Prediction of Melanoma Risk in a Southern European Population Based on a Weighted Genetic Risk Score

Katerina P. Kypreou[1,11], Irene Stefanaki[1,11], Kyriaki Antonopoulou[1], Fani Karagianni[1], Georgios Ntritsos[2], Alexios Zaras[1], Vasiliki Nikolaou[1], Iro Kalfa[3], Vasiliki Chasapi[1], Dorothea Polydorou[1], Helen Gogas[4], George M. Spyrou[5], Lars Bertram[6,7], Christina M. Lill[8], John P.A. Ioannidis[9], Christina Antoniou[1], Evangelos Evangelou[2,10,12] and Alexander I. Stratigos[1,12]

Many single nucleotide polymorphisms (SNPs) have been described as putative risk factors for melanoma. The aim of our study was to validate the most prominent genetic risk loci in an independent Greek melanoma case-control dataset and to assess their cumulative effect solely or combined with established phenotypic risk factors on individualized risk prediction. We genotyped 59 SNPs in 800 patients and 800 controls and tested their association with melanoma using logistic regression analyses. We constructed a weighted genetic risk score (GRS_{GWS}) based on SNPs that showed genome-wide significant (GWS) association with melanoma in previous studies and assessed their impact on risk prediction. Fifteen independent SNPs from 12 loci were significantly associated with melanoma ($P < 0.05$). Risk score analysis yielded an odds ratio of 1.36 per standard deviation increase of the GRS_{GWS} ($P = 1.1 \times 10^{-7}$). Individuals in the highest 20% of the GRS_{GWS} had a 1.88-fold increase in melanoma risk compared with those in the middle quintile. By adding the GRS_{GWS} to a phenotypic risk model, the C-statistic increased from 0.764 to 0.775 ($P = 0.007$). In summary, the GRS_{GWS} is associated with melanoma risk and achieves a modest improvement in risk prediction when added to a phenotypic risk model.

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INTRODUCTION

The development of cutaneous melanoma (CM) is a complex process involving the interplay of environmental, phenotypic, and genetic risk factors. Highly penetrant susceptibility genes include CDKN2A (Hussussian et al., 1994; Kamb et al., 1994), CDK4 (Puntervoll et al., 2013; Soufir et al., 1998; Zuo et al., 1996), and the recently described genes BAP1, MITF, TERT, POT1 and other shelterin complex genes (ACD and TERF2IP) (Aoude et al., 2015; Bertolotto et al., 2011; Harbour et al., 2010; Horn et al., 2013; Robles-Espinoza et al., 2014; Shi et al., 2014; Yokoyama et al., 2011). Genome-wide association studies (GWASs) and candidate gene studies have also revealed numerous common single nucleotide polymorphisms (SNPs) exerting more modest risk effects. A recent GWAS meta-analysis highlighted more than 20 genome-wide significant (GWS) (i.e., $P < 5 \times 10^{-8}$) risk loci, including five novel regions (Law et al., 2015). These findings corroborate the association of CM with pigmentation-associated (MC1R, TYR, SLC45A2) and nevi-associated genes (MTAP, PLA2G6), as well as with loci potentially implicated in apoptosis (CASP8), DNA repair (PARP-1, ATM), metabolism (FTO), and telomerase maintenance (TERT/CLPTM1L) (Barrett et al., 2011; Iles et al., 2013; Ward et al., 2012).

This growing list of melanoma risk loci needs to be validated in large independent datasets from other populations. In this context, the Greek population is of particular interest because it reportedly has a low incidence of melanoma compared to other European countries despite a high degree of ambient ultraviolet exposure year-round (Ferlay et al., 2013). The aim of this study was to validate the extensive set of SNPs previously associated with CM risk in an independent sample of melanoma patients and healthy controls from Greece. In addition, we assessed the cumulative impact of the genetic variants on melanoma risk prediction by

