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Association of the 16q24.3 region gene variants rs1805007 and rs4785763 with heightened risk of melanoma in Latvian population

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ABSTRACT

Chromosome region 16q24.3 has been shown to modify the risk for developing melanoma in genome-wide association studies (GWAS). This region includes at least three SNPs for which significant independent effects on melanoma risk have been demonstrated: rs258322 (*CDK10* intron), rs4785763 (pseudogene *AFG3L1P*), and rs8059973 (flanking 5′UTR of *DBNDD1*). Also variants within the *MC1R* gene, located in the same region, are known to be associated with an increased melanoma risk. However, the exact risk these variants convey has never been estimated in the population of Latvia. Also the haplotypes of the 16q24.3 region and their relationship with melanoma have not been studied in this population before. To elucidate the associations of the variants from the 16q24.3 region with melanoma alongside their mutual interactions, we performed direct sequencing of the *MC1R* gene and genotyped the rs258322, rs4785763, and rs8059973 SNPs. In total, the study subjects included 479 individuals, comprising 255 melanoma patients and 224 controls. Univariate analyses of genotypes showed that only rs1805007 variant from *MC1R* gene, and two chromosome 16 SNPs, rs258322 and rs4785763, were nominally associated with an increased risk of melanoma. Multivariate models built by stepwise regression revealed that the contributions of rs1805007 and rs4785763 to melanoma risk are independent. Haplotype analyses demonstrated that rs1805007 and rs4785763 are independently associated with melanoma, whereas the impact of rs258322 to melanoma risk is related to rs1805007.

1. Introduction

Cutaneous melanoma is a skin cancer that arises from an increased division of pigment-producing cells of neuro-ectodermal origin—melanocytes. The incidence of melanoma is continually increasing in Western countries (Whiteman et al., 2016) as well as in Latvia (Azarjana et al., 2013). According to the European Network of Cancer Registries, the age-adjusted incidence rate of cutaneous melanoma in Latvia is 7.6/100,000 per annum, which is lower than the estimated rate in Europe (11.1/100,000 per annum) (Ferlay et al., 2013). Significant progress in the therapy for advanced melanoma has been reached in recent years—several molecular inhibitors targeting *BRAF* and *MEK* signaling pathways as well as immune checkpoint inhibitors targeting *CTLA4* and *PD1* have been developed. However, an acquired resistance and a short response duration hinders the application of these approaches (Davey et al., 2016; Michielin and Hoeller, 2015), thus bringing prevention and ascertaining of genetic predisposition to the forefront (Corrie et al., 2014).

The most frequently encountered melanoma-associated gene is the cyclin-dependent kinase inhibitor 2A gene (*CDKN2A*) located on

Abbreviations: ACD, adrenocortical dysplasia protein homolog gene; *AFG3L1P*, AFG3-like matrix AAA peptidase subunit 1 of the pseudogene; *BAP1*, BRCA1 associated protein-1 gene; BRAF, serine/threonine-protein kinase B-Raf; *CDK4*, cyclin-dependent kinase 4; *CDK10*, cyclin-dependent kinase 10 gene; *CDKN2A*, cyclin-dependent kinase inhibitor 2A gene; CI, confidence interval; CTLA4, cytotoxic T-lymphocyte associated protein 4; *DBNDD1*, dysbindin domain containing 1 gene; GWAS, genome wide association study; LD, linkage disequilibrium; LGDB, Latvian Genome Data Base; MAF, minor allele frequency; *MC1R*, melanocortin 1 receptor gene; MEK, mitogen-activated protein kinase kinase; *MITF*, melanogenesis associated transcription factor gene; OR, odds ratio; p, *p*-value; PD1, programmed death receptor 1; *POT1*, protection of telomeres protein 1 gene; r^2, squared correlation coefficient; rs1110400, SNP p.Ile155Thr in *MC1R*; rs1805007, SNP p.Arg151Cys in *MC1R*; rs1805008, SNP p.Arg160Trp in *MC1R*; rs2228479, SNP p.Val92Met in *MC1R*; rs258322, SNP c.160 + 171A > G in *CDK10*; rs4785763, SNP n.1682A > C in *AFG3L1P*; rs8059973, SNP c.32-3696T > C in *DBNDD1*; *SLC45A2*, solute carrier family 45 member 2 gene; SNP, single nucleotide polymorphism; *TERF2IP*, telomeric repeat-binding factor 2-interacting protein 1 gene; *TERT*, telomerase reverse transcriptase gene

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chromosome 9p21.3. It accounts for 20%–40% of familial melanomas (Read et al., 2016; Goldstein et al., 2006). Another well-known gene that conveys a high melanoma risk is the cyclin-dependent kinase 4 gene (*CDK4*) located on chromosome 12q14.1 (Puntervoll et al., 2013). Several other high-risk genes for melanoma—BRCA1-associated protein-1 gene (*BAP1*), protection of telomeres protein 1 gene (*POT1*), adrenocortical dysplasia protein homolog gene (*ACD*), telomeric repeat-binding factor 2-interacting protein 1 gene (*TERF2IP*), and telomerase reverse transcriptase gene (*TERT*)—have also been identified recently; however, mutations in the latter genes are rare and are found in few families worldwide (Aoude et al., 2015).

