ISSN 1022-7954, Russian Journal of Genetics, 2009, Vol. 45, No. 3, pp. 351–355. © Pleiades Publishing, Inc., 2009.
Original Russian Text © B.A. Malyarchuk, M.A. Perkova, M.V. Derenko, 2009, published in Genetika, 2009, Vol. 45, No. 3, pp. 401–405.

HUMAN GENETICS

Polymorphism of Pigmentation Genes (*OCA2* and *ASIP*) in Some Populations of Russia

B. A. Malyarchuk, M. A. Perkova, and M. V. Derenko

Institute of Biological Problems of the North, Russian Academy of Sciences, Magadan, 685000 Russia;
e-mail: malyarchuk@ibpn.ru

Received October 3, 2007

Abstract—In Russian populations, polymorphism of two pigmentation system genes, *OCA2* (loci 305, 355, and 419, tested in Russians, Buryats, Chukchi, Koryaks, and Evens) and *ASIP* (locus 8818, tested in Russians and Buryats) was examined. Pairwise comparisons of the F_{ST} distances between the populations showed that only the populations from Northeast Asia (Chukchi, Koryaks, and Evens) were statistically significantly different from all other populations, at least relative to one of the *OCA2* locus. In Russians from Pskov oblast and Novgorod oblast, increased frequency (up to 6%) of the *OCA2* allele 419A was revealed. In earlier studies, as association of this allele with green eye color was demonstrated. The data obtained in terms of their application for ethnic population genetics.

DOI: 10.1134/S1022795409030144

INTRODUCTION

In recent years, in addition to traditional analysis of variation of mitochondrial DNA and Y-chromosome, the genetic systems widely used in evolutionary and population genetics, there is a growing interest to autosomal loci. Allelic variants of these loci are characterized by ethnic and racial specificity. Because of this, they can be used for determination of the ancestry of certain individuals, subpopulations, and populations, as well as for determination of the degree of interracial/interethnic admixture in the populations [1–3]. Such markers, called AIMs (Ancestry Informative Markers), are also widely used in genome mapping, analysis of linkage disequilibrium, and associative relationships between different genome regions in normal and pathological states. The AIMs panels are usually constructed using markers associated with certain phenotypic manifestation. The most thoroughly studied markers are those represented by the pigmentation system (of skin, eyes, and hair), and some other anthropologically informative characters, like specific features of craniofacial skeleton. The most popular model phenotypes are the systems of skin and eye pigmentation [4, 5].

It is known that genetic system, determining the variety of hues of skin, eyes, and hair pigmentation, is extremely complex in terms of the number of genes involved and the patterns of interactions between them [2, 5, 6]. By now, a set of pigmentation informative genes has been formed (for instance, *OCA2*, *ASIP*, *DCT*, *MC1R*, *MYO5A*, *TYR*, and *TYRP1*) [4]. Approximately a half of these genes, including the gene for oculocutaneous albinism type 2 (*OCA2*), was mapped to the region q11.2-q12 of chromosome 15. The *OCA2* gene plays an important role in the functioning of pigmentation system. The defects in OCA2 protein can result in the change of the pattern of oculocutaneous pigmentation, and as a consequence, to the risk of skin cancer [7]. At the same time, population studies of normally pigmented individuals showed that *OCA2* allelic variation was associated with variability of human eye color [4, 6, 8].

One of anthropologically informative markers associated with human pigmentation is polymorphism (G> A transition) in position 8818 of the 3' untranslated region of the *ASIP* gene, encoding agouti signaling protein in humans [9]. In earlier studies, it was demonstrated that highest frequencies of allele 8818G (higher than 0.8) were typical of individuals of the African origin. Substantially lower frequencies (below 0.3) were observed among the individuals of Asian and Caucasoid origins [5]. It was also demonstrated that the 8818G allele was ancestral to humans and G>A mutation was thought to mark some adaptive reactions, associated with human adaptation and the independent skin lightening in some regions of the world [10]. At the same time, the data on the prevalence of the *ASIP* 8818 allelic variants in world populations are rather fragmentary [4, 5]. Analysis of pigmentation genes polymorphism in the populations of the Russian Federation seems to be an important problem, especially taking into consideration the fact that populations of Russia are poorly investigated in this respect.

