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Genetic analysis of melanocortin 1 receptor red hair color variants in a Russian population of Eastern Siberia

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The melanocortin 1 receptor is a G_s protein-coupled receptor implicated in melanogenesis regulation. The receptor gene is highly polymorphic, which accounts for the association of several of its single-nucleotide polymorphisms (SNPs) with an increased risk of melanoma. The present study aimed to evaluate the distribution of melanocortin 1 receptor gene variants R151C, R160W, and D294H within the Russian population of Eastern Siberia and its association with melanoma development. Melanoma patients ($n = 95$) admitted to Krasnoyarsk Territorial Oncological Center and healthy controls ($n = 334$) were enrolled in the study. A clinical examination of patients was performed to evaluate the phenotypic features of melanoma patients. SNPs were analyzed by real-time PCR. Clinical examination indicated a more frequent occurrence of fair skin type, blue eyes, blonde and red hair, and more frequent localization of freckles on the neck, trunk, and extremities in the melanoma group of patients. The R151C melanocortin 1 receptor gene variant was found in 18% of melanoma patients and associated with an increased likelihood of melanoma development (odds ratio = 6.4; 95% confidence interval: 2.8–14.3; $P = 0.0001$). The two remaining variant alleles of the melanocortin 1 receptor gene occurred with low frequency both in controls and in the melanoma group. The R160W SNP was identified neither in controls nor in melanoma patients. The D294H heterozygous variant was observed in 0.3% of individuals in the control group and in 1.1% of the patients in the melanoma group. Such an asymmetric distribution of the melanocortin 1 receptor within red hair color genotypes in the population under study compared with other populations may be because of Russian genetic homogeneity. Carriers of the mutant R151C allele should exercise caution in terms of exposure to the sun to avoid the risk of melanoma development. *European Journal of Cancer Prevention* 27:192–196 Copyright © 2018 The Author(s). Published by Wolters Kluwer Health, Inc.

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Introduction

The melanocortin 1 receptor (MC1R) is a G_s protein-coupled receptor that is triggered by adrenocorticotropic hormone or α-melanocyte-stimulating hormone, resulting in activation of the cyclic AMP (cAMP) and protein kinase A signaling pathways through which MC1R induces cell differentiation, eumelanin production, antioxidant reactions, and DNA repair (Rodriguez and Setaluri, 2014; Jarrett *et al.*, 2015). The melanocortin 1 receptor gene (*MC1R*) is highly polymorphic and several of its single-nucleotide polymorphisms (SNPs) are associated with increased ultraviolet-induced skin damage and the risk of melanoma development (Chatzinasiou *et al.*, 2011).

It was 1996 when Valverde *et al.* reported on the first study about MC1R variants and the risk of melanoma. They showed that *MC1R* allelic variants carriers occurred more frequently in patients with melanoma than in a control group. This observation suggested a correlation between certain *MC1R* SNPs and melanoma (Valverde *et al.*, 1996). Since then, numerous studies have provided evidence on the relations between *MC1R* variant alleles and the risk of melanoma in Dutch, South European/Mediterranean, French, Australian, US and Canadian, and several other populations (Kennedy *et al.*, 2001; Matichard *et al.*, 2004; Landi *et al.*, 2005; Kanetsky *et al.*, 2006).

Extensive investigations showed that Val60Leu, Arg142His, and Arg151Cys variants of *MC1R* are associated with the risk of melanoma in Southern European populations (Stratigos *et al.*, 2006). The R160W variant was found to correlate with sporadic melanoma cases in US melanoma patients; D84E, R142H, R151C, and R160W were related to a significantly increased risk of melanoma in the German population (Council *et al.*, 2009; Scherer *et al.*, 2009).

