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HUMAN GENETICS

Allelic Polymorphism of Short Tandem Repeats Located in *HUMF13A01* and *HUMCD4* Loci in the Russian Populations of Moscow and Tomsk

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Abstract—In samples from populations of the cities of Moscow and Tomsk, analysis of allelic polymorphism of microsatellite loci *HUMF13A01* and *HUMCD4* was performed by polymerase chain reaction (PCR). Eight *HUMCD4* alleles (115–165 bp) and nine alleles (180–230 bp) of locus *HUMF13A01* were identified. In both populations, the distributions of allelic frequencies for these loci did not differ significantly. The distribution of observed genotype frequencies fitted Hardy–Weinberg equilibrium in both populations. Mendelian inheritance of these tandem repeats was demonstrated by analysis of two large families. Parameters of polymorphism information content (PIC) for the loci studied were detected; comparative analysis of allelic frequencies with similar data on several populations was performed. These short tandem repeats (STR) were proposed for use in personal identification and paternity tests.

INTRODUCTION

Multiple hypervariable regions that consist of tandemly repeated motifs and are characterized by the presence of allelic polymorphism are present in human genome DNA. Short tandem repetitious DNAs (short tandem repeats; STRs), or microsatellites, are an example of these sequences. STRs consist of repeated 2- to 5-bp units and usually are 100 to 350 bp long [1]. Recently, microsatellites have been widely used in medical genetic investigations as polymorphic markers for studying many hereditary diseases and for genome mapping [2, 3]. Microsatellites with high levels of allelic polymorphism and information content are used in forensic medicine and identification testing.

In many countries (e.g., Germany, Great Britain, and the United States), panels comprising independent, highly polymorphic microsatellites have been developed and recommended for use in genomic fingerprinting. In Germany, the test system includes 14 STR; in the United States and Great Britain, panels of 13 and 6 microsatellites, respectively, have been developed [4, 5]. These panels have a high resolution, and their use allows attaining of the lowest values of probability for random coincidence of individual parameters (of the order of 10^{-8} to 10^{-10}) for personal identification. STRs have smaller sizes than minisatellite repeats (which are hundreds and even thousands of bp in length) and, therefore, have a substantial advantage for analysis of degraded DNA and microscopic samples of human DNA [5].

Earlier, we developed a test system including six polymorphic minisatellite loci for identification purposes [6, 7]. In addition to this test system, we developed a similar panel comprising eight microsatellite loci [8, 9]. Simultaneous use of two panels will allow us to considerably increase the resolution of the identification tests and extend the possibilities of analysis of degraded specimens and microscopic samples of human DNA.

Microsatellites located in *HUMF13A01* and *HUMCD4* loci are included in an identification panel developed in the United States [4].

The gene for subunit A of human coagulation factor XIII (*HUMF13A01* locus) is located in chromosome region 6p24-p25 [10]. Two A subunits united with two B subunits form protein factor XIII. This factor operates in the final stage of blood coagulation and acts as fibrinogenase-generating intermolecular bonds between amino-acid residues of lysine and glutamine acid in the neighboring fibrin molecules. The catalyst function itself is fulfilled by A subunit [11].

In intron A of the *HUMF13A01* gene, a polymorphic tandem repeat $(AAAG)_n$ is located, beginning from position 248 of the gene [12]. In a small sample of North American Caucasoids (24 individuals), Polymeropoulos *et al.* [13] revealed eight alleles of this microsatellite, 180–230 bp in size. Hammond *et al.* [4]

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studied allelic polymorphism of the *HUMF13A01* locus in representatives of different races and demonstrated the presence of a maximum of 13 alleles.

The *CD4* gene of the surface antigen is located in 12pter-p12 [14, 15]. The protein product of this gene is present on the cellular surface of T-helpers/inducers, while its mRNA is also found in B-cells, macrophages, and granulocytes [16]. This antigen serves as a T-cell receptor for molecular recognition at the surface of specific target cells and is also recognized by the immune deficiency virus [16].

In the gene of surface antigen *CD4*, a polymorphic microsatellite consisting of tandem repeats $(AAAAG)_n$ has been revealed [17]. Edwards *et al.* [18] were first to study the allelic polymorphism of this gene in two North American populations by means of polymerase chain reaction (PCR). Later, Hammond *et al.* [4] performed similar studies in different races and revealed as many as 11 alleles of locus *HUMCD4* ranging from 125 to 175 bp. Zamani *et al.* [19] detected allelic frequencies of this microsatellite in some western European populations with the use of different primers: they demonstrated a maximum of eight alleles 88–128 bp in length.

