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Replication of associations between GWAS SNPs and melanoma risk in the Population Architecture using Genomics and Epidemiology (PAGE) study

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To the Editor

Melanoma is a considerable public health burden, with an estimated 76,690 new diagnoses and 9,480 deaths from melanoma in the United States in 2013 alone (Howlader *et al.*, 2013). Multiplex families have pointed to important genetic factors for melanoma, including high-penetrance risk loci such as *CDKN2A* or *CDK4* (Gruber and Armstrong, 2006). In sporadic disease, genome-wide association studies (GWAS) have also successfully identified at least 8 single nucleotide polymorphisms (SNPs) associated with melanoma (Gerstenblith *et al.*, 2010). Our study aimed to replicate these existing GWAS findings within the large Population Architecture using Genomics and Epidemiology (PAGE) study in order to further evaluate their association with melanoma.

In addition to genetic factors, other risk factors for melanoma include exposure to natural and artificial ultraviolet radiation, larger numbers of nevi, pigmentation traits (light versus dark hair, eye, and skin color), race/ethnicity (European versus non-European ancestry), skin response to UV exposure (burn versus tan), older age, and male sex (Gruber and Armstrong, 2006). Anatomic location of melanoma also tends to vary by sex, arising most commonly on the back, abdomen, and chest in males, and on the lower leg, hip, and thigh in females (Gruber and Armstrong, 2006). Females also appear to have lower risk of metastases and longer melanoma-specific survival than males (Joosse *et al.*, 2011). As melanoma risk, anatomic location, and survival have been shown to vary by sex, this study also aimed to evaluate whether genetic associations with melanoma differed by sex as well.

To answer these questions, we evaluated 2,131 invasive melanoma cases and 20,353 melanoma-free controls from five study populations (Table S1). Three studies collaborated through their participation in the PAGE study (Matise *et al.*, 2011): the Multiethnic Cohort (MEC); the Women's Health Initiative (WHI); and Epidemiological Architecture for Genes Linked to Environment (EAGLE), accessing BioVU, the Vanderbilt biorepository linked to de-identified electronic medical records. Two non-PAGE studies also contributed: the Nurses' Health Study (NHS) and the Health Professionals Follow-up Study (HPFS). Additional details for these studies are provided in the Supplementary Materials. All analyses were performed using Stata version 13 (StataCorp LP, College Station, TX).

Study-specific logistic regression estimates evaluated the association between each SNP and melanoma, coded additively for each copy of the purported risk allele. These results were combined using fixed effect inverse-weighted meta-analysis to obtain overall effect estimates. The association between a SNP and melanoma was considered statistically significant if the Bonferroni-corrected p-value was below 0.006 (=0.05/8). In order to evaluate for potential sex-specific genetic effects, we also evaluated the association between each SNP and melanoma risk stratified by sex. We performed meta-regression to obtain p-heterogeneity values for the difference between sex-specific regression estimates, using a statistical significance threshold of p-heterogeneity<0.05. All participants were of European

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ancestry. HPFS is a male-only study. Since NHS and WHI are female-only studies, the overall analysis included roughly twice as many females as males (Table S1). Melanoma cases tended to be of similar or older age than controls (overall mean age of 65 in cases vs. 63 in controls), except for in EAGLE-BioVU where controls were younger (mean age 64 in cases vs. 56 in controls).

We evaluated 8 SNPs previously identified by GWAS for an association with melanoma risk (Bishop *et al.*, 2009; Brown *et al.*, 2008; Falchi *et al.*, 2009; Fernandez *et al.*, 2008; Gerstenblith *et al.*, 2010). These SNPs are in or near genes which are likely to be important to melanoma pathways through their potential impact on melanogenesis (*TYR, SLC45A2/MATP, AFG3L1P/MC1R, PIGU/ASIP*), cell cycle regulation (*CDK10*), cell growth and apoptosis (*PLA2G6*), or tumor suppression (*MTAP/CDKN2A*). Results from the meta-analyses across 3-5 studies showed 7 SNPs statistically significantly associated with melanoma at Bonferroni-corrected levels (meta-analysis $p<0.006$), while the eighth SNP was nominally significant ($p=0.02$; Table 1). All 8 SNPs showed an association in the same direction and of similar magnitude as previously reported. Six of the 7 significant SNPs showed a modest increase in melanoma risk (OR=1.17−1.55), while rs16891982 showed a much larger effect (OR=3.11).

Sex-stratified analyses showed similar results, with 4 SNPs significantly associated with melanoma in both male-only and female-only meta-analyses at Bonferroni-corrected levels, and 3 SNPs nominally associated in each (meta-analysis $p<0.05$; Table S2). Only one of these SNPs, rs16891982, showed a potential difference in effect by sex (p-heterogeneity=0.02), with a stronger association in males (OR=5.50, 95% CI: 2.94–10.28) than females (OR=2.37, 95% CI: 1.69–3.31; Table 2, Figure S1). This non-synonymous SNP in the *SLC45A2* gene has previously been associated with melanoma (Duffy *et al.*, 2010; Fernandez *et al.*, 2008; Guedj *et al.*, 2008) and pigmentation traits such as skin and hair color (Stokowski *et al.*, 2007). Also known as *MATP*, this gene encodes an ion transporter protein in the melanosome. Ion and small molecule transport is functionally important to melanogenesis and the pigmentation pathway (Scherer and Kumar, 2010), with ion exchange predicted to impact melanogenesis by playing an important role in regulating melanosome pH levels (Kondo and Hearing, 2011).