[1]1st Department of Dermatology, Andreas Syggros Hospital, Medical School, National and Kapodistrian University of Athens, Athens, Greece; [2]Clinical and Molecular Epidemiology Unit, Department of Hygiene and Epidemiology, School of Medicine, University of Ioannina, Ioannina, Greece, GR; [3]Blood Donation Unit, Laikon Hospital, Athens, Greece; [4]Department of Internal Medicine, University of Athens, Laikon Hospital, Athens, Greece; [5]Biomedical Informatics Unit, Biomedical Research Foundation, Academy of Athens, Athens, Greece; [6]Platform for Genome Analytics, Institutes of Neurogenetics & Integrative and Experimental Genomics, University of Lübeck, Lübeck, Germany; [7]School of Public Health, Faculty of Medicine, Imperial College London, London, United Kingdom; [8]Institute of Neurogenetics, University of Lübeck, Lübeck, Germany; [9]Stanford Prevention Research Center, Department of Medicine and Department of Health Research and Policy, Stanford University School of Medicine, and Department of Statistics, Stanford University School of Humanities and Sciences, Stanford, USA; and [10]Department of Epidemiology and Biostatistics, Imperial College London, St. Mary's Campus, London, United Kingdom

[11]These authors contributed equally to this work.

[12]These authors contributed equally to this work.

Correspondence: Alexander J. Stratigos, 1st Department of Dermatology, University of Athens, A. Syggros Hospital, 5 I. Dragoumi Street, Athens 16121, Greece. E-mail: alstrat@hol.gr

Abbreviations: AUC, area under the receiver operating characteristic curve; CM, cutaneous melanoma; GRS, genetic risk score; GWAS, genome-wide association study; GWS, genome-wide significant; OR, odds ratio; SNP, single nucleotide polymorphism

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calculating a weighted genetic risk score (GRS) and combined this GRS with phenotypic risk factors.

RESULTS

Demographics and phenotypic traits of the 800 patients with CM and 800 control subjects are given in Supplementary Table S1 (online). Fifty-five of 59 SNPs were genotyped with call rates ≥97%. Fifty-three SNPs were considered in the final analysis, of which 26 were genome-wide significantly associated with CM based on the MelGene field synopsis and meta-analysis (Antonopoulou et al., 2015; Chatzinasiou et al., 2011) or on independent GWAS (details in Supplementary Materials online).

Association between putative risk SNPs and melanoma

Univariate logistic regression analyses assuming an additive model revealed 15 SNPs with nominally significant ($P < 0.05$) effect size estimates showing the same direction of effect as previously described (Table 1 and Supplementary Table S2 online). This included 10 previously reported GWS SNPs, specifically rs16891982, rs1805007, rs401681, rs1885120, rs4636294, and rs10931936 (Antonopoulou et al., 2015), as well as rs12918773, rs10739221, rs4778138, and rs17119490 (Barrett et al., 2011; Bishop et al., 2009; Law et al., 2015). Among the five new loci identified in the most recent GWAS meta-analysis (Law et al., 2015), the intergenic SNP with rs10739221 near TMEM38B, ZNF462, and RAD23B as well as the SNP with rs4778138 (OCA2) were also significantly associated with CM in our dataset (rs10739221: odds ratio [OR] = 1.21, $P = 0.015$; rs4778138: OR = 0.83, $P = 0.014$; Table 1) (Law et al., 2015).

The additive ORs of the eligible SNPs with melanoma risk in our study as well as the ORs reported in the original reference source are summarized in Supplementary Figure S1 (online) and Supplementary Table S2. Overall, we observed a modest correlation of our effect size estimates and those reported previously ($r^2 = 0.41$, $P = 0.038$ for the previously GWS SNPs; $r^2 = 0.34$, $P = 0.0130$ for all 53 SNPs). The correlation of risk allele frequencies between the Greek population and a set of European population derived from the 1000 Genomes (1KG) project was high ($r^2 = 0.97$) (see Supplementary Figure S2 online and Supplementary Table S3 online).

