Russian Journal of Genetics, Vol. 33, No. 2, 1997, pp. 203–208. Translated from Genetika, Vol. 33, No. 2, 1997, pp. 262–268.


HUMAN GENETICS

# Distribution of Alleles of Microsatellite Loci *HUMCYAR04* and *D19S253* in Population Samples of Two Russian Cities

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Received May 23, 1996

**Abstract**—In population samples of Moscow and Tomsk, the allelic polymorphism of microsatellite loci *HUMCYAR04* and *D19S253* was studied by polymerase chain reaction. Seven *HUMCYAR04* alleles (181–205 bp) and nine alleles (208–240 bp) of the *D19S253* locus were identified. In both population samples, the absence of statistically significant differences in the distribution of allele frequencies for these loci was demonstrated. The distribution of the observed genotype frequencies was shown to correspond to the Hardy–Weinberg equilibrium in both populations. Mendelian inheritance of these tandem repeats was demonstrated by an analysis of two large families. The parameters of polymorphism information content for the loci studied were determined; comparative analysis of allele frequencies with corresponding data for a number of populations was performed. These short tandem repeats were proposed for use in personal identification and paternity tests.

## INTRODUCTION

Short tandem repeats (STRs), or microsatellites, are currently used widely in various fields of genetics and medicine. STRs are used as genetic markers for genome mapping and for studying numerous inherited diseases [1, 2]. Microsatellites with high levels of allelic polymorphism and information content are used in forensic medicine and identification applications. STRs have smaller sizes (on average 100–350 bp) than microsatellite repeats and, therefore, have a substantial advantage for analysis of degraded DNA and microamounts of human DNA [3].

In the United States and Great Britain, panels including, respectively, 13 and 6 highly polymorphic and independent microsatellites have been developed and recommended for use in forensic medicine [4–6]. These panels are shown to be of high resolution and their usage allows the lowest values of probability for random coincidence of individual parameters to be attained (of order $10^{-8}$–$10^{-10}$) in personal identification.

Earlier, we developed a panel from six polymorphic microsatellite loci for use in genome fingerprinting [7, 8]. At present, in addition to the already existing panel, development of an analogous panel from six to seven microsatellite loci is in progress [9]. Simultaneous use of two panels would allow the resolution ability of identification studies to sharply increase and would broaden the possibilities of analysis of degraded specimens and microamounts of human DNA.

Loci *HUMCYAR04* and *D19S253* are included in the above-mentioned foreign microsatellite panels [4, 5]. Microsatellite *CYP19* of locus *HUMCYAR04* is localized in the aromatase cytochrome P450 gene in chromosome region 15q21.1 [6]. Eight alleles 181–209 bp in size, including five to twelve AAAT repeats, were detected [4]. In Americans, Negroids, and Mexicans, a rare allele of intermediate size, compared to alleles with six and seven repeats, was revealed; this allele was absent in Caucasoids and Mongoloids [4].

The polymorphic locus *D19S253* was shown to have $(CA)_n$ structure and up to ten alleles 204–240 bp in size including 3–21 repeats [5]. Note that the repeated monomer of these alleles is 4 bp in size and consists of two dinucleotide motives. Eight alleles of the locus 212–240 bp were revealed in Caucasoids [10].

In the present work, we perform a study of population samples from two Russian cities to determine allele frequencies of the loci *HUMCYAR04* and *D19S253*, to compare obtained frequencies with analogous data on other populations, and to estimate the suitability of these loci for identification studies.

## MATERIALS AND METHODS

DNA polymerase $Taq^R$ was received from NPK Biotekh, Moscow. Oligonucleotide primers were syn-

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thesized by V.P. Veiko, State Research Institute of Genetics and Selection of Industrial Microorganisms, Moscow.

Genome DNA isolation from human venous blood was performed according to the standard technique [11]. DNA from saliva and blood spots was extracted by chelate polymer Chelex$^R$-100 (Bio-Rad, USA) [12].