In this study, we analyzed population samples from two Russian cities, Moscow and Tomsk, to determine allelic frequencies for loci *HUMCD4* and *HUMF13A01* and compare them with similar data for other populations, as well as to estimate the suitability of these loci for identification tests.

MATERIALS AND METHODS

DNA polymerase Taq^R was obtained from Biotekh (Moscow). Oligonucleotide primers were synthesized by Evios-Ros (Institute of Agricultural Biotechnology, Russian Academy of Agricultural Sciences, Moscow).

Isolation of genomic DNA from human venous blood was performed by the standard method [20]. DNA from saliva and blood spots was extracted with the use of chelate polymer Chelex^R-100 (Bio-Rad, USA) [21].

Blood samples from unrelated representatives of the Moscow population were collected at traumatology stations; the Institute of Rheumatology, Russian Academy of Medical Sciences; and the Forensic Medicine Investigation Bureau. Blood samples from unrelated representatives of the Tomsk population were collected at maternity hospitals and obtained from healthy individuals.

PCR was performed in PHC-2 (Techne, Great Britain) or PolyChainII (Polygen, Germany) thermal cyclers in a 50-μl reaction mixture of the following composition: 67 mM of Tris–HCl, pH 8.8; 16.6 mM of ammonium sulfate; 0.01% Tween 20; 1.0 and 2.0 mM of magnesium chloride for *HUMF13A01* and *HUMCD4*, respectively; 0.2 mM of each dNTP; 2.5 units of Taq^R polymerase; 50–100 ng of genomic DNA or 20 μl of DNA extracted using Chelex^R-100. For amplification of *HUMF13A01* and *HUMCD4* alleles, we used 66 ng of each oligonucleotide primer, the sequences of which are shown in [4] and [13], respectively. We performed 30–35 cycles of PCR according to the following scheme: 94°C/1 min, 55°C (*HUMF13A01*) or 65°C (*HUMCD4*)/1 min, 72°C/1 min, with initial denaturation for 3 min and final extension for 10 min.

To exactly identify the alleles in DNA samples, allelic "ladders" were synthesized for both loci. To 100 μl of the reaction mixture, including 10 pmol of each primer, we added 1 μl of an equimolar mixture of the total spectrum of alleles after diluting it 10^4 times.

Amplification products were analyzed by electrophoresis in 12% polyacrylamide gels (the length of the gel was 16 cm; the thickness, 0.7 mm, with an additional 7% of glycerol); 10 μl of the reaction mixture were placed into each gel hole. After electrophoresis, the gel was stained with silver [22].

The observed genotypic frequencies of the loci studied were tested for Hardy–Weinberg equilibrium using the χ^2 test and G-statistics with the aid of R × C (Rows × Columns) computer software [23]. The software was also used to compare distributions of allelic frequencies of the loci studied in samples from different populations.

The expected heterozygosity (H_{exp}) and the following parameters for polymorphic microsatellites were studied: 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 informativeness or polymorphism information content (PIC). We estimated these parameters using computer software based on generally known algorithms reported earlier [7].

RESULTS AND DISCUSSION

The polymorphism of locus *HUMF13A01* was studied in two population samples of 109 (Moscow) and 75 unrelated individuals (Tomsk). Nine alleles ranging from 180 to 230 bp (Figs. 1, 2) were revealed. These alleles were classified according to the number of tandem repeats that they contained. According to Polymeropoulos *et al.* [13], the 180-bp allele contained three AAAG repeats. This allele was designated number 3, the next allele (184 bp) was designated number 4, etc.

However, 226- and 230-bp alleles were also revealed; they contained nonintegral numbers of repeats. These alleles were enumerated according to the recommendations of the Commission for Standardization of Nomenclature of Microsatellite Loci, which were accepted at the symposium of the International Society of Forensic Hemogenetics (ISFH) in October 1993, Venice (Italy). A 226-bp allele was assigned

number 14.2, because it included 14 whole repeats and two additional nucleotides. Correspondingly, a 230-bp allele was assigned number 15.2. Alleles of intermediate size were revealed earlier for other tetranucleotide motifs, e.g., *HUMCYAR04* and *HUMTH01* [4, 5].