Association between GRS and melanoma

Analyses of the GRS_{GWS} yielded OR = 1.36 (95% confidence interval [CI]: 1.21−1.52) per standard deviation increase ($P = 1.1 \times 10^{-7}$). This result was similar for GRS_{ALL} [OR = 1.39 (95% CI: 1.23−1.55), $P = 3.2 \times 10^{-8}$] (see Supplementary Table S4 online). The adjusted ORs for melanoma showed a linear relationship with increasing percentiles of the GRS (trend test for GRS_{GWS} quintiles: $P = 1.4 \times 10^{-7}$, GRS_{ALL}: $P = 3.2 \times 10^{-9}$) (Figure 1 and Supplementary Table S5 online). The OR for individuals in the lowest quintile was 0.73 (95% CI: 0.50−1.05) and for participants in the highest quintile was 1.88 (95% CI: 1.29−2.74) compared with study participants in the middle quintile (see Supplementary Table S5).

The discriminative ability of GRS_{GWS} was modest, with C-statistic = 0.575 (95% CI: 0.549−0.604). When we considered traditional phenotypic risk factors only (i.e., sex, age, eye color, hair color, skin color, phototype, and tanning ability), the C-statistic was 0.764 (95% CI: 0.741−0.787). Upon combination of all genetic and phenotypic risk factors, the C-statistic including GRS_{GWS} increased to 0.775 (95% CI: 0.752−0.797, P for area under the receiver operating characteristic curve [AUC] comparison = 0.007). The results

Table 1. Statistically significant results from univariate analysis of the 53 eligible SNPs

			Univariate analysis		
SNP	***Nearest gene*[1]**	***MAF***	***P value***	***OR (95% CI)***	***Function***
rs12918773[2]	(CDK10)	0.031	1.63×10^{-6}	2.28 (1.61-3.22)	Pigmentation
rs16891982[2]	SLC45A2	0.135	3.82×10^{-6}	0.59 (0.47-0.74)	Pigmentation
rs1805007[2]	MC1R	0.024	8.22×10^{-6}	2.34 (1.59-3.43)	Pigmentation
rs11547464[2]	MC1R	0.009	1.04×10^{-4}	3.13 (1.71-5.75)	Pigmentation
rs401681[2]	CLPTM1L	0.416	2.23×10^{-4}	1.30 (1.13-1.50)	Nevi
rs12913832[2]	HERC2	0.368	7.78×10^{-4}	1.28 (1.11-1.47)	Pigmentation
rs1805005	MC1R	0.141	2.56×10^{-3}	1.34 (1.11-1.62)	Pigmentation
rs1885120	MYH7B	0.019	3.09×10^{-3}	1.94 (1.24-3.04)	Pigmentation
rs35390	SLC45A2	0.089	3.46×10^{-3}	0.67 (0.51-0.88)	Pigmentation
rs10739221[3]	(TMEM38B, ZNF462, RAD23B)	0.271	0.015	1.21 (1.04-1.41)	Intergenic locus
rs4778138[3]	OCA2	0.370	0.014	0.83 (0.72-0.96)	Pigmentation
rs3768080	NID1	0.4095	0.026	1.17 (1.02-1.35)	Basement membrane
rs10931936	CASP8	0.307	0.030	1.18 (1.02-1.37)	Apoptosis
rs17119490	LOC101927549	0.01757	0.033	1.67 (1.04-2.68)	Intergenic locus
rs4636294	MTAP	0.4044	0.030	0.85 (0.74-0.98)	Nevi

Abbreviations: CI, confidence interval; MAF, minor allelic frequency; OR, odds ratio; SNP, single nucleotide polymorphism.

[1]Nearest gene denotes the gene in the respective locus or one proximal gene in the respective locus (denoted with parenthesis) if the SNP itself does not map into a gene region. It should be noted that these genes are not necessarily the genes that are functionally affected by the genetic association finding in this locus.

[2]SNPs that survived Bonferroni correction.

[3]SNPs derived from genome-wide association study meta-analysis (Law et al., 2015) and replicated to our cohort.