The present study was focused on the analysis of the *OCA2* and *ASIP* polymorphism in some populations of Russian Federation from Eastern Europe (Russians), Southern Siberia (Buryats), and Northeast Asia (Chukchi, Koryaks, and Evens).

Table 1. Characteristics of the *OCA2* and *ASIP* loci tested in the present study

Locus, amino acid substitution (code)	Nucleotide substitution	Localization	Accession number in dbSNP (www.ncbi.nlm.nih.gov)	Restriction polymorphism, literature source
Gene *OCA2*				
Arg305Trp (R305W)	C305T	Exon 9	rs1800401	*Msp*I [8]
Ala355Ala (A355A)	G355A	Exon 10	rs1800404	*Hae*III [1]
Arg419Gln (R419Q)	G419A	Exon 13	rs1800407	*Ama*871 [8]
Gene *ASIP*				
G8818A	G8818A	3'-untranslated region	rs6058017	*Bsr*BI [3]

Table 2. Genotype frequency distribution for three *OCA2* loci in some populations of Russia

Three-locus genotypes (in brackets, polymorphism type)			Regions/populations						
305 (C/T)	419 (G/A)	355 (G/A)	Southern Siberia	Northeastern Asia			Eastern Europe (Russians)		
			Buryats (n = 96)	Chukchi (n = 15)	Evens (n = 14)	Koryaks (n = 32)	Belgorod (n = 56)	Velikii Novgorod (n = 71)	Pskov (n = 70)
CC	GG	GG	0.06 (6)	0.07 (1)	0	0	0	0.01 (1)	0.01 (1)
CC	GG	GA	0.29 (28)	0.20 (3)	0.29 (4)	0.38 (12)	0.23 (13)	0.35 (25)	0.21 (15)
CC	GG	AA	0.51 (49)	0.73 (11)	0.71 (10)	0.62 (20)	0.59 (33)	0.41 (29)	0.61 (43)
CC	GA	GA	0.03 (3)	0	0	0	0.04 (2)	0.13 (9)	0.09 (6)
CT	GG	GG	0.01 (1)	0	0	0	0	0	0
CT	GG	GA	0.09 (9)	0	0	0	0.11 (6)	0.10 (7)	0.04 (3)
CC	GA	GG	0	0	0	0	0.04 (2)	0	0.01 (1)
CC	GA	AA	0	0	0	0	0	0	0.01 (1)

MATERIALS AND METHODS

The samples examined were composed of normally pigmented individuals, representing different populations of Russia: Russians from Belgorod oblast (n = 56), Pskov oblast (n = 70), and Novgorod oblast (n = 73); Buryats (n = 95), Chukchi (n = 15), Koryaks (n = 32), and Evens (n = 14). Blood Samples from Russian individuals were collected during expeditions in 2000 through 2001 on the basis of oblast hospitals (the city of Belgorod) and blood transfusion centers (the city of Velikii Novgorod). DNA samples of Russians from Pskov oblast were obtained from the DNA samples bank of the Laboratory of Human Genetics, Vavilov Institute of General Genetics, Russian Academy of Sciences. Blood samples from the indigenous populations of Siberia were collected during expeditions to the Republic of Buryatia (Buryats), Magadan oblast (Koryaks and Evens), and Chukotskii Autonomous Okrug (Chukchi).

Polymorphism of three *OCA2* loci, corresponding to codons 305, 355, and 419 was typed (Table 1). The conditions of polymerase chain reaction and restriction analysis of amplified DNA fragments are presented elsewhere [1, 8] (Table 1). Polymorphism of the *ASIP* position 8818 was analyzed as described in [3].

