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Inherited Genetic Variants Associated with Melanoma *BRAF*/*NRAS* Subtypes

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BRAF and *NRAS* mutations arise early in melanoma development, but their associations with low-penetrance melanoma susceptibility loci remain unknown. In the Genes, Environment and Melanoma Study, 1,223 European-origin participants had their incident invasive primary melanomas screened for *BRAF*/*NRAS* mutations and germline DNA genotyped for 47 single-nucleotide polymorphisms identified as low-penetrant melanoma-risk variants. We used multinomial logistic regression to simultaneously examine each single-nucleotide polymorphism's relationship to *BRAF* V600E, *BRAF* V600K, *BRAF* other, and *NRAS*+ relative to *BRAF*−/*NRAS*− melanoma adjusted for study features. *IRF4* rs12203592*T was associated with *BRAF* V600E (odds ratio [OR] = 0.59, 95% confidence interval [CI] = 0.43−0.79) and V600K (OR = 0.65, 95% CI = 0.41−1.03), but not *BRAF* other or *NRAS*+ melanoma. A global test of etiologic heterogeneity (P_{global} = 0.001) passed false discovery (P_{global} = 0.0026). *PLA2G6* rs132985*T was associated with *BRAF* V600E (OR = 1.32, 95% CI = 1.05−1.67) and *BRAF* other (OR = 1.82, 95% CI = 1.11−2.98), but not *BRAF* V600K or *NRAS*+ melanoma. The test for etiologic heterogeneity (P_{global}) was 0.005. The *IRF4* rs12203592 associations were slightly attenuated after adjustment for melanoma-risk phenotypes. The *PLA2G6* rs132985 associations were independent of phenotypes. *IRF4* and *PLA2G6* inherited genotypes may influence melanoma *BRAF*/*NRAS* subtype development.

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Abbreviations: CI, confidence interval; GEM, Genes, Environment, and Melanoma; OR, odds ratio; SNP, single-nucleotide polymorphism; WT, wild type (BRAF−/NRAS−; without BRAF exon 15 or NRAS exon 2 and 3 mutations)

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INTRODUCTION

Genome-wide association studies and candidate pathway studies have identified low-penetrant genetic variants associated with melanoma risk. Many of these variants are in gene regions associated with pigmentation, such as *TYRP1, TYR, HERC2/OCA2, SLC45A2,* and *ASIP*; nevi, such as *PLA2G6, MTAP,* and *NID1*; or both, such as *IRF4*, whereas others are in genes, including *ATM, MX2, PARP1, ARNT,* and *CASP8*, not associated with melanoma-risk phenotypes (Amos et al., 2011; Barrett et al., 2011; Bishop et al., 2009; Fernandez et al., 2008; Gudbjartsson et al., 2008; Han et al., 2008; Jannot et al., 2005; Law et al., 2012; Macgregor et al., 2011; Nan et al., 2011; Zhang et al., 2012). In parallel but separate studies, we and Hacker et al. found that the increased number of nevi was associated with melanoma *BRAF* V600E and V600K subtypes (Hacker et al., 2016; Thomas et al., 2007, 2017), and we found that *BRAF* V600E was associated with blond and/or light brown hair and *BRAF* V600K with less freckling (Thomas et al., 2017). *MC1R* has been inconsistently associated with *BRAF* V600E cases (Fargnoli et al., 2008; Hacker et al., 2010, 2013, 2016; Landi et al., 2006; Thomas et al., 2010a). Our recent work indicates that *MC1R* variants are positively associated with *BRAF* V600E cases in people with darker phenotypes, but inversely associated with *BRAF* V600K cases with no significant effect modification by phenotype (Thomas et al., 2017). To our knowledge, the associations between melanoma *BRAF/NRAS* subtypes and other low-penetrant genetic variants besides *MC1R* have not been investigated.

We studied low-penetrant melanoma-risk variants in the Genes, Environment and Melanoma (GEM) Study, a large international population-based study of incident melanoma (Begg et al., 2006; Millikan et al., 2006), in relationship to melanoma *BRAF/NRAS* subtypes. Participants' germline DNA was genotyped for 47 single-nucleotide polymorphisms (SNPs) from 21 distinct genomic regions, and their invasive primary melanomas were analyzed for *BRAF* and *NRAS* mutations. For each SNP, we used multinomial logistic regression to simultaneously assess its association with *BRAF* V600E, *BRAF* V600K, *BRAF* other, and *NRAS*+, compared with wild-type (WT; *BRAF*−/*NRAS*−) melanoma adjusted for study features: age at diagnosis, sex, study center, and whether first- or higher-order primary melanoma.

