). A similar picture was observed in Finns (FO 0.220 and 0.368) [20] and in Frenchmen (0.210 and 0.430) [13]. Among the genotype variants, the most frequent were the following allele combinations in Russians: 4–4 and 5–5 (FO are 0.166 and 0.125, Table 7).

In general, despite some distinctions, the alleles of each hypervariable region studied in this work have very close distribution patterns among different Caucasian groups. The allele distribution probably differs among representatives of different races: for example, pMCT118 alleles were shown to be distributed among US Caucasians otherwise than among Negroids living in the same country [25]. Within the same race the genetic distinctions are expressed weakly; this is confirmed, in particular, by the uniformity of different VNTR allele distribution among Caucasians.

The data on allele distribution of four hypervariable regions obtained by us are important because they may serve as one of the criteria for selecting a certain VNTR for use in genome dactyloscopy based on the PCR technique. Other criteria for this selection are the following: high polymorphism level, large value of heterozygosity index; specificity of PCR conditions allowing identification of the complete set of alleles for a given population, and inheritance independent of other hypervariable regions [27].

The VNTRs in Table 1 suit the genetic individual identification procedure to different extents. For example, the hypervariable region of interleukin-6 gene is poorly appropriate because of a low polymorphism level (only four alleles of this VNTR are known [17]). Region HVR of Ha-*ras* protooncogene is poorly appropriate

TABLE 4. Distribution of Genotypes in Locus D117S30 (tandem repeat pYNZ22) Among 120 Unrelated Representatives of Russian Population

No	Genotype (in allele number)	Frequency of occurrence	No	Genotype (in allele number)	Frequency of occurrence
1	1 – 1	1/0.008	19	4 – 7	2/0.016
2	1 – 4	4/0.033	20	4 – 8	1/0.008
3	1 – 6	1/0.008	21	4 – 9	3/0.025
4	2 – 2	3/0.025	22	4 –10	2/0.016
5	2 – 3	2/0.016	23	5 – 5	3/0.025
6	2 – 4	9/0.075	24	5 – 6	1/0.008
7	2 – 6	1/0.008	25	5 – 8	1/0.008
8	2 – 9	3/0.025	26	5 – 9	2/0.016
9	2 –10	1/0.008	27	6 – 6	2/0.016
10	3 – 3	6/0.050	28	6 – 8	2/0.016
11	3 – 4	10/0.083	29	6 – 9	1/0.008
12	3 – 5	4/0.033	30	7 – 8	1/0.008
13	3 – 6	1/0.016	31	8 – 9	1/0.008
14	3 – 9	3/0.025	32	8 –10	1/0.008
15	3 –10	3/0.025	33	9 – 9	2/0.008
16	4 – 4	28/0.233	34	9 –10	1/0.008
17	4 – 5	2/0.016	35	9 –12	1/0.008
18	4 – 6	5/0.042	36	10–10	5/0.042

TABLE 5. Distribution of Genotypes in Locus IGHJ Among 120 Unrelated Representatives of Russian Population

No	Genotype (in allele number)	Frequency of occurrence	No	Genotype (in allele number)	Frequency of occurrence
1	1 – 1	2/0.016	11	3 – 5	26/0.217
2	1 – 2	5/0.042	12	3 – 6	5/0.042
3	1 – 3	13/0.108	13	3 – 9	9/0.075
4	1 – 5	6/0.050	14	3 –10	1/0.008
5	1 – 6	1/0.008	15	5 – 5	15/0.125
6	2 – 2	2/0.016	16	5 – 6	1/0.008
7	2 – 3	1/0.008	17	5 – 9	5/0.042
8	2 – 5	5/0.042	18	6 – 6	1/0.008
9	2 – 6	2/0.016	19	9 –10	1/0.008
10	3 – 3	19/0.158			

TABLE 6. Distribution of Genotypes in Locus D1S80 (tandem repeat pMCT118) Among 120 Unrelated Representatives of Russian Population

