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Association of *IRF4* SNP rs12203592 with melanoma-specific survival

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Dear Editor,

We have previously shown that the functional melanoma risk variant rs12203592 in interferon regulatory factor-4 (*IRF4*) was associated with increased Breslow thickness, an

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important prognostic indicator for melanoma.¹ In line with this, a recent study found evidence for an association between the minor T allele of this single nucleotide polymorphism (SNP) and melanoma-specific survival in patients from two European hospitals.²

We therefore sought to extend our previous work and replicate these hospital-based findings by investigating the association of rs12203592 with melanoma-specific survival, overall and by anatomic site. We used 3,303 melanoma cases of European ancestry from two large and representative population-based studies; the Genes, Environment and Melanoma (GEM) Study and the Western Australian Melanoma Health Study (WAMHS), both of which have been described in detail previously.^{3, 4}

The GEM cases (n=2,298) were diagnosed with an incident, first primary melanoma between 2000 and 2001 in Australia, Canada, Italy or the United States of America (mean follow-up=7.4 years). DNA was extracted from buccal brushes and genotyping conducted using the MassArray iPLEX chemistry and platform (Agena Bioscience, San Diego, CA, USA). Histopathology data were confirmed by pathologist's review of hematoxylin-eosin stained tumour sections.

The WAMHS cases (n=1,005) were diagnosed in Western Australia between 2006 and 2009, also with an incident, first primary melanoma (mean follow-up=8.8 years). DNA was obtained from peripheral blood samples and genotyping performed using the Illumina OmniExpressExome-v1 chip (San Diego, CA, USA). Histopathology data were obtained from pathology reports.

Cox proportional hazards regression analyses estimated the hazard ratios (HRs) and 95% confidence intervals (CIs) for the association of *IRF4* rs12203592 (per minor T-allele) with melanoma-specific survival. Analyses were conducted in each separate study sample and then in the combined GEM-WAMHS sample, with baseline adjustments made for age at diagnosis, sex and study centre. A second model also adjusted for Breslow thickness, which was log transformed to normalize the left-skewed distribution. Analyses were further stratified by anatomic site of tumour occurrence, categorized as melanoma of the head and neck, trunk or extremities.

In the combined GEM-WAMHS sample, a total of 208 melanoma-specific deaths had occurred. The male to female ratio was 1.05:1 and the mean age was 56.0 years (standard deviation=15.0 years). The mean Breslow thickness was 1.22 mm (standard deviation=1.87 mm) and the majority of individuals had either melanoma of the upper and lower limbs (42.3%) or the trunk (42.1%).

In each separate study, a significantly increased risk of melanoma-specific death was observed for each copy of the T allele in the baseline adjusted model (Table 1). In the combined sample, the HR estimate also indicated that rs12203592*T was significantly associated with increased melanoma-specific death (HR: 1.35, 95% CI: 1.09–1.67, P=0.006).

When adjusted for Breslow thickness, no associations remained significant and the HRs were attenuated (combined sample: HR: 1.12, 95% CI: 0.87–1.45, $P=0.38$). Further, no significant SNP-by-anatomic-site interactions were observed in any of the models. When stratified by anatomic site in the pooled cohort, the direction of association was the same for each site.

Sub-distribution HRs from the Fine-Gray⁵ model for competing risk also showed that rs12203592*T was significantly associated with melanoma death in the GEM-WAMHS sample, prior to adjustment for Breslow thickness (sub-distribution HR: 1.38, 95% CI: 1.10–1.72, $P=0.02$). Following adjustment, the association was just above the threshold for statistical significance (sub-distribution HR: 1.26, 95% CI: 0.99–1.59, $P=0.057$).

A mediation analysis found that 70.2% of the observed association between rs12203592*T and melanoma-specific survival was mediated through Breslow thickness, with significant natural indirect effects identified (HR: 1.13, 95% CI: 1.06–1.20, $P<0.0001$).

Our results suggest that *IRF4* rs12203592 is associated with poorer melanoma-specific survival, largely mediated through Breslow thickness, reinforcing our previous findings related to rs12203592*T and tumour thickness.¹ This variant's adverse effect on survival may occur through its functional effect on *IRF4* expression. The SNP is known to modulate *IRF4* enhancer-mediated transcriptional regulation in melanocytes, and it may play a similar role in immune cells⁶. Increased *IRF4* expression in immune cells and the subsequent activation of the TERT promoter has been shown to increase telomerase activity, which in turn has been associated with increased Breslow thickness and tumour progression.^{7, 8} In lymphocytes, rs12203592*T results in higher *IRF4* expression and it has been suggested that this may enable suppressive regulatory T cells to inhibit the immune response, enabling an acceleration in tumour growth and progression.²

In conclusion, *IRF4* rs12203592 appears to play a role in melanoma prognosis, which is seemingly mediated by the SNP's association with Breslow thickness at diagnosis. The effect on survival does not appear to be site-specific. Further studies are now required to confirm the pathway and mechanism by which this SNP affects survival, and determine the potential clinical and prognostic utility of these findings.

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Association between *IRF4* SNP rs12203592 and melanoma-specific survival, in each individual study and in a pooled sample of single primary melanoma participants from the Genes, Environment and Melanoma (GEM) Study and Western Australian Melanoma Health Study (WAMHS), (n=3,303)

Table 1:

	N total/N deaths ^a	Baseline adjusted model ^b			Fully adjusted model ^c		
		HR	95% CI	P _{trend} ^d	HR	95% CI	P _{trend} ^d
Pooled GEM and WAMHS (n=3,303)							
CC	1874 / 108	1.00	Reference		1.00	Reference	
CT	1041 / 80	1.30	(0.97, 1.74)		1.24	(0.88, 1.77)	
TT	180 / 20	1.91	(1.18, 3.11)		1.08	(0.56, 2.08)	
per T allele		1.35	(1.09, 1.67)	0.006	1.12	(0.87, 1.45)	0.38
<i>P_{interaction} =0.79 (baseline model) and P_{interaction}=0.72 (adjusted model) for SNP-by-anatomic site interaction</i>							
GEM (n=2,298)							
CC	1353/77	1.00	Reference		1.00	Reference	
CT	694 /59	1.40	(0.99, 1.97)		1.24	(0.87, 1.77)	
TT	104 / 11	1.72	(0.90, 3.28)		1.08	(0.56, 2.08)	
per T allele		1.35	(1.04, 1.75)	0.02	1.12	(0.87, 1.45)	0.38
<i>P_{interaction} =0.39 (baseline model) and P_{interaction}=0.08 (adjusted model) for SNP-by-anatomic site interaction</i>							
WAMHS (n=1,005)							
CC	521/31	1.00	Reference		1.00	Reference	
CT	347/21	1.11	(0.67, 1.85)		0.93	(0.56, 1.55)	
TT	76 / 9	2.31	(1.21, 4.44)		1.96	(1.02, 3.77)	
per T allele		1.40	(1.02, 1.95)	0.04	1.26	(0.90, 1.76)	0.17
<i>P_{interaction} =0.60 (baseline model) and P_{interaction}=0.39 (adjusted model) for SNP-by-anatomic site interaction</i>							

Abbreviations: SNP: single nucleotide polymorphism; HR: Hazard Ratio; CI: Confidence Interval

^aN total: total number of subjects, N deaths: number of melanoma-specific deaths

^bAdjusted for baseline variables: age at diagnosis (years, continuous), sex, and study centre.

^cAdjusted for baseline variables plus logged Breslow thickness (mm).

^dCox proportional hazards models estimated the per-allele HRs, 95% CIs and trend P-value. Bold type indicates statistical significance (P<0.05).