In the Russian population, the most frequent alleles were 5, 6, and 7 (Fig. 2). In Russians, allele 11 was revealed; however, this allele was not reported by Polymeropoulos *et al.* [13] for Caucasoids of the United States. At the same time, intermediate allele 3.2, revealed by Hammond *et al.* [4] in North American Caucasoids, was absent in the studied samples.

From 91 possible genotype variants, 25 genotypes (27% of the total amount) were revealed in Moscow inhabitants and 22 genotypes (25%), in Tomsk inhabitants. As expected, in both samples, homo- and heterozygous combinations of the most frequent alleles 5, 6, and 7 were observed (Table 1).

DNAs from 102 representatives of the Moscow population and 75 inhabitants of Tomsk were typed for locus *HUMCD4*. Eight alleles, from 115 to 165 bp in length, were revealed in Moscow inhabitants; seven alleles were detected in the Tomsk population (the 115-bp allele revealed in the Moscow population was absent in Tomsk) (Figs. 3, 4). For this microsatellite, alleles were numbered according to the number of repetitive monomers. According to Hammond *et al.* [4], the 125-bp allele included six pentanucleotide repeats and, respectively, was designated number 6, etc. Alleles 7, 8, and 12 were most frequent (Fig. 3). In the Moscow population, we revealed a new 115-bp allele 4, which was not found in the studied foreign populations [4, 18, 19]. However, recently, Tishkoff *et al.* [24] described the presence of alleles of locus *HUMCD4*, including those comprising 4 to 15 repeats, in an African Negroid population. In the Russian population, a 125-bp allele was found earlier in American Mongoloids, but not in Caucasoids; however, allele 11 reported for American Caucasoids was absent.

From 91 possible allelic combinations of locus *NUMCD4*, in the Moscow sample, 13 allelic combinations were found; in the Tomsk sample, this value was two less. In both samples, heterozygotes 7/8 and 7/12 and homozygotes 7/7 were most frequent.

It was demonstrated that the observed distributions of genotype frequencies of the loci studied in both population samples fitted Hardy–Weinberg equilibrium (Table 3). Therefore, allelic distributions in the urban population samples were unbiased, and the studied samples were sufficiently homogeneous.

We compared experimental values of allelic frequencies of locus *HUMF13A01* for both Russian samples with the published data for foreign populations (Fig. 5). Allelic distributions in the Russian samples were similar. We found neither statistically significant differences between the pooled Russian populations nor differences between the pooled Russian population sample and North American Caucasoids. The common

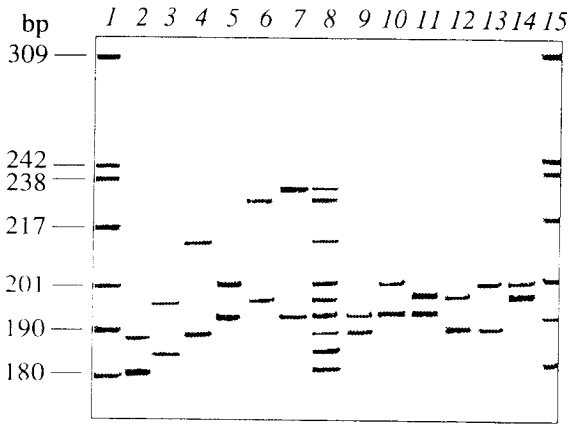

Fig. 1. Separation of the amplified alleles of locus *HUMF13A01* in 12% PAAG: (*1* and *15*) fragments of plasmid DNA pBR322 digested by restriction endonuclease *Msp*I; (*2–7*) genotyping of six unrelated individuals: (*2*) 3/5; (*3*) 4/7; (*4*) 5/11; (*5*) 6/8; (*6*) 7/14.2; (*7*) 6/15.2; (*8*) allelic "ladder"; (*9–14*) family analysis revealing the following genotypes: (*9*) 5/6 (father), (*10*) 6/8 (child 1), (*11*) 6/7 (child 2), (*12*) 5/7 (child 3), (*13*) 5/8 (child 4), and (*14*) 7/8 (mother).