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According to Gerstenblith *et al.* (2007) there are seven SNPs among Caucasian populations that have shown significant differences in allele distribution, namely V60L, V92M, D84E, R151C, R160W, R163Q, and D294H. Several *MC1R* gene variants are strongly associated with specific phenotypes such as red hair and fair skin (R160W, D294H) or red hair only (D84E, p.R142H, and p.R151C) (Raimondi *et al.*, 2008), which have been further linked to increased ultraviolet sensitivity and melanoma predisposition. Several mechanisms may contribute toward the increased melanoma risk of carriers of *MC1R* variants as the experimental functional studies showed lower cell surface expression of the receptor and its impaired desensitization (Sanchez-Laorden *et al.*, 2007). cAMP production is dependent on the level of cell surface receptor, a decrease in which accounts for a reduction of the cAMP intercellular concentration, and may lead to modification of downstream signaling (Beaumont *et al.*, 2005). A recent international study based on 5160 melanoma cases and 12 119 healthy controls discovered that *MC1R* polymorphisms associated with red hair color correlate with an increased melanoma risk only in darker-pigmented Europeans carrying no typical features such as blonde hair and fair skin color. The rather unexpected results were explained by the significant impact of MC1R on intercellular signaling pathways including mitogen-activated protein kinase and the proinflammatory NF-κβ pathway triggered by MC1R (Pasquali *et al.*, 2015).

The aim of this study was to evaluate the frequency of *MC1R* gene variants R160W, D294H, and R151C in an Eastern Siberian population of Russia among melanoma patients and healthy controls residing in the region and the relation of polymorphisms to melanoma predisposition and the patients' phenotype.

Patients and methods

Patients

The study was approved by the local ethics committee of Krasnoyarsk State Medical University (protocol 51/2013 of 28 October 2013) and by the ethics committee of Krasnoyarsk Territorial Oncological Center (protocol 18/2013 of 2 December 2013); it was carried out in accordance with the ethical standards as stated in the Declaration of Helsinki. Melanoma patients ($n = 95$) admitted to Krasnoyarsk Territorial Oncological Center in 2013–2015 and healthy voluntaries ($n = 334$) were recruited into the study. The participants of the study underwent a clinical skin examination, a detailed questionnaire, and analysis of the distribution of *MC1R* R160W, R151C, and D294H allele variants distinguished as wild type, homozygous, and heterozygous carriers. Informed consent was obtained from all individual participants for inclusion in the study. Age at diagnosis, family history of melanoma, and phenotypic data (hair and eye color, Fitzpatrick skin phototype, nevi count, distribution of freckles) were evaluated by the dermatologist for all the participants in the study.

The data obtained by a clinical examination of hair color were coded as blonde, brown, black, and red. Eye color was coded as blue, gray, green, and brown. Skin color was evaluated according to Fitzpatrick's phototype classification (I, II, III, IV) (Fitzpatrick, 1988). The number of nevi covering the entire body and the presence of freckles and their localization (face, neck, back, chest, and upper and lower extremities) were evaluated by a dermatologist. Breslow thickness of the tumor was provided by the pathologist report of Krasnoyarsk Territorial Oncological Center and Krasnoyarsk Pathologic Anatomy Bureau.

DNA isolation and real-time PCR

DNA was extracted from peripheral leukocyte whole-blood samples according to the manufacturer's protocol for Ampli-prime DNA-sorb-B (NextBio, Moscow, Russia). The concentration of extracted DNA was measured using a NanoVue Plus spectrophotometer (Biochrom LTD, Cambridge, England for GE Healthcare Bio-Sciences AB).

The MC1R gene was genotyped using TaqMan SNP Genotyping Assays. Three red hair color SNPs in the *MC1R* gene were studied: R151C (rs1805007); R160W (rs1805008); and D294H (rs1805009). All primers and probes were designed by Applied Biosystems (Foster City, California, USA) and genotyping analyses were carried out on an ABI StepOne Real-Time PCR system (Applied Biosystems, Warrington, UK) according to the manufacturer's protocol: 95°C for 15 min, followed by 40 cycles of 92°C for 15 s and 60°C for 60 s, and analyzed using StepOne, version 2.3 software (Applied Biosystems). The final reaction volume was 20 μl, containing 8 μl of PCR reaction mix with ROX (M-430; Syntol, Moscow, Russia), 1 μl, of TaqMan probe mix, and 10 ng of genomic DNA. For genotyping quality control, negative controls were included in all SNPs and 15% of samples were randomly selected and analyzed in duplicate to check the accuracy of genotyping. The concordance rate was 100%.