At least three genes are considered moderate-risk genes for melanoma: melanogenesis-associated transcription factor gene (*MITF*) located on chromosome 3p13 (Bertolotto et al., 2011; Yokoyama et al., 2011), solute carrier family 45 member 2 gene (*SLC45A2*) located on chromosome 5p13.2 (Fernandez et al., 2008; Guedj et al., 2008), and melanocortin 1 receptor gene (*MC1R*) located on chromosome 16q24.3 (Williams et al., 2011; Raimondi et al., 2008). The latter is highly polymorphic (Pérez Oliva et al., 2009) and encompasses several variants that are associated with an increased melanoma risk and also with various pigmentation traits (Williams et al., 2011; Raimondi et al., 2008). The 16q24.3 region has also been shown to be implicated in modifying melanoma risk in genome-wide association studies (GWAS) (Bishop et al., 2009; Barrett et al., 2011). In these studies, three non-coding SNPs in this region were associated with melanoma—rs258322 (residing within an intron of the cyclin-dependent kinase 10 gene, *CDK10*), rs4785763 (located within the *AFG3*-like matrix AAA peptidase subunit 1 of the pseudogene *AFG3L1P*), and rs8059973 (within flanking 5′UTR of dysbindin domain containing 1 gene, *DBNDD1*) (Bishop et al., 2009; Barrett et al., 2011). However, none of the *MC1R* variants were present on the genotyping arrays used in the GWAS. Moreover, neither the effects nor the significance of the 16q24.3 region variants are known for the Baltic region.

This study aims at gaining an understanding of the associations between the variants residing in the 16q24.3 region and melanoma in the population of Latvia. To achieve this goal, we juxtaposed non-coding variants in the 16q24.3 region with the *MC1R* variants and evaluated the contribution of the *MC1R* variants to the risk conveyed by variants in the 16q24.3 region. Furthermore, we assessed the associations of the haplotypes within the 16q24.3 region with melanoma.

2. Materials and methods

2.1. Study population

We conducted this study using DNA samples and data on cofactors (age and sex) from the Latvian Genome Data Base (LGDB), a government-funded biobank (briefly described in Ciganoka et al. (2011)). All cases and controls included in this study are Latvian nationals and have European ancestry. In addition, the Latvian population has been shown to be genetically homogenous (Pliss et al., 2015). In total, 479 samples were selected, including 224 unrelated healthy volunteers and 255 melanoma patients with histopathologically confirmed cutaneous melanoma (ICD-10 diagnosis code C43).

Written, informed consent was acquired from all LGDB participants. The Central Medical Ethics Committee of Latvia approved the protocols for sample collection. These protocols were No. A-3/2006 and No. A-7/2007, which were part of the project “Creation of Genome Data Base of Latvian population,” and the protocol No. 01-29/2016-1-1 for this particular study.

2.2. Genotyping

The entire coding region of the *MC1R* gene was sequenced as described in Ozola et al. (2013) with at least two-fold coverage (at least one-fold of each strand). Chromosome 16 SNPs—rs258322, rs4785763, and rs8059973—were genotyped using TaqMan SNP Genotyping Assays (C___653812_1_, C__2875849_10 and C_29970391_10, respectively) on ViiA™ 7 Real-Time PCR Instrument (Thermo Scientific Molecular Biology, Waltham, MA) as per manufacturer's instructions. The genotyping included at least two random replicates and a no template control test in each assay.

2.3. Statistical analyses

The minor allele frequency (MAF) of each variant was estimated using all controls having the genotype information for this variant (224 controls for the *MC1R* gene, 203 for rs258322, 204 for rs8059973 and 205 for rs4785763). In the subsequent analyses, we considered only the variants with $\geq 4\%$ MAF and with at least one homozygote of two minor alleles in controls that did not significantly deviate from the Hardy-Weinberg equilibrium. Next, we carried out univariate analyses with and without cofactors (age and sex) by fitting a logistic regression model for each SNP and a melanoma case/control indicator. We assumed an additive model of the contribution of alleles to the disease. We measured the significance of a genotype-phenotype association by the Wald test applied to the genotype term with $\alpha = 0.05$. All logistic regression models were fitted by the function *glm* in R environment. In addition, we carried out a permutation test to obtain empirical *p*-values of genotype-phenotype associations (only individuals without any missing genotypes for all SNPs were considered here and the case/control status for all individuals was permuted 999 times; the minimum *p*-values across all SNPs in a single permuted data set were compared with the real association *p*-value for a SNP).

To gain an understanding of the potential interactions of variants on chromosome 16, we built multivariate models with and without cofactors (age and sex) by stepwise regression. Models were built by the function *stepAIC* from the R package *MASS* starting with a model without any SNPs. We used a generalized linear model throughout all multivariate analyses, together with the additive model of allele contributions.

We also assessed associations of various haplotypes with the disease status. Haplotypes were identified for combinations of SNPs that were either moderately significant within univariate models (empirical *p*-value ≤ 0.1) or were included in any of the multivariate models. Haplotype associations were detected using the R library *haplo.stats* (Lake et al., 2002; Schaid et al., 2002). Initially, genotypes were recoded by functions *geno1to2* and *setupGeno*, and then haplotype association tests were performed by *haplo.glm* (we used logit link for a binomial family of models, and we set the threshold for rare haplotypes to 0.01).

In addition, we estimated a linkage disequilibrium (LD) for each pair of associated variants and reported the squared correlation coefficient (r^2); the computation was based on the European population haplotype data from the 1000 Genomes Project and carried out by the *Ldmatrix* module from the web-based application suite LDlink 2.0 (Machiela and Chanock, 2015).

3. Results

3.1. Demographic characteristics of the study population

The demographic characteristics of the study participants are listed in Table 1. Melanoma patients and controls did not differ significantly in terms of sex (Chi-squared test $p = 0.149$); however, the control group was significantly younger than patients (Mann-Whitney test $p = 2.36 \times 10^{-8}$).

3.2. Genotyping results

Within the *MC1R* gene, we detected 26 different variants: 18 non-synonymous variants, seven synonymous, and one insertion. However, in our set of controls, only four non-synonymous *MC1R* variants (rs2228479, rs1805007, rs1110400, rs1805008) and two of the three