Unrelated Moscow residents were represented by specimens obtained in traumatological stations, the Rheumatological Institute, the Bureau of Forensic Medical Expertize, and from the staff of the State Research Institute of Genetics and Selection of Industrial Microorganisms. A sample from unrelated representatives of the Tomsk population was made up of specimens received from healthy donors.

Polymerase chain reaction (PCR) was performed on a PHC-2 thermal cycler (Techne, Great Britain) or the PolyChainII (Polygen, Germany) in a 50-μl reaction mixture of the following composition: 67 mM Tris–HCl, pH 8.8; 16.6 mM ammonium sulfate; 0.01% Tween 20; 1.0 and 2.0 mM magnesium chloride for *HUMCYAR04* and *D19S253*, respectively; 0.2 mM of each dNTP; 2.5 units *Taq*$^R$ polymerase; 50–100 ng genome DNA or 20 μl DNA extracted by means of the Chelex$^R$-100. For amplification of *HUMCYAR04* and *D19S253* alleles, we used 66 ng of each oligonucleotide primer, the sequence of which is shown in [4] and [5], respectively. We performed 30–35 PCR cycles as follows: 94°C for 1 min, 55°C (*D19S253*) or 65°C (*HUMCYAR04*) for 1 min, 72°C for 1 min; initial denaturation for 4 min and final extension 10 min.

For correct identification of alleles in DNA specimens, allelic "stairs" were synthesized for both loci: to the 100-μl reaction mixture, including 10 pmol of each primer, 1 μl of equimolar mixture of the total spectrum of alleles was added after it was diluted $10^4$ times.

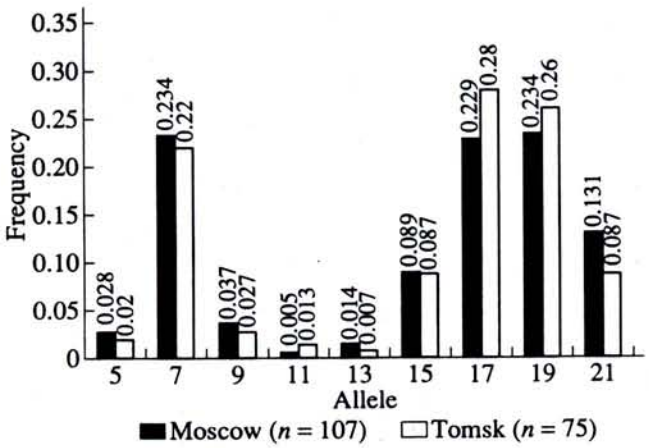


**Fig. 1.** Allele frequencies of locus *D19S253* in the populations of Moscow and Tomsk. Allelic numbers correspond to the number of tandem repeats included in the alleles; (*n*) number of individuals in the sample studied (for Fig. 1 and 3).

The amplification products were analyzed by electrophoresis in 12% polyacrylamide gel (length of gel, 16 cm; thickness, 0.7 mm) with an additional 7% glycerol; 10 μl of reaction mixture were applied to gel slots. After electrophoresis, the gel was stained with silver [13].

The observed genotype frequencies of the loci studied were tested for deviation from Hardy–Weinberg equilibrium by $\chi^2$ and G-statistics by means of the R × C (Rows × Columns) computer program based on the algorithm described earlier [14]. The algorithm allows estimation of the statistical significance of deviations from the expected frequency distribution when the number of observations in many of the classes is less than five and the standard $\chi^2$ test cannot be used. The R × C program was also used to compare allelic frequencies of the studied loci in samples from different populations.

Expected heterozygosity ($H_{exp}$) and parameters of polymorphism informativeness of the polymorphic microsatellites studied were calculated with a computer program based on the algorithms presented earlier [8]. We estimated the following parameters of polymorphism informativeness: probability of random coincidence of genotypes of two unrelated individuals (probability of match, *pM*), mean probability of exclusion of the specimen studied by target genotype (mean exclusion chance, *W*), and polymorphism information content (*PIC*).