Statistical analysis

The collected data and the clinical characteristics were statistically analyzed using Statistica software, version 10.0 (Statsoft, Moscow, Russia). Quantitative data were expressed as mean values ± SE. Percentage frequency was used to characterize the population. The relation between risk factors and melanoma was expressed as an odds ratio (OR) with a confidence interval (CI) of 95%. For each polymorphism, departure of the genotype distribution from that expected from Hardy–Weinberg equilibrium was assessed using the standard χ^2-test. The difference in the mean nevi count between groups was evaluated using an unpaired *t*-test. A nonparametric

Mann–Whitney *U*-test was used for analysis of the Breslow thickness of the tumor in melanoma patients. To analyze the relationship between the individual pairs of qualitative features, Fisher's exact test was used, with the construction of contingency tables for all possible pairs of the given attributes. *P* values less than 0.05 were considered significant.

Results

The analysis of phenotypic characteristics of individuals recruited to the study showed their specific features in the group of melanoma patients. Skin phototype I was more frequent in melanoma patients, whereas phototype III was more frequent in the control group. Melanoma patients had blue eyes more frequently and, more rarely, gray and brown. In addition, melanoma patients had blonde and red hair color more often and, more rarely, black and brown. No differences in the total number of nevi were found between the two groups studied. Freckle localization was more frequent on the neck, chest, back, and upper and lower extremities in melanoma patients compared with the control group (Table 1).

The distribution of the genotypes R160W and D294H did not deviate from the Hardy–Weinberg equilibrium.

The frequencies of MC1R R151C, R160W, and D294H genotypes in patients with melanoma and in healthy controls are shown in Table 2. A higher frequency of the R151C CT allele was found among melanoma patients compared with healthy controls (18 vs. 3.3%; OR = 6.356; 95% CI: 2.831–14.27; $P = 0.0001$). The allele frequencies of R160W were identical for melanoma patients and healthy controls. The CT and TT carriers of the R160W allelic variant were identified in our study in neither the melanoma patients nor the control group. The D294H heterozygous GC allelic variant was identified in one melanoma patient (1.1%) and in one healthy control individual (0.3%). Homozygous D294H CC allelic variant carriers were not found among the melanoma patients or the control group.

Table 1 Melanoma patients' and healthy controls' phenotypic characteristics

	Controls ($n = 334$) [*n* (%)]	Melanoma ($n = 95$) [*n* (%)]	*P*
Phototype			
I	0 (0)	7 (7.4)	0.0001
II	88 (26.3)	30 (31.6)	0.3620
III	237 (71)	53 (55.8)	0.0063
IV	9 (2.7)	5 (5.2)	0.2050
Eye color			
Blue	65 (19.5)	38 (40)	0.0001
Gray	44 (13.2)	5 (5.3)	0.0425
Green	113 (33.8)	36 (37.9)	0.4662
Brown	112 (33.5)	16 (16.8)	0.0014
Hair color			
Blonde	161 (48.2)	59 (62.1)	0.0199
Brown	121 (36.3)	24 (25.3)	0.0497
Black	47 (14)	5 (5.3)	0.0200
Red	5 (1.5)	7 (7.3)	0.0064
Total number of nevi	54 ± 19.2	56 ± 28.6	0.8220
Freckles			
Number of individuals with freckles localized on			
Face	74 (22.1)	28 (29.4)	0.1714
Neck	15 (4.5)	18 (18.9)	0.0001
Chest	21 (6.3)	41 (43.1)	0.0001
Back	27 (8.0)	64 (67.4)	0.0001
Upper extremities	30 (8.9)	30 (31.5)	0.0001
Lower extremities	0 (0)	8 (8.4)	0.0001
Without freckles	238 (71.3)	19 (20)	0.0001

The R151C CT genotype was associated with a decreased number of individuals with brown eyes in melanoma patients compared with the control group (Table 3). No significant associations were found between the *MC1R* gene R151C polymorphism and skin phototype, although freckle localization on the back and upper extremities was higher in patients of the melanoma group. The *MC1R* gene D294H polymorphism was not associated with any of the phenotypic characteristics evaluated. We observed no associations between any *MC1R* genotype determined in this study and Breslow thickness of the tumor in melanoma patients.