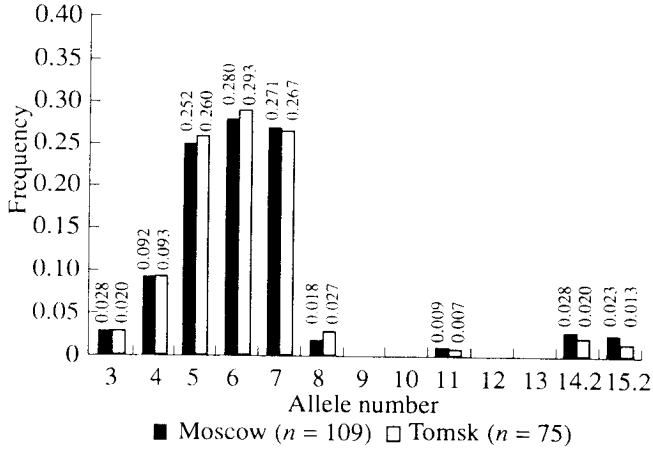

Fig. 2. Allelic frequencies of locus *HUMF1301* in urban populations of Moscow and Tomsk. The allele numeration corresponds to the numbers of tandem repeats contained in alleles; *n*, number of individuals in the sample studied.

pattern of allelic distribution in Caucasoid populations included, e.g., the prevalence of the same alleles (5, 6 and 7). The remaining North American populations (Latin Americans, Negroids, and Mongoloids) significantly differed from Russians. In Latin Americans, five alleles had frequencies higher than 10%, of which alleles 3.2, 6, and 7 prevailed [4]. In Negroids, the maximum number of *HUMF13A01* alleles were observed (13), with alleles 5 and 7 being most frequent. In North American Mongoloids, allele 16 was found, but it was not revealed in other populations; the prevailing alleles

Table 1. Genotype frequencies of locus *HUMF13A01* in urban populations of Moscow and Tomsk

Allelic number	Moscow		Tomsk	
	observed	expected	observed	expected
3-3	0.009	0.001	0	0.000
3-4	0.009	0.005	0	0.004
3-5	0.018	0.014	0.013	0.010
3-6	0	0.015	0.013	0.012
3-7	0.009	0.015	0.013	0.011
4-4	0.018	0.008	0.027	0.009
4-5	0.046	0.046	0.040	0.049
4-6	0.046	0.051	0.053	0.055
4-7	0.037	0.050	0.040	0.050
4-14.2	0.009	0.005	0	0.004
5-5	0.092	0.064	0.093	0.068
5-6	0.119	0.141	0.133	0.153
5-7	0.101	0.137	0.107	0.139
5-8	0	0.009	0.013	0.014
5-11	0.049	0.038	0.080	0.040
5-14.2	0.018	0.014	0.013	0.010
5-15.2	0.009	0.012	0	0.007
6-6	0.101	0.078	0.107	0.086
6-7	0.138	0.151	0.120	0.156
6-8	0.018	0.010	0.027	0.016
6-14.2	0.018	0.015	0.013	0.012
6-15.2	0.018	0.013	0.013	0.008
7-7	0.101	0.073	0.107	0.071
7-8	0.018	0.010	0.013	0.014
7-11	0.009	0.005	0	0.004
7-14.2	0.009	0.015	0.013	0.014
7-15.2	0.018	0.012	0.013	0.007

were alleles 3.2 and 6. Allele 6 was considerably more common than others (frequency, 0.508) [4].

Comparative analysis of the observed distributions of allelic frequencies of locus *HUMCD4* in inhabitants of Moscow and Tomsk demonstrated that they were almost identical (Table 4). We did not observe statistically significant differences between Russians and the European populations, nor between Russians and two North American populations (Caucasoids and Latin Americans) [4, 18, 19]. The same three prevailing alleles (7, 8, and 12) were typical for these populations. In Latin Americans, the rare allele 10 was present, but alleles 4 and 6, which were detected in the Russian population, were not revealed. However, North American Caucasoids and Latin Americans differed to a lesser extent than Russians and Latin Americans. Obviously, this provides evidence for gene exchange between the two former populations. Drastic differences with respect to the allelic distributions for locus *HUMCD4* were revealed between American Negroids and Mongoloids compared to the Moscow population. Negroids exhibited the most complete allelic spectrum of the given locus (10 alleles, from allele 7 to 16). In Negroids, five alleles had frequencies higher than 10%, while, in Caucasoids and Latin Americans, only three such alleles were revealed [4]. Eight alleles were revealed in Mongoloids. The prevalence of only two alleles (7 and 12) was characteristic of *HUMCD4* allelic distribution in this population group, with allele 7 prevailing markedly (frequency, 0.564) [4].

