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Functional melanoma-risk variant *IRF4* rs12203592 associated with Breslow thickness: a pooled international study of primary melanomas

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Dear Editor

Breslow thickness is considered to be the most important prognostic tumor feature in melanoma patients and is associated with age, sex, and phenotypic risk factors for melanoma such as number of nevi. However, its association with inherited genetic variants in recently identified melanoma risk loci is largely unknown.

In a Western Australian Melanoma Health Study (WAMHS) study, published in the *British Journal of Dermatology*, *IRF4* rs12203592, *OCA2* rs1800401 and *TP53* rs1042522 were significantly associated ($P < 0.05$) with Breslow thickness; however, these associations did not pass false discovery.¹ Within the large, international population-based Genes, Environment, and Melanoma (GEM) Study, we previously investigated the association of 47 SNPs in melanoma risk loci with multiple primary melanoma occurrence and found *IRF4* rs12203592 to predict histologic markers of the hypothesized dual melanoma pathways.^{2, 3}

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In the present study, we pooled WAMHS and GEM data to investigate the association of those melanoma risk SNPs genotyped in both WAMHS and GEM with Breslow thickness adjusting for patient characteristics and potential confounding variables.

Details concerning study population and genotyping have been published previously for GEM³ and WAMHS.¹ Each study's institutional review board approved the study. Genotype information for each SNP included in this study ($n = 22$) is presented in Table S1 (see Supporting Information). In GEM, 2,458 patients of European origin were diagnosed with an incident first primary melanoma in the year 2000 (Table 1). Patient characteristics were collected via phone interviews and self-completed questionnaires. DNA was collected from buccal swab kits. SNPs were genotyped using the MassArray iPLEX platform (Sequenom, San Diego, CA) with quality control measures described previously.⁴

In WAMHS, 1,215 incident cases of cutaneous melanoma in patients of European origin with genotyping information were included for this study (Table 1). All cases were diagnosed between the years 2006 and 2010 and recruited through the Western Australian Cancer Registry (WACR). Patient characteristics were obtained from questionnaires administered by phone interview, and histopathology data were obtained from WACR records. DNA samples were extracted from peripheral blood samples and genotyping was performed on an Illumina OmniExpressExome-v1 chip, using standard quality control measures (Illumina, San Diego CA).

The left-skewed distribution of Breslow thickness was normalized using a log transformation. Within GEM, *IRF4* rs12203592*T, *CCND1* rs11263498*T, and *MX2* rs45430*G were nominally associated ($P < 0.05$) with increased Breslow thickness in linear regression models adjusted for age, sex and GEM study center (Table 2). Within WAMHS, *IRF4* rs12203592*T was also significantly associated with increased Breslow thickness adjusting for age and sex ($P = 0.005$), consistent with previous WAMHS results.¹ In a pooled analysis with both studies, *IRF4* rs12203592*T was associated with increased Breslow thickness ($\beta = 0.09$, $P = 5.47 \times 10^{-5}$) adjusting for age, sex, and study center, and was the only SNP to pass the false discovery threshold ($P = 0.0026$) adjusted for multiple comparisons using the method described by Lin *et al.*⁵ The association between rs12203592*T and Breslow thickness was similar ($\beta = 0.08$, $P = 8.66 \times 10^{-4}$) in a pooled analysis further adjusting for phenotypic characteristics associated with rs12203592 (number of nevi, hair color, eye color, ability to tan) (results not shown).

Interferon regulatory factor-4 (IRF4) is a transcription factor that regulates B- and T-cell differentiation and is a histological marker detected in hematological malignancies and melanoma.^{6, 7} rs12203592 is a functional, intronic variant affecting *IRF4* promoter activity and *IRF4* expression in immune cell lines and melanocytes.^{6, 7} In immune cells, rs12203592*T is associated with increased *IRF4* expression, which has been shown to activate the *TERT* promoter and lead to increased telomerase activity.⁸ Telomerase activity is associated with increased ulceration, mitotic rate and Breslow thickness of melanomas; however, its association with *IRF4* expression in melanomas has not been reported.⁹

Several studies have investigated the association of genetic variants in well-characterized melanoma risk loci (e.g., *MC1R*, *CDKN2A*, *VDR*) with Breslow thickness; however, the prognostic importance of inherited variants in recent GWAS-identified loci is uncertain.¹ In a hospital-based study in Spain ($n = 493$), rs12203592*T was associated with worse melanoma survival, but was not associated with Breslow thickness.¹⁰ The association between *IRF4* rs12203592 and Breslow thickness in this study is a novel finding.

Strengths of this study include its large sample size and international, population-based recruitment. Potential limitations are that not all GWAS-identified SNPs in melanoma risk loci were genotyped in both GEM and WAMHS and different genotyping platforms were used in each study. Another potential limitation is inter-study heterogeneity; however, the I^2 value for the majority of SNPs, including rs12203592, was zero (Table 2).