## RESULTS AND DISCUSSION

We typed 107 and 75 unrelated representatives of the populations of Moscow and Tomsk, respectively, by the *D19S253* locus and revealed nine alleles ranging in size from 208 to 240 bp (Figs. 1 and 2). Alleles of this locus were classified according to the number of dinucleotide repeats included in them. The allele of the lowest molecular weight (204 bp) had three repeats and was, therefore, designated with the number 3. The next allele (208 bp) had five repeats and received the number 5, and so on. In both Russian populations studied, allele 3 was not found, but all the remaining alleles were detected. The most frequent were alleles 7, 17, and 19 (Fig. 1). From 45 possible genotypes, 25 were revealed in Moscow and 20 in Tomsk. In the Moscow population, the most frequent genotype was 7-19 (14%); in the Tomsk population, the most frequent genotype was 7-17 (20%) (Table 1).

DNA of 101 representatives of the Moscow population and 75 representatives of the Tomsk population was studied for the *HUMCYAR04* locus. Seven alleles 181–205 bp were revealed (Figs. 3 and 4). For this microsatellite, alleles were numbered according to the number of repeated monomers. The 181-bp allele had five tetranucleotide repeats and was given the number 5, and so on. Alleles 5 and 10 were the most frequent (Fig. 3). Allele 12, revealed in a number of foreign populations, was not found in the Russian samples [4, 6].

Twenty one from 28 possible genotypes of microsatellite *CYP19* were present in the Moscow population. In the Tomsk population, 22 genotypes were revealed. In both populations studied, heterozygotes 5–10 were the most frequent (21% in Moscow and 20% in Tomsk) (Table 2).

In both populations, the observed distributions of genotype frequencies of the loci studied conformed to Hardy–Weinberg expectations (Table 3). This demonstrates the unbiased character of allele distribution in the Tomsk sample, which was not random. Correspondence of the observed distribution of genotype frequencies for both loci to Hardy–Weinberg equilibrium (significance 0.934–1.000) indicates the absence of inner heterogeneity in the populations studied.

In the Moscow and Tomsk populations, the observed distribution of allele and genotype frequencies of the *D19S253* locus had a similar pattern (Table 4). Comparison of the distribution of microsatellite allele frequencies with existing data on Caucasoids from the United States was also performed. In 100 Americans of European origin, as in Russians, the prevailing alleles were 7, 17, and 19 (with frequencies of 0.29, 0.26, and 0.24, respectively); however, allele 5 was absent. A similar picture of allelic distribution of *D19S253* was observed in Indians and inhabitants of the Caribbean basin—migrants from Africa [5]. In Negroids from the Caribbean, rare alleles 3 and 5 were revealed [5].

According to the data in Table 4, differences in the distribution of *D19S253* allele frequencies between samples of Moscow and samples of Tomsk and North American (Caucasoids) populations are statistically nonsignificant. At the same time, significance levels for $\chi^2$ and G-statistics indicate more marked differences in the distribution of allele frequencies between Russians and Americans than between the Russian populations.

We compared distributions of allele frequencies of microsatellite *CYP19* in the Russian samples with analogous data for different races (Fig. 5). In earlier population studies, Polymeropoulos *et al.* [6] examined a small sample (23 individuals) of Caucasoids from the United States using different primers than the ones used in our work and demonstrated the presence of five alleles of the *HUMCYAR04* locus. In this sample, the prevalent alleles had five, six, and ten repeats (frequencies of 0.28, 0.24, and 0.37, respectively) [6]. Later, in a larger sample (190 individuals), Hammond *et al.* succeeded in revealing three more alleles in North American Caucasoids [4]. In Americans of European origin, as in the Russian populations, a noticeable predominance of alleles 5 and 10 was demonstrated (frequencies 0.342 and 0.353, respectively), but the most prominent allele was allele 10. In addition, an allele with 12 repeats, which was not found in Russians, was detected. In Mongoloids, the same alleles prevailed, as in Europeans; however, allele 10 was more prominent (0.403) and allele 8 was absent. American Negroids and