RESULTS

Subject characteristics

In GEM, 1,223 participants of European origin had their incident cutaneous invasive primary melanomas analyzed for *BRAF* and *NRAS* mutations. The median age was 60 years; 59.5% were male; and 61.5% were from Australia and 38.5% from the United States (Table 1). These 1,223 melanomas (all from different patients) were from 908 patients (74.2%) who had only one melanoma at the time of recruitment (a first primary melanoma) and 315 patients (25.8%) who had more than one melanoma at the time of recruitment. For the latter group of patients, we retrieved and utilized for these analyses the 315 second- or higher-order primary melanomas that prompted their recruitment into GEM. The melanomas were 17.9% *BRAF* V600E, 5.6% *BRAF* V600K, 2.9% *BRAF* other, 13.5% *NRAS*+, and 60.2% WT. Each of these mutations was exclusive of the others. The predominant subtype was superficial spreading melanoma (67.9%). The median Breslow thickness was 0.70 mm. Of the 1,223 participants, 24.7% had 0−4, 21.7% had 11−25, and 17.9% had >25 nevi on their back. Blond or light brown was the natural hair color of a majority (63.3%) of the participants; 9.1% had red hair. A few freckles were found in 44.4% of the participants; 12.6% had many.

Table 1. Characteristics of 1,223 participants with incident primary invasive cutaneous melanoma analyzed for *BRAF/NRAS* subtype in the GEM Study[1]

Characteristic	No. (%)
Age at diagnosis, y	
Median (IQR)	60 (24)
Sex	
Male	728 (59.5)
Female	495 (40.5)
Country	
North America	471 (38.5)
Australia	752 (61.5)
Lesion status	
First primary melanoma	908 (74.2)
Second- or higher-order primary melanoma	315 (25.8)
NRAS/BRAF subtype	
BRAF+	322 (26.3)
BRAF V600E	219 (17.9)
BRAF V600K	68 (5.6)
BRAF other[2]	35 (2.9)
NRAS+	165 (13.5)
WT *(BRAF−/NRAS−)*	736 (60.2)
Breslow thickness, mm	
Median (IQR), mm	0.70 (0.83)
Histologic subtype	
Superficial spreading melanoma	830 (67.9)
Nodular melanoma	104 (8.5)
Lentigo maligna melanoma	184 (15.0)
Other	105 (8.6)
No. of back nevi	
0−4	430 (35.7)
5−10	297 (24.7)
11−25	261 (21.7)
>25	215 (17.9)
Hair color	
Dark brown/black	337 (27.6)
Blonde/light brown	772 (63.3)
Red	111 (9.1)
Freckling	
None	505 (43.0)
Few	521 (44.4)
Many	148 (12.6)

Abbreviations: GEM, Genes, Environment and Melanoma; IQR, interquartile range; WT, wild type.
[1]Limited to individuals of European origin. Counts may not sum to the total number of study subjects due to missing data.
[2]*BRAF* other included L584F (n = 1), D594G (n = 2), D594N (n = 6), L597K (n = 1), L597R (n = 1), L597S (n = 1), V600D (n = 4), V600R (n = 9), K601E (n = 5), K601N (n = 2), R603Q (n = 1), G606E (n = 1), and the compound deletion VKS600-602D (n = 1).

Relationship of inherited genetic variants to *BRAF/NRAS* subtypes

Details of those successfully genotyped for each of the 47 SNPs—number of participants (out of 1,223), chromosomal location, minor/major alleles, minor allele frequency—are reported in Supplementary Table S1 online.

For each SNP, associations with melanoma subtypes are given in Table 2. *IRF4* rs12203592 was associated with *BRAF/NRAS* mutational subtype (P_{global} = 0.001), and this association passed the false discovery threshold (P_{global} = 0.0026). The odds ratio (ORs) for the association of rs12203592*T were 0.59 (95% confidence interval [CI] = 0.43−0.79) for *BRAF* V600E, 0.65 (95% CI = 0.41−1.03) for *BRAF* V600K, 1.57 (95% CI = 0.93−2.65) for *BRAF* other, and 0.99 (95% CI = 0.75−1.30) for *NRAS*+, relative to WT melanoma.