Therefore, comparative analysis of allelic distributions for the two studied loci demonstrated the homo-

Table 2. Genotype frequencies of locus *HUMCD4* in urban populations of Moscow and Tomsk

Allelic number	Moscow		Tomsk	
	observed	expected	observed	expected
4-7	0.039	0.018	0	0.000
6-6	0.010	0.000	0	0.000
6-7	0	0.009	0.013	0.006
7-7	0.216	0.217	0.227	0.224
7-8	0.255	0.247	0.280	0.259
7-12	0.186	0.183	0.173	0.189
7-13	0	0.000	0.027	0.025
7-14	0.020	0.014	0	0.006
8-8	0.069	0.070	0.067	0.075
8-9	0	0.003	0.027	0.025
8-12	0.098	0.104	0.067	0.109
8-13	0.029	0.013	0.027	0.015
8-14	0.010	0.008	0.013	0.004
9-13	0.010	0.000	0	0.001
12-12	0.049	0.038	0.080	0.040
12-13	0.010	0.010	0	0.011

Table 3. Estimates of polymorphism criteria and χ^2 and G-statistic tests for Hardy–Weinberg equilibrium for loci *HUMF13A01* and *HUMCD4* in urban populations of Moscow and Tomsk

Parameter	*HUMF13A01*		*HUMCD4*	
	Moscow	Tomsk	Moscow	Tomsk
H_{obs}	0.679	0.667	0.657	0.627
H_{exp}	0.774	0.770	0.673	0.660
pM	0.087	0.094	0.160	0.173
W	1.005	0.974	0.653	0.602
PIC	0.427	0.391	0.198	0.144
χ^2	9.6043	4.9957	11.1737	6.5611
Probability	1.0000	1.0000	0.9750	0.9650
±*S.E.**	0	0	0.0049	0.0058
G-statistic	11.5885	5.7527	14.7162	8.1437
Probability	1.0000	1.0000	0.9680	0.9650
±*S.E.**	0	0	0.0056	0.0058

* Standard error.

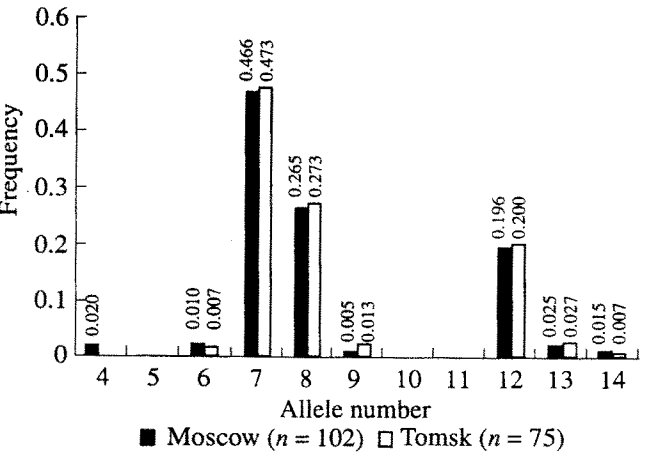

Fig. 3. Allelic frequencies of locus *HUMCD4* in urban populations of Moscow and Tomsk. Allele numeration corresponds to the numbers of tandem repeats contained by alleles; *n*, number of individuals in the sample studied (for Figs. 1, 3).

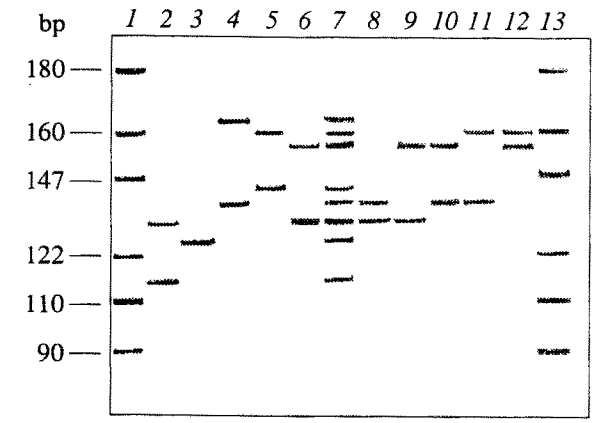

Fig. 4. Separation of the amplified alleles of locus *HUMCD4* in 12% PAAG: (*1* and *13*) fragments of plasmid DNA pBR322 digested by restriction endonuclease *Msp*I; (*2–6*) genotyping of five unrelated individuals: (*2*) 4/7, (*3*) 6/6, (*4*) 8/14, (*5*) 9/13, (*6*) 7/12, (*7*) allelic "ladder"; (*8–12*) family analysis revealing the following genotypes: (*8*) 7/8 (father), (*9*) 7/12 (child 1), (*10*) 8/12 (child 2), (*11*) 8/13 (child 3), and (*12*) 12/13 (mother).

geneity of their distribution in populations of the same race, while racial differences were significant.