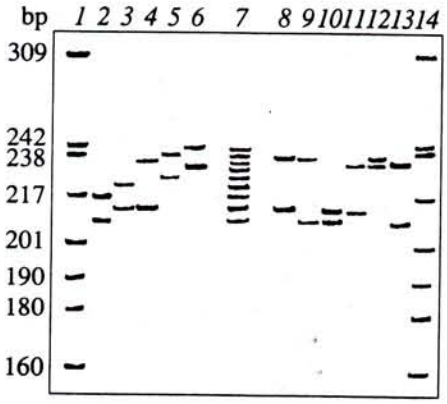


**Fig. 2.** Separation of the amplified alleles of locus *D19S253*; (*1*) and (*14*) fragments of plasmid DNA pBR322 digested by restriction endonuclease *Msp*I; (*2*)–(*6*) genotyping of five unrelated individuals: (*2*) 5-9, (*3*) 7-11, (*4*) 7-17, (*5*) 13-19, (*6*) 15-21; (*7*) allelic "stairs"; (*8*)–(*13*) family analysis revealing the following genotypes: (*8*) 7-19 (father), (*9*) 5-19 (child 1), (*10*) 5-7 (child 2), (*11*) 7-17 (child 3), (*12*) 17-19 (child 4), (*13*) 5-17 (mother).

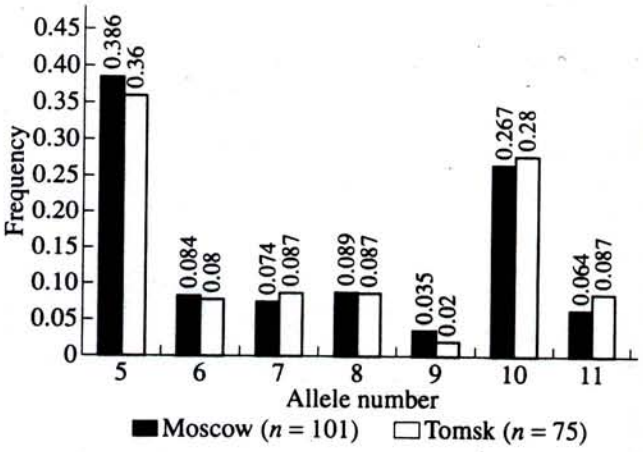


**Fig. 3.** Allele frequencies of locus *HUMCYAR04* in the populations of Moscow and Tomsk.

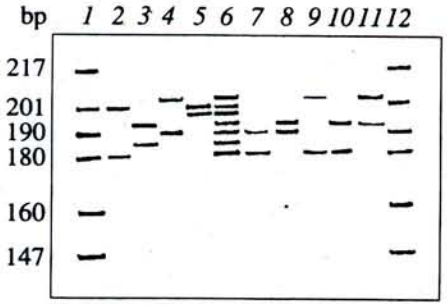


**Fig. 4.** Separation of the amplified alleles of locus *HUMCYAR04*; (*1*) and (*12*) fragments of plasmid DNA pBR322 digested by restriction endonuclease *Msp*I; (*2*)–(*5*) genotyping of four unrelated individuals: (*2*) 5-10, (*3*) 6-8, (*4*) 7-11, (*5*) 9-10; (*6*) allelic "stairs"; (*7*)–(*11*) family analysis revealing the following genotypes: (*7*) 5-7 (father), (*8*) 7-8 (child 1), (*9*) 5-11 (child 2), (*10*) 5-8 (child 3), (*11*) 8-11 (mother).