No	Genotype (in allele number)	Frequency of occurrence	No	Genotype (in allele number)	Frequency of occurrence
1	1– 1 (18–18)	11/0.092	25	5–10(22–27)	1/0.008
2	1– 2 (18–19)	1/0.008	26	5–12(22–29)	1/0.008
3	1– 4 (18–20)	4/0.033	27	6–6 (23–23)	1/0.008
4	1– 5 (18–22)	2/0.016	28	6–7 (23–24)	2/0.016
5	1– 7 (18–24)	25/0.208	29	6–8 (23–25)	1/0.008
6	1– 8 (18–25)	3/0.025	30	6–11 (23–28)	1/0.008
7	1– 9 (18–26)	1/0.008	31	7–7 (24–24)	14/0.117
8	1– 1 (18–28)	5/0.042	32	7–8 (24–25)	1/0.008
9	1– 1 (18–29)	1/0.008	33	7–9 (24–26)	2/0.016
10	1–14(18–31)	8/0.067	34	7–10(24–27)	2/0.016
11	2– 3 (19–20)	1/0.008	35	7–11(24–28)	2/0.016
12	2– 4 (19–21)	1/0.008	36	7–12(24–29)	1/0.008
13	2– 7 (19–24)	1/0.008	37	7–13(24–30)	1/0.008
14	2– 1 (19–29)	1/0.008	38	7–14(24–31)	4/0.033
15	3– 4 (20–21)	1/0.008	39	8–8 (25–25)	2/0.016
16	3– 5 (20–22)	1/0.008	40	8–9 (25–26)	1/0.008
17	3– 6 (20–23)	1/0.008	41	8–14(25–31)	1/0.008
18	3– 7 (20–24)	1/0.008	42	9–9 (26–26)	1/0.008
19	3–12(20–29)	1/0.008	43	9–12(26–29)	1/0.008
20	4– 6 (21–23)	1/0.008	44	10–10(27–27)	1/0.008
21	4– 7 (21–24)	2/0.016	45	11–11(28–28)	1/0.008
22	4– 8 (21–25)	1/0.008	46	12–12(29–29)	1/0.008
23	5– 5 (22–22)	1/0.008	47	14–14(31–31)	1/0.008
24	5– 7 (22–24)	1/0.008			

Footnote. Allele nomenclature owing to Budowle et al. [24].

TABLE 7. Distribution of Genotypes in Locus APOB Among 120 Unrelated Representatives of Russian Population

No	Genotype (in allele number)	Frequency of occurrence	No	Genotype (in allele number)	Frequency of occurrence
1	1 – 4 (29–35)	2/0.016	19	5 – 5 (37–37)	21/0.175
2	1 – 5 (29–37)	3/0.025	20	5 – 6 (37–39)	1/0.008
3	1 – 7 (29–41)	1/0.008	21	5 – 7 (37–41)	2/0.016
4	1 – 9 (29–45)	1/0.008	22	5 – 9 (37–45)	5/0.042
5	2 – 2 (31–31)	1/0.008	23	5 –10 (37–47)	5/0.042
6	2 – 4 (31–35)	1/0.008	24	5 –11 (37–49)	5/0.042
7	2 – 5 (31–37)	2/0.016	25	5 –12 (37–51)	2/0.016
8	2 – 7 (31–41)	1/0.008	26	6 – 6 (39–39)	6/0.050
9	2 –10 (31–47)	2/0.016	27	6 – 8 (39–43)	1/0.008
10	3 – 3 (33–33)	1/0.008	28	6 –11 (39–49)	2/0.016
11	3 – 5 (33–37)	4/0.033	29	6 –12 (39–51)	1/0.008
12	3 –11 (33–49)	1/0.008	30	7 – 7 (41–41)	5/0.042
13	4 – 4 (35–35)	12/0.100	31	7 – 9 (41–45)	1/0.008
14	4 – 5 (35–37)	15/0.125	32	7 –10 (41–47)	2/0.016
15	4 – 6 (35–39)	1/0.008	33	7 –11 (41–49)	1/0.008
16	4 – 7 (35–41)	3/0.025	34	10 –12 (47–51)	1/0.008
17	4 – 9 (35–45)	1/0.008	35	11 –11 (49–49)	1/0.008
18	4 –10 (35–47)	2/0.016	36	12 –12 (51–51)	4/0.033

Footnote. Allele numbers in parentheses according to Boervinkle et al. [13].

because of a low heterozygosity index [14]. Locus COL2A1 is of little convenience because its repeated units sometimes differ in length only by 2–3 bp, which significantly complicates the typing in this locus [17].

To obtain reliable results, it is necessary to use a complex approach consisting of simultaneous usage of a number of VNTR for genetic typing of an individual. Preference should be given to highly polymorphic minisatellite sequences with a high level of heterozygosity and more uniform allele distribution, for example pMCT118, pYNZ22, and p602 at the first stage of the typing procedure. It is expedient to use a second additional stage of investigation with VNTRs of less informative allele distribution, for example HVR-Ig_H, APOB, p33.6.

The typing process can be accelerated by coamplification of a number of VNTRs in a single sample [19]. To perform these experiments, it is necessary to use hypervariable regions capable to be amplified in the same reaction conditions and not overlapping in allele length. In the cases of overlapping, subsequent hybridization experiments with probes specific to definite minisatellite repeats will allow differentiation between different alleles.

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