On the model of two large families with 3 and 4 children, we demonstrated an independent inheritance of the alleles of two microsatellites (Figs. 1, 4).

Comparison of the informativeness of loci *HUMCD4* and *HUMF13A01* allows us to consider the latter substantially more informative (Table 3). Alleles of both microsatellites comprise approximately the same number of repeats; however, *HUMF13A01* is characterized by a higher polymorphic level (nine alleles vs. seven or eight for locus *HUMCD4*) and higher heterozygosity, which increases PIC for this locus (Table 3). The values of informativeness parameters of these loci in the Moscow population are comparable with similar data for most of the six microsatellites that we studied earlier, namely, for hypervariable regions of retinoblastoma and heavy chains of immunoglobulin [6, 7]. This demonstrates the importance of the given microsatellites for identification tests.

In the framework of developing the microsatellite panel for personal identification, we performed analysis of allelic polymorphism of four tetranucleotide microsatellites (*D6S366, D19S253, HUMvWFII,* and *HUMCYAR04*) in the Russian population [8, 9]. In the Moscow population, the summary value *pM* for all six microsatellites studied is 6.9×10^{-7}. This allows us to guarantee precise personal identification for a population of more than 1.4 million. However, resolution of our identification panel is an order of magnitude lower than the test system of six microsatellites, developed in Great Britain by Urquhart *et al.* [5], which has *pM* of 2.94×10^{-8}. The higher resolution ability of this panel is due to the higher polymorphism of the microsatellites, which include 9–11 alleles [5]. To attain exact personal identification at the scale of the total population of Russia, we need a panel with a summary value of *pM* of an order of 6.7×10^{-9}, which would increase the resolution of the existing test system by 100 times. Therefore, it is necessary to add two more highly polymorphic loci to the microsatellites used in our panel.

Russian (Moscow) (Tomsk)	Caucasoid (USA) [13]	Caucasoid (USA) [4]	Latin American (USA) [4]	Negroid (USA) [4]	Mongoloid (USA) [4]	
0.9705	3.9375	11.2519	22.1171	26.3328	67.4256	χ^2
(1.0000±0.0000)	(0.8900±0.0099)	(0.1660±0.0118)	(0.0120±0.0034)	(0)	(0)	Probability ±*S.E.*
0.9810	4.4202	12.1451	25.1003	30.7621	80.7446	G-statistic
(1.0000±0.0000)	(0.8930±0.0098)	(0.2260±0.0132)	(0.0140±0.0037)	(0)	(0)	Probability ±*S.E.*

Fig. 5. Comparative analysis of allelic distribution of locus *HUMF13A01* in the Moscow population and other populations.

Table 4. Comparison of distributions of allelic frequencies for locus *HUMCD4* in the Moscow population and other Caucasoid populations

Parameter	Russian (Tomsk)	Caucasoid (USA)		Dutch [19]	Belgian [19]	French [19]	Latin American (USA) [4]	Mongoloid (USA) [4]	Negroid (USA)	
		[18]	[14]						[18]	[14]
Sample size, number of individuals	75	100	191	308	208	100	193	78	140	190
Set, R × C	2 × 11	2 × 11	2 × 11	2 × 11	2 × 11	2 × 11	2 × 11	2 × 11	2 × 13	2 × 13
χ^2	2.2895	8.6771	9.0678	10.105	10.505	10.919	12.4150	34.226	45.946	66.296
Probability	0.9640	0.5090	0.4480	0.1310	0.1110	0.0900	0.0870	0	0	0
±*S.E.**	0.0059	0.0158	0.0157	0.0107	0.0099	0.0090	0.0089	0	0	0
G-statistic	3.0682	10.613	11.427	12.437	12.437	12.607	14.4361	40.410	56.645	83.757
Probability	0.9550	0.5650	0.4490	0.1220	0.1060	0.1120	0.1110	0	0	0
±*S.E.**	0.0066	0.0157	0.0157	0.0103	0.0097	0.0100	0.0099	0	0	0

* Standard error.

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