**Table 1.** Genotype frequencies of locus *D19S253* in the populations of Moscow and Tomsk

| Genotype | Frequency | | | |
|---|---|---|---|---|
| | observed | expected | observed | expected |
| | Moscow | | Tomsk | |
| 5-5 | 0.009 | 0.001 | 0 | 0.000 |
| 5-7 | 0.009 | 0.013 | 0 | 0.009 |
| 5-9 | 0.009 | 0.002 | 0 | 0.001 |
| 5-11 | 0 | 0.000 | 0.013 | 0.001 |
| 5-13 | 0 | 0.001 | 0 | 0.000 |
| 5-15 | 0 | 0.005 | 0.027 | 0.003 |
| 5-17 | 0.019 | 0.013 | 0 | 0.011 |
| 5-19 | 0 | 0.013 | 0 | 0.010 |
| 5-21 | 0 | 0.007 | 0 | 0.003 |
| 7-7 | 0.056 | 0.055 | 0.013 | 0.048 |
| 7-9 | 0 | 0.017 | 0 | 0.012 |
| 7-11 | 0.009 | 0.002 | 0 | 0.006 |
| 7-13 | 0 | 0.007 | 0 | 0.003 |
| 7-15 | 0.037 | 0.041 | 0.027 | 0.038 |
| 7-17 | 0.121 | 0.107 | 0.200 | 0.123 |
| 7-19 | 0.140 | 0.109 | 0.147 | 0.114 |
| 7-21 | 0.037 | 0.061 | 0.040 | 0.038 |
| 9-9 | 0.009 | 0.001 | 0 | 0.001 |
| 9-11 | 0 | 0.000 | 0 | 0.001 |
| 9-13 | 0 | 0.001 | 0 | 0.000 |
| 9-15 | 0 | 0.007 | 0 | 0.005 |
| 9-17 | 0.009 | 0.017 | 0.013 | 0.015 |
| 9-19 | 0.009 | 0.017 | 0.013 | 0.014 |
| 9-21 | 0.028 | 0.010 | 0.027 | 0.005 |
| 11-11 | 0 | 0.000 | 0 | 0.000 |
| 11-13 | 0 | 0.000 | 0 | 0.000 |
| 11-15 | 0 | 0.001 | 0 | 0.002 |
| 11-17 | 0 | 0.002 | 0.013 | 0.007 |
| 11-19 | 0 | 0.002 | 0 | 0.007 |
| 11-21 | 0 | 0.001 | 0 | 0.002 |
| 13-13 | 0 | 0.000 | 0 | 0.000 |
| 13-15 | 0 | 0.002 | 0 | 0.001 |
| 13-17 | 0 | 0.006 | 0 | 0.004 |
| 13-19 | 0.009 | 0.007 | 0 | 0.003 |
| 13-21 | 0.019 | 0.004 | 0.013 | 0.001 |
| 15-15 | 0.019 | 0.008 | 0.013 | 0.008 |
| 15-17 | 0.019 | 0.041 | 0.067 | 0.049 |
| 15-19 | 0.056 | 0.041 | 0.027 | 0.045 |
| 15-21 | 0.028 | 0.023 | 0 | 0.015 |
| 17-17 | 0.056 | 0.052 | 0.040 | 0.078 |
| 17-19 | 0.084 | 0.107 | 0.147 | 0.146 |
| 17-21 | 0.093 | 0.060 | 0.040 | 0.049 |
| 19-19 | 0.056 | 0.055 | 0.067 | 0.068 |
| 19-21 | 0.056 | 0.061 | 0.053 | 0.045 |
| 21-21 | 0 | 0.017 | 0 | 0.008 |

Mexicans were characterized both by the presence of an intermediate-sized allele between alleles 6 and 7, which was absent in other races, and by the most frequent alleles 5, 6, and 10. Allele 12 was also absent in these populations. Alleles 6 and 5 prevailed in American Negroids and Mexicans, respectively [4].

The data in Fig. 5 demonstrate the possibility of statistically significant differences in allelic distribution of the *HUMCYAR04* locus between the Russian populations and other populations. Differences in this loci, which are quantitatively comparable with interracial differences, were shown for Caucasoid populations isolated from each other; for example, the values of $R \times C$ parameters are rather close in the following pairs: Russians–Caucasoids of the United States and Russians–Mongoloids of the United States (Fig. 5).