Discussion

The present study considers the distribution of the three most frequent red hair color SNPs of *MC1R* in healthy controls and in melanoma patients of Krasnoyarsk Territory belonging to the Siberian Federal District of the Russian Federation. Caucasian individuals compose more than 90% of the Siberian Federal District population, which led us to identify the way in which the *MC1R* gene variants are linked to melanoma development.

Melanoma age-adjusted incidence rates in Krasnoyarsk Territory increased from 2.6 in 1996 to 4.32 in 2009 per 100 000 inhabitants and correspond to all-Russian and eastern European indices (Gyrylova *et al.*, 2014). However, the increase in age-adjusted mortality rates from 1.56 to 2.02 in 1999–2009 in the region points to the importance of further evaluation of the impact of risk factors on melanoma development.

Red hair color *MC1R* gene polymorphisms showed associations with melanoma risk in several eastern European populations: R151C was found to be linked with the highest risk of melanoma in a Latvian population, and was correlated with melanoma risk in Poland's population (Debniak *et al.*, 2006; Ozola *et al.*, 2013). Hence, the aim of the present study was to identify the distribution of *MC1R* gene polymorphisms R151C, R160W, and D294H, referred to as the 'red hair color phenotype,' in the population of a Siberian Territory and its relationship with melanoma development.

We found that, in an eastern Siberian population, melanoma patients have a special phenotype expressed as a more frequent I skin phototype, blue color of the eyes, blonde/red color of hair, and more frequent freckles on the neck, trunk, and extremities. Our observation is in line with numerous previous reports on the higher risk of

Table 2 Prevalence of MC1R genotypes in melanoma patients and healthy controls in the Krasnoyarsk Territory

MC1R genotype	SNP_rs	Allelic variant	Controls [*n* (%)]	Melanoma patients [*n* (%)]	OR (95% CI)	*P*
R151C	1805007	CC	319 (96.7)	73 (82)		0.0001
		CT	11 (3.3)	16 (18)	6.356 (2.831–14.27)	0.0001
		TT	0 (0)	0 (0)	–	–
R160W	1805008	CC	328 (100)	88 (100)	–	–
		CT	0 (0)	0 (0)	–	–
		TT	0 (0)	0 (0)	–	–
D294H	1805009	GG	322 (99.7)	88 (98.9)		0.3858
		GC	1 (0.3)	1 (1.1)	3.659 (0.227–59.091)	0.3858
		CC	0 (0)	0 (0)	–	–

CI, confidence interval; *MC1R*, the melanocortin 1 receptor gene; OR, odds ratio; SNP, single-nucleotide polymorphism.

Table 3 Phenotype comparative analysis in CT R151C *MC1R* gene carriers

	Variant R151C		
	Controls ($n=11$) [*n* (%)]	Melanoma patients ($n=16$) [*n* (%)]	*P*
Phototype			
I	0 (0)	1 (6.25)	1.0
II	3 (27.3)	6 (37.5)	0.5796
III	8 (72.7)	8 (50)	0.2376
IV	0 (0)	1 (6.25)	1.0
Eye color			
Blue	1 (9)	5 (31.25)	0.1736
Gray	1 (9)	0 (0)	0.4074
Green	5 (45.6)	10 (62.5)	0.3811
Brown	4 (36.4)	1 (6.25)	0.0478
Hair color			
Blonde	4 (36.4)	9 (56.25)	0.3096
Brown	4 (36.4)	4 (25)	0.5252
Black	3 (27.2)	0 (0)	0.0564
Red	0 (0)	3 (18.75)	0.2479
Number of individuals with freckles localized on			
Face	2 (18.2)	7 (43.7)	0.1661
Neck	0 (0)	2 (12.5)	0.4986
Chest	1 (9)	5 (31.2)	0.3110
Back	3 (27.3)	13 (81.2)	0.0050
Upper extremities	2 (18.2)	10 (62.5)	0.0228
Lower extremities	0 (0)	3 (18.7)	0.2479
Without freckles	7 (63.6)	1 (6.3)	0.0013