Population genetic differentiation was evaluated using the F_{ST} statistics (AMOVA, the ARLEQUIN 3.0 software package [11]). Statistical significance of F_{ST} statistic differences upon pairwise population comparisons was tested using nonparametric permutation approach (10100 permutations) [11]. Expected heterozygosity (H_e) and the fit of genotype distribution to Hardy–Weinberg proportions, evaluated using Fisher's exact test, were calculated using the ARLEQUIN 3.0 software package [11]. Genetic differences between the samples were also evaluated with the help of pseudoprobability χ-square test, realized in the CHIRXC program [12].

RESULTS

Three *OCA2* polymorphisms were typed in populations from three regions of North Eurasia, including Eastern Europe (Russians), South Siberia (Buryats), and Northeast Siberia (Evens, Koryaks, and Chukchi) (Table 2). In the population samples analyzed a total of

Table 3. Heterozygosity of three *OCA2* loci in populations from Russia

Regions/populations	Loci (in brackets, polymorphism type)		
	305 (C/T)	419 (G/A)	355 (G/A)
Southern Siberia (Buryats)	0.1 ± 0.03	0.03 ± 0.02	0.41 ± 0.03
Northeastern Asia (Chukchi, Evens, and Koryaks)	0	0	0.29 ± 0.04
Russians (Belgorod)	0.1 ± 0.04	0.07 ± 0.03	0.35 ± 0.04
Russians (Velikii Novgorod)	0.09 ± 0.03	0.12 ± 0.04	0.43 ± 0.03
Russians (Pskov)	0.04 ± 0.02	0.11 ± 0.03	0.32 ± 0.04

eight combinations of three-locus genotypes were identified. Among these, the *CC/GG/AA* genotype for the 305, 419, and 355 loci, respectively, was the most frequent (Table 2). In the samples of indigenous populations of Northeast Eurasia only three genotypes, differing in polymorphism of the 355 locus were detected. As the samples of Chukchi, Koryaks, and Evens were characterized by similar distributions of the *OCA* genotype frequencies ($P > 0.09$, pseudoprobability test), they were pooled in one sample in further analyses. In the population samples from Eastern Europe (Russians) and South Siberia (Buryats) seven and six genotypes were identified, respectively. Taken together, the level of the *OCA2* diversity (heterozygosity) varied from 48.3% in Eastern Europe (Russians) and 49% in South Siberia (Buryats) to 29% in Northeast Asia (Chukchi, Koryaks, and Evens). The differences between the regional population groups examined constituted approximately 1% ($F_{ST} = 0.99\%$, $P = 0.02$).

Analysis of pairwise F_{ST} distances showed that only populations from Northeast Asia (Chukchi, Koryaks, and Evens) were statistically significantly different from other populations (the F_{ST} values constituted from 4 to 6%). Genetic differentiation among the populations (in terms of the F_{ST} values) over individual *OCA2* loci constituted 0.78% ($P = 0.076$), 0.95% ($P = 0.05$), and 1.49% ($P = 0.015$) for the loci 305, 355, and 419, respectively. The values of expected heterozygosity (H_e) for three *OCA2* loci are demonstrated in Table 3. Locus 355 was found to be most diverse, while for the other loci lower heterozygosity values ($H_e < 0.15$) were obtained. Statistically significant deviation from the Hardy–Weinberg equilibrium was observed only in one case, in Novgorod population for the locus 355. The reason for this deviation probably lies in statistically significantly increased ($P < 0.03$, exact Fisher's test) frequency of the GA heterozygotes in this sample compared to other populations examined (Table 2).

Pairwise F_{ST} comparisons of the populations showed that populations from Northeast Asia were statistically significantly different from Buryats at the loci 305 and 355, and at the locus 419 from each of Russian population samples from Eastern Europe. In turn, Buryats, being compared with Russians, appeared to be statistically significantly different only from Novgorod Russians at locus 419. This locus deserves special interest, since in earlier studies an association of the 419 (G/A) polymorphism with eye color differences was demonstrated. Specifically, the 419A allele was statistically significantly more often in green-eyed individuals [6, 8]. The samples examined were also different in the frequencies of the 419A allele (Table 4). In Russians from Pskov oblast and Novgorod oblast, the frequency of this allele constituted 0.06, on average, while in Russians from Belgorod oblast it was 0.036, and in Buryats, 0.016.