However, in the samples from two distant Russian city populations, a virtually undistinguishable distribution of allele frequencies was demonstrated. Note that this similarity was also revealed for two earlier studied microsatellite repeats (*D6S366* and *HUMvWFII*) [9]. Therefore, the data obtained for distinct population samples on allele frequencies might be extrapolated to the entire Russian population. At the same time, allele frequencies typical for one representative sample could be reliably extrapolated to the total Russian population for probability calculations in experiments on personal identification.

We showed the independent character of inheritance of alleles for both microsatellites in the analysis of two big families with three and four children (Figs. 2 and 4). Mutant alleles were not detected.

Comparison of information parameters for loci *HUMCYAR04* and *D19S253* showed that the latter locus was substantially more informative (Table 3). This locus was shown to have a higher level of polymorphism (nine alleles, compared to seven alleles in the *CYP19* microsatellite) and heterozygosity (about 80% for the Russian population). Estimates of the information parameters for these loci in the Moscow population are comparable with analogous data on six earlier studied minisatellites [7, 8]. This demonstrates that the microsatellites are undoubtedly useful and informative for identification analysis.

In the Moscow population, the combined *pM* value for the four microsatellites that we studied (*D6S366, D19S253, HUMvWFII,* and *HUMCYAR04*) was $4.96 \times 10^{-5}$. This is within one order higher than the *pM* value ($5.55 \times 10^{-6}$) reported by Urquhart *et al.* [5] for a panel from four STRs. The higher resolution ability of the foreign panel is explained by the higher level of polymorphism of the included microsatellites, which contain from nine to eleven alleles [5]. To reach *pM* values of the order $10^{-8}$–$10^{-9}$, it is necessary to add three more highly polymorphic microsatellite loci to our STR panel.

Foreign test systems for personal identification frequently have dinucleotide microsatellites with over ten

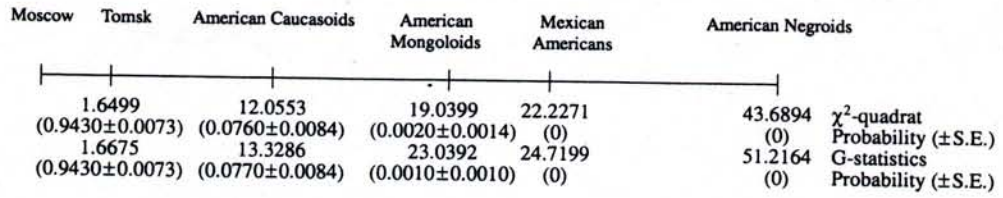


**Fig. 5.** Comparative analysis of allelic distribution of locus *HUMCYAR04* in the Moscow population and other population groups.

alleles [5, 15]. High informativeness and heterozygosity are obvious advantages of such an STR, but for correct detection of these STR alleles, it is necessary to have expensive equipment with high resolution (automatic sequencer and apparatus for capillary electrophoresis). To develop the microsatellite panel, we prefer tetranucleotide nucleotides, because their alleles can be successfully separated and identified by means of the simple and cheap technique of electrophoresis in polyacrylamide gel.