Providing biological plausibility for a potential sex difference in effect at this SNP is evidence that skin pigmentation processes can be up- or down-regulated by sex hormones. In a recent study of the hyperpigmentation condition melasma, findings supported the role of several ion transporters, including *SLC26A3*, in the estrogen-induced expression of tyrosinase (Kim *et al.*, 2012). In another study, androgens were shown to have an inhibitory effect on tyrosinase activity (Tadokoro *et al.*, 2003). Tyrosinase is considered the rate-limiting enzyme in melanin synthesis, and regulation of its activity can influence skin pigmentation through the levels of eumelanin and phenomelanin produced (Kondo and Hearing, 2011). Importantly, both tyrosinase levels and tyrosinase activity have also been associated with rs16891982 genotype (Cook *et al.*, 2009). As males and females differ in their circulating levels of sex hormones, it is possible that these hormones impact ion exchange or tyrosinase activity in a way that modifies the effect of this *SLC45A2* variant on melanoma risk, perhaps through alterations to melanogenesis or skin pigmentation.

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Interestingly, sex differences in the genetic effect of solute carrier genes have also been seen for other phenotypes, such as *LYPLAL1/SLC30A10* with waist-hip ratio (Randall *et al.*, 2013). Further research is needed to evaluate these potential sex differences in genetic contributions to melanoma risk.

This study was strengthened by the collaboration of five large studies, which provide sizable samples to evaluate the melanoma GWAS SNP association with melanoma. Limitations included two SNPs that were not available in HPFS and NHS (rs16891982 and rs910873), though both still replicated. An additional limitation is that we were unable to test whether some of our findings are independently associated with melanoma, or are due to an association with pigmentation characteristics. Additional work will be needed to explore the relationships between these genetic variants, pigmentation characteristics, and melanoma.

In summary, this large meta-analysis of five studies successfully replicated seven of eight previous melanoma findings, with the eighth SNP still showing a suggestive effect in the expected direction. Additionally, we observed potential differences in effect by sex for SNP rs16891982 in *SLC45A2*, with a larger effect in males than females. This study reinforces previous evidence that these genetic variants are important for melanoma risk, and for one SNP provides suggestive evidence for a potential sex difference in effect. These results implicate a complex interaction between genetic variants, ion transport, hormones, and pigmentation on melanoma etiology, and demonstrate the potential utility of evaluating sex-specific associations to further elucidate these relationships.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

EAGLE-BioVU	Epidemiologic Architecture of Genes Linked to Environment, accessing BioVU, the Biorepository of Vanderbilt University
GWAS	genome-wide association study
HPFS	Health Professionals Follow-up Study
MEC	Multiethnic Cohort Study
NHS	Nurses' Health Study
PAGE	Population Architecture using Genomics and Epidemiology
SNP	single nucleotide polymorphism
WHI	Women's Health Initiative

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Table 1

Meta-analysis results for the association between eight melanoma GWAS SNPs and melanoma.

SNP	Gene	Chromosome / Risk allele	n	# Studies	OR	95% CI	P-value	Study P-heterogeneity
rs258322	*CDK10*	16 / A	22,082	5	1.55	(1.41 - 1.70)	**8.54E-19**	0.62
rs4785763	*AFG3L1P* (near *MC1R*)	16 / A	21,993	5	1.31	(1.22 - 1.40)	**1.01E-14**	0.73
rs16891982	*SLC45A2* (*MATP*)	5 / G	15,949	3	3.11	(2.31 - 4.18)	**7.39E-14**	0.43
rs1393350	*TYR*	11 / A	22,009	5	1.25	(1.17 - 1.35)	**6.21E-10**	0.80
rs4636294	*MTAP* (near *CDKN2A*)	9 / A	22,053	5	1.18	(1.11 - 1.27)	**5.51E-07**	0.18
rs7023329	*MTAP* (near *CDKN2A*)	9 / A	22,114	5	1.17	(1.10 - 1.25)	**1.93E-06**	0.36
rs910873	*PIGU* (near *ASIP*)	20 / A	15,937	3	1.31	(1.15 - 1.48)	**2.46E-05**	1.00
rs2284063	*PLA2G6*	22 / G	22,087	5	1.09	(1.01 - 1.16)	0.019	0.27

Bold p-values are statistically significant for replication at a Bonferroni-corrected threshold of 0.05/8=0.006. SNPs rs16891982 and rs910873 were not available in HPFS or NHS. SNPs are ordered by p-value.

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Table 2

Sex-stratified meta-analysis of the association between rs16891982 and melanoma.

SNP	Gene	Chromosome / Risk allele	Group	n	# Studies	OR	95% CI	P-value	Study P-heterogeneity	Sex P-heterogeneity
rs16891982	*SLC45A2*	5 / G	Female	10,160	3	2.37	(1.69 - 3.31)	**4.67E-07**	0.45	0.02
			Male	5,789	2	5.50	(2.94 - 10.28)	**9.53E-08**	0.34	

Bold p-values are statistically significant for replication at a Bonferron-corrected threshold of 0.05/8=0.006. SNP rs16891982 was not available in HPFS (male only) or NHS (female only).