melanoma development in fair-skinned individuals than in dark-skinned ones. Eye color, the presence of dysplastic nevi, and a high degree of freckling as a cutaneous ultraviolet response were also shown to be associated with increased melanoma risk (Baccarelli and Landi, 2002; Markovic *et al.*, 2007; Quereux *et al.*, 2011). No difference was found between the number of nevi in healthy controls and that in melanoma patients in the present study, which is in agreement with controversial data on the role of nevi as a marker of melanoma. The multicenter case–control study of Garbe *et al.* (1994) showed that the presence of more than 100 melanocytic nevi increases the risk of melanoma development sevenfold versus individuals who have only 10 melanocytic nevi. The more recent study by Geller *et al.* (2016) showed a rather small group of melanoma patients to have more than 50 nevi over the entire body. Therefore, it is not recommended to consider a patient's at-risk status with only nevi taken into account. We did not find any associations between Breslow thickness of the tumor and *MC1R* status. Similar data were described recently by Taylor *et al.* (2015).

A study of genetic variation in 1679 melanoma cases in a Mediterranean population assessed the frequency of R151C, R160W, and D294H *MC1R* polymorphisms, respectively. Puig-Butille *et al.* (2013) showed a similar distribution within three red hair *MC1R* polymorphisms studied. The *MC1R* gene polymorphism identification study carried out in the Netherlands determined frequencies of ~5, 11, and 1% for R151C, R160W, and D294H *MC1R* polymorphisms, respectively, in healthy individuals (Bastiaens *et al.*, 2001). In the Russian population of the Siberian region, the frequency of mutant alleles in healthy individuals was lower than that in the above-mentioned populations and was 3.3, 0, and 0.3% for R151C, R160W, and D294H, respectively.

In our study, we did not find R160W mutant allele carriers among the melanoma group and the control group, and identified one (0.3%) carrier of the D294H heterozygous variant of *MC1R* in the control group and one (1.1%) in the melanoma group. At the same time, we identified an 18% CT allele frequency of R151C in melanoma patients.

This tendency may be relevant to another genotyping study in cancer patients of a Russian population: Suspitsin *et al.* described *BRCA1* germ-line mutation carriers where the 5382incC mutation occurred in 9.7% of ovarian cancer patients in North-Western and 17.2% in South Russian regions, whereas other *BRCA1* mutations that were previously shown to have an impact on hereditary breast/ovarian cancer development in other populations had insignificant values (Suspitsin *et al.*, 2009). These data were considered an indication of greater genetic homogeneity of the Russian population. The same phenomenon was presumably identified in *MC1R* gene polymorphism distribution in the present study, although the final conclusion should be made after a representative study on *MC1R* sequencing results in the Russian population.

In our study, R151C *MC1R* was associated with an increased risk of melanoma (OR = 6.4; 95% CI: 2.8–14.3; $P=0.0001$) that was also previously established,

including in a meta-analysis survey (Debniak *et al.*, 2006; Raimondi *et al.*, 2008). The R151C variant of the *MC1R* gene is a diminished-function SNP that results in reduced MC1R signaling (Beaumont *et al.*, 2005). In conclusion, the phenotype of melanoma patients of a Russian population is similar to that generally observed in other populations of European origin. At the same time, the distribution of *MC1R* red hair genotypes in the control group and melanoma patients is characterized by specific features such as the relatively high similarity of genotypes in melanoma patients that may correspond to genetic homogeneity of the Russian population.

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Conflicts of interest

There are no conflicts of interest.

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