**Table 2.** Genotype frequencies of locus *HUMCYAR04* in the populations of Moscow and Tomsk

| Genotype | Frequency | | | |
|---|---|---|---|---|
| | observed | expected | observed | expected |
| | Moscow | | Tomsk | |
| 5-5 | 0.119 | 0.149 | 0 | 0.000 |
| 5-6 | 0.030 | 0.065 | 0 | 0.009 |
| 5-7 | 0.059 | 0.057 | 0 | 0.001 |
| 5-8 | 0.149 | 0.069 | 0.013 | 0.001 |
| 5-9 | 0.040 | 0.027 | 0 | 0.000 |
| 5-10 | 0.208 | 0.206 | 0.027 | 0.003 |
| 5-11 | 0.050 | 0.050 | 0 | 0.011 |
| 6-6 | 0.020 | 0.007 | 0 | 0.010 |
| 6-7 | 0.010 | 0.012 | 0 | 0.003 |
| 6-8 | 0.010 | 0.015 | 0.013 | 0.048 |
| 6-9 | 0.010 | 0.006 | 0 | 0.012 |
| 6-10 | 0.050 | 0.045 | 0 | 0.006 |
| 6-11 | 0.020 | 0.011 | 0 | 0.003 |
| 7-7 | 0.010 | 0.006 | 0.027 | 0.038 |
| 7-8 | 0 | 0.013 | 0.200 | 0.123 |
| 7-9 | 0 | 0.005 | 0.147 | 0.114 |
| 7-10 | 0.059 | 0.040 | 0.040 | 0.038 |
| 7-11 | 0 | 0.010 | 0 | 0.001 |
| 8-8 | 0 | 0.008 | 0 | 0.001 |
| 8-9 | 0 | 0.006 | 0 | 0.000 |
| 8-10 | 0.010 | 0.048 | 0 | 0.005 |
| 8-11 | 0.010 | 0.011 | 0.013 | 0.015 |
| 9-9 | 0 | 0.001 | 0.013 | 0.014 |
| 9-10 | 0.020 | 0.019 | 0.027 | 0.005 |
| 9-11 | 0 | 0.004 | 0 | 0.000 |
| 10-10 | 0.089 | 0.071 | 0 | 0.000 |
| 10-11 | 0.020 | 0.034 | 0 | 0.002 |
| 11-11 | 0.010 | 0.004 | 0.013 | 0.007 |

**Table 3.** Estimates of polymorphism parameters and $\chi^2$ tests and G-statistics at Hardy–Weinberg equilibrium for loci *HUMCYAR04* and *D19S253* in the populations of Moscow and Tomsk

| Parameter | *HUMCYAR04* | | *D19S253* | |
|---|---|---|---|---|
| | Moscow | Tomsk | Moscow | Tomsk |
| $H_{obs}$ | 0.743 | 0.733 | 0.794 | 0.867 |
| $H_{exp}$ | 0.754 | 0.763 | 0.811 | 0.789 |
| *pM* | 0.094 | 0.090 | 0.062 | 0.076 |
| *W* | 0.724 | 0.738 | 0.842 | 0.787 |
| *PIC* | 0.565 | 0.578 | 0.681 | 0.645 |
| $\chi^2$ | 17.2840 | 4.3586 | 22.1728 | 18.2968 |
| Probability | 0.9340 | 1.0000 | 0.9710 | 0.9690 |
| (±S. E.)* | (0.0079) | (0) | (0.0053) | (0.0055) |
| G-statistics | 20.4146 | 5.1654 | 28.4687 | 23.4858 |
| Probability | 0.9500 | 1.0000 | 0.9650 | 0.9630 |
| (±S. E.)* | (0.0069) | (0) | (0.0058) | (0.0060) |

* Standard error

**Table 4.** Comparison of distributions of allele frequencies for locus *D19S253* in the Moscow population with other Caucasoid populations

| Parameter | Russians (Tomsk) | Caucasoids, USA |
|---|---|---|
| Sample size | 75 | 100 |
| Array R × C | 9 × 2 | 9 × 2 |
| $\chi^2$ | 1.7613 | 9.8572 |
| Probability ±S.E.* | 0.9840 ± 0.0040 | 0.2830 ± 0.0142 |
| G-statistics | 1.7681 | 11.2893 |
| Probability ±S.E. | 0.840 ± 0.0040 | 0.2730 ± 0.0141 |

* Standard error.

## ACKNOWLEDGMENTS

The authors are grateful to J. Karmodi (Carleton University, Ottawa) for the R × C computer program and O.V. Vishnichenko (All-Russia Research Institute of Space Instruments, Moscow) for developing the other software.

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