While future studies are warranted, these results indicate that an inherited functional variant in *IRF4* may independently predict Breslow thickness - the most important prognostic indicator for melanoma.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Cadby G, Ward S, Cole J, Moses E, Millward M, Palmer L. The association of host and genetic melanoma risk factors with Breslow thickness in the Western Australian Melanoma Health Study. *British Journal of Dermatology*. 2014; 170(4):851–7. [PubMed: 24405011]
2. Gibbs DC, Orlov I, Bramson JI, Kanetsky PA, Luo L, Kricker A, et al. Association of Interferon Regulatory Factor-4 Polymorphism rs12203592 With Divergent Melanoma Pathways. *Journal of the National Cancer Institute*. 2016; 108(7):djw004. [PubMed: 26857527]
3. Gibbs DC, Orlov I, Kanetsky PA, Luo L, Kricker A, Armstrong BK, et al. Inherited genetic variants associated with occurrence of multiple primary melanoma. *Cancer Epidemiology Biomarkers & Prevention*. 2015; 24(6):992–7.
4. Orlov I, Roy P, Reiner AS, Yoo S, Patel H, Paine S, et al. Vitamin D receptor polymorphisms in patients with cutaneous melanoma. *International journal of cancer Journal international du cancer*. 2012; 130(2):405–18. [PubMed: 21365644]
5. Lin DY. An efficient Monte Carlo approach to assessing statistical significance in genomic studies. *Bioinformatics*. 2005; 21(6):781–7. [PubMed: 15454414]
6. Visser M, Palstra R-J, Kayser M. Allele-specific transcriptional regulation of *IRF4* in melanocytes is mediated by chromatin-looping of the intronic rs12203592-enhancer to the *IRF4*-promoter. *Human molecular genetics*. 2015:ddv029.
7. Do TN, Ucisik-Akkaya E, Davis CF, Morrison BA, Dorak MT. An intronic polymorphism of *IRF4* gene influences gene transcription in vitro and shows a risk association with childhood acute lymphoblastic leukemia in males. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease*. 2010; 1802(2):292–300. [PubMed: 19897031]

8. Hrdliková R, Nehyba J, Bose HR. Regulation of telomerase activity by interferon regulatory factors 4 and 8 in immune cells. *Molecular and cellular biology*. 2009; 29(3):929–41. [PubMed: 19047367]
9. Carvalho L, Lipay M, Belfort F, Santos I, Andrade J, Haddad A, et al. Telomerase activity in prognostic histopathologic features of melanoma. *Journal of Plastic, Reconstructive & Aesthetic Surgery*. 2006; 59(9):961–8.
10. Potrony M, Rebollo-Morell A, Giménez-Xavier P, Zimmer L, Puig-Butille JA, Tell-Marti G, et al. IRF4 rs12203592 functional variant and melanoma survival. *International Journal of Cancer*. 2017; 140(8):1845–9. [PubMed: 28103633]

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Table 1

Characteristics of patients diagnosed with a first primary melanoma in the GEM Study ($n = 2,458$) and WAMHS ($n = 1,215$)^a

Characteristic	GEM Study <i>n</i> (%) ^b	WAMHS <i>n</i> (%) ^b
Age at diagnosis, years		
Median (IQR)	55 (14)	62 (17)
Sex		
Male	1274 (52)	705 (58)
Female	1184 (48)	510 (42)
Breslow thickness, mm		
Median (IQR)	0.73 (0.84)	0.60 (0.70)
0.01 to 1.00	1598 (65)	866 (72)
1.01 to 2.00	479 (19)	182 (15)
2.01 to 4.00	224 (9)	87 (7)
>4.00	124 (5)	45 (4)
Missing	33 (1)	35 (3)
Number of back nevi		
0–10	1390 (57)	611 (50)
>10	1017 (41)	579 (48)
Missing	51 (2)	25 (2)
Hair color		
Dark hair (dark brown, black)	766 (31)	398 (33)
Light hair (light brown, blonde)	1454 (59)	662 (54)
Red	212 (9)	128 (11)
Missing	26 (1)	27 (2)
Eye color		
Dark eyes (brown, black)	476 (19)	174 (14)
Light eyes (blue, grey, green, hazel)	1957 (80)	1033 (85)
Missing	25 (1)	8 (1)
Ability to tan		
Deep/moderate tan	1419 (58)	698 (57)
Mild/no tan	981 (40)	509 (42)
Missing	58 (2)	8 (1)

GEM, Genes, Environment and Melanoma; IQR, Interquartile range; WAMHS, Western Australia Melanoma Health Study.

^aLimited to whites of European origin diagnosed with a first primary melanoma.

^bMedian (IQR) reported for continuous variables; *n* (%) reported for categorical variables.

Association of genotypes with Breslow thickness in patients diagnosed with a first primary melanoma in the GEM Study ($n = 2,458$) and WAMHS ($n = 1,215$)^a

Bold type indicates P -values < 0.05 (two-sided). a, minor allele; A, major allele; CI, confidence interval; MAF, minor allele frequency; SNP, single nucleotide polymorphism.

^aLimited to whites of European origin diagnosed with a first primary melanoma.

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^b Adjusted for age (continuous), sex, and study center.

^c Adjusted for age (continuous) and sex.

^d Pooled analysis combining GEM and WAMHS for SNPs genotyped in both studies; adjusted for age (continuous), sex and study center (nine GEM centers plus WAMHS coded as one center).

^e As the outcome, Breslow thickness, was log-transformed, the values here are presented as $100 \times (e^{\text{Estimated Beta Coefficient}} - 1)$ which may be interpreted as the *percent* change in the estimated mean of Breslow thickness per minor allele.