Position 8818 of 3' untranslated region of the *ASIP* gene, encoding agouti signaling protein in humans, is another locus, for which the existence of associations with brown eyes and dark skin was reported [5]. Analysis of G/A polymorphism at this locus in Russians from Belgorod oblast ($n = 56$) and Pskov oblast ($n = 70$) showed that the frequency of the 8818G allele in Russians was 0.115, on average. This value was consistent with those reported for other European populations, from 0.07 to 0.16 [5]. At the same time, in Buryats, which represented indigenous population of Southern Siberia, similar frequency of this allele (0.14) was

Table 4. Allele frequencies at three *OCA2* loci in populations of Russia

Regions/populations	Sample size	Allele frequencies		
		305T	355A	419A
Northeastern Asia				
Chukchi	15	0	0.833	0
Evens	14	0	0.857	0
Koryaks	32	0	0.813	0
Southern Siberia				
Buryats	96	0.052	0.719	0.016
Eastern Europe				
Russians (Belgorod)	56	0.054	0.777	0.036
Russians (Velikii Novgorod)	71	0.049	0.697	0.063
Russians (Pskov)	70	0.021	0.800	0.057

Table 5. Genotype and allele frequency distributions at the *ASIP* 8818 locus in some populations of Russia

Population	Genotype			Allele	
	AA	AG	GG	A	G
Buryats (n = 96)	0.73 (70)	0.26 (24)	0.01 (1)	0.854	0.146
Russians (Belgorod; n = 56)	0.77 (43)	0.23 (13)	0	0.884	0.116
Russians (Pskov; n = 70)	0.77 (54)	0.23 (16)	0	0.886	0.114

Note: In brackets are the numbers of individuals.

revealed. These data conflict with the earlier investigations reporting higher frequencies of this allele for the populations of Eastern Asia, ranging from 0.17 in Chinese to 0.28 in Japanese [5]. At the same time in Yakuts, the only Siberian population investigated so far, extremely low frequency of the 8818G allele was observed (only 0.02 [5]). Genotype frequency distributions for the *ASIP* 8818 locus in Russians and Buryats are presented in Table 5. The populations studied were characterized by similar frequency distributions and diversity at this locus. The value of heterozygosity H_e was 0.24 ± 0.04 in Buryats, 0.21 ± 0.05 in Russians from Belgorod oblast, and 0.20 ± 0.04 in Russians from Pskov oblast. Analysis of the F_{ST} distances performed showed the absence of statistically significant differences in all comparison pairs of Russians and Buryats. It seems likely that despite of the value of this *ASIP* locus in terms of investigation of the evolution of skin pigmentation in humans [5, 9], application of this marker for gene geographic investigations of the populations of Northern Eurasia is rather limited.

DISCUSSION

The investigation performed made it possible to evaluate the levels of polymorphism of the two genes, associated with the functioning of human pigmentation system, in some populations of Russia, and thereby to plan further investigations in this field of population genetics. The data on the existence of genetic differences among Russian populations from Eastern Europe in the distribution of the alleles and genotypes of the *OCA2* gene, playing an important role in human pigmentation system, seem to be the most valuable. Although in Russian populations examined the differences in the 419A allele frequency distributions did not reach statistically significant levels ($P > 0.4$, pseudoprobability test), the data obtained can be treated in terms of the existing interethnic and interpopulation differences in eye color in the populations of North Eurasia, specifically, in Slavs. For instance, according to anthropological data, Slavs are characterized by rather wide range of eye color variation (in the points of chromaticity), from 0.3 to 0.9 [13]. In conformity with anthropological data, the population of Novgorod oblast and Pskov oblast, where the increased frequency of the 419A allele was discovered, abut on the less pigmented population of the Baltic States and the Onega–Dvina basin. At the same time, Russian population of Belgorod oblast, characterized by lower frequency of the 419A allele, abut on the moderately dark-eyed population of the Ukraine. Recent studies showed that the 419A variant was associated with green eye color [6]. Similar highly statistically significant association was also demonstrated for the 355A allele [6]. However, the data of the present study did not allow revealing statistically significant interpopulation differences in the allele frequency distributions of the 355 locus. Because of this, further investigations of human pigmentation system in populations of Russia seem to be expedient.

ACKNOWLEDGMENTS

We thank I.K. Dambueva and S.Yu. Rychkov for their help in realization of this research.

This work was supported by the Russian Foundation for Basic Research (grant no. 06-04-48136).

REFERENCES

1. Parra, E.J., Marcini, A., Akey, J., et al., Estimating African American Admixture Proportions by Use of Population-Specific Alleles, *Am. J. Hum. Genet.*, 1998, vol. 63, pp. 1839–1851.
2. Shriver, M.D., Parra, E.J., Dios, S., et al., Skin Pigmentation, Biogeographical Ancestry and Admixture Mapping, *Hum. Genet.*, 2003, vol. 112, pp. 387–399.
3. Bonilla, C., Shriver, M.D., Parra, E.J., et al., Ancestral Proportions and Their Association with Skin Pigmentation and Bone Mineral Density in Puerto Rican Women from New York City, *Hum. Genet.*, 2004, vol. 115, pp. 57–68.
4. Frudakis, T., Thomas, M., Gaskin, Z., et al., Sequences Associated with Human Iris Pigmentation, *Genetics*, 2003, vol. 165, pp. 2071–2083.

5. Norton, H.E., Kittles, R.A., Parra, E., et al., Genetic Evidence for the Convergent Evolution of Light Skin in Europeans and East Asians, *Mol. Biol. Evol.*, 2007, vol. 24, pp. 710–722.
6. Duffy, D.L., Montgomery, G.W., Chen, W., et al., A Three-SNP Haplotype in the First Intron of OCA2 Explains Most Human Eye Color Variation, *Am. J. Hum. Genet.*, 2007, vol. 80, pp. 241–252.
7. Lee, S.T., Nicholls, R.D., Bundey, S., et al., Mutations of the *P* Gene in Oculocutaneous Albinism, Ocular Albinism, and Prader–Willi Syndrome Plus Albinism, *N. Engl. J. Med.*, 1994, vol. 330, pp. 529–534.
8. Rebbeck, T.R., Kanetsky, P.A., Walker, A.H., et al., *P* Gene as an Inherited Biomarker of Human Eye Color, *Cancer Epidemiol. Biomarkers Prev.*, 2002, vol. 11, pp. 782–784.
9. Kanetsky, P.A., Swoyer, J., Panossian, S., et al., A Polymorphism in the Agouti Signaling Protein Gene Is Associated with Human Pigmentation, *Am. J. Hum. Genet.*, 2002, vol. 70, pp. 770–775.
10. Bonilla, C., Boxill, L., Donald, S., et al., The *8818G* Allele of the Agouti Signaling Protein (*ASIP*) Gene Is Ancestral and Is Associated with Darker Skin Color in African Americans, *Hum. Genet.*, 2005, vol. 116, pp. 402–406.
11. Excoffier, L., Laval, G., and Schneider, S., Arlequin Ver. 3.0: An Integrated Software Package for Population Genetics Data Analysis, *Evol. Bioinform. Online*, 2005, vol. 1, pp. 47–50.
12. Zaykin, D.V. and Pudovkin, A.J., Two Programs to Estimate Significance of Chi-Square Values Using Pseudo-Probability Test, *J. Hered.*, 1993, vol. 84, p. 152.
13. Alekseeva, T.I., *Etnogenez vostochnykh slavyan* (Ethnogenesis of Eastern Slavs), Moscow: Mosk. Gos. Univ., 1973.