PLA2G6 rs132985 and rs738322 had low *P*-values for their associations with *BRAF/NRAS* mutational subtype (P_{global} = 0.005 and 0.02, respectively), which did not pass the false discovery threshold. rs132985*T was associated with *BRAF* V600E (OR = 1.32, 95% CI = 1.05−1.67) and *BRAF* other (OR = 1.82, 95% CI = 1.11−2.98), but not *BRAF* V600K or *NRAS*+ melanoma. Similarly, rs738322*G was associated with *BRAF* V600E (OR = 1.28, 95% CI = 1.02−1.60) and *BRAF* other (OR = 1.66, 95% CI = 1.01−2.72), but not *BRAF* V600K or *NRAS*+ melanoma. In a stepwise logistic regression model including these two SNPs and adjusting for study features, rs132985 remained associated with melanoma mutational subtype and was selected for further modeling.

Of the haplotypes examined for the genes with at least two SNPs genotyped belonging to the same haplotype block, no haplotypes reached global significance

Table 2. Association of SNPs with melanoma *BRAF/NRAS* subtypes in the GEM Study[1]

				Compared with WT melanoma				
				***BRAF* V600E**	***BRAF* V600K**	***BRAF* other**	***NRAS*+**	**Global**
Chrom	**Gene region**	**SNP**	**a/A**	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	***P*-value**
1	*ARNT*	rs7412746	C/T	1.12 (0.89–1.40)	1.00 (0.70–1.43)	0.65 (0.38–1.11)	1.11 (0.87–1.42)	0.37
1	*PARP1*	rs3219090	A/G	1.00 (0.78–1.27)	1.03 (0.70–1.51)	0.78 (0.45–1.35)	0.99 (0.76–1.30)	0.93
1	*PARP1*	rs2695238	C/G	0.96 (0.75–1.23)	0.90 (0.61–1.32)	0.69 (0.39–1.23)	1.02 (0.78–1.32)	0.75
1	*NID1*	rs3768080	G/A	1.06 (0.83–1.33)	1.03 (0.72–1.47)	0.90 (0.55–1.48)	1.01 (0.79–1.29)	0.98
1	*NID1*	rs10754833	C/T	1.03 (0.81–1.30)	0.99 (0.70–1.42)	0.87 (0.53–1.43)	1.01 (0.79–1.29)	0.98
2	*CASP8*	rs6735656	G/T	1.08 (0.84–1.40)	1.28 (0.87–1.87)	1.70 (1.02–2.81)	1.11 (0.85–1.46)	0.23
2	*CASP8*	rs13016963	A/G	1.04 (0.82–1.31)	1.00 (0.69–1.43)	1.52 (0.95–2.44)	0.90 (0.70–1.15)	0.39
5	*TERT*	rs2242652	T/C	1.06 (0.80–1.42)	1.26 (0.82–1.93)	1.12 (0.61–2.07)	0.69 (0.49–0.97)	0.14
5	*TERT*	rs2853676	A/G	0.98 (0.76–1.25)	1.25 (0.86–1.81)	0.96 (0.56–1.65)	0.91 (0.69–1.18)	0.69
5	*TERT*	rs13356727	G/A	0.95 (0.75–1.20)	0.65 (0.45–0.94)	0.87 (0.53–1.41)	0.92 (0.72–1.18)	0.24
5	*TERT;CLPTM1L*	rs4975616	G/A	0.95 (0.75–1.22)	0.60 (0.41–0.89)	0.85 (0.50–1.44)	0.94 (0.73–1.22)	0.15
5	*TERT;CLPTM1L*	rs401681	T/C	1.00 (0.79–1.26)	0.60 (0.42–0.88)	0.74 (0.45–1.22)	0.96 (0.75–1.23)	0.08
5	*SLC45A2*	rs16891982	C/G	0.84 (0.40–1.79)	*Nonestimable*	*Nonestimable*	0.41 (0.10–1.69)	0.81
5	*SLC45A2*	rs35391	T/C	0.54 (0.19–1.56)	*Nonestimable*	*Nonestimable*	0.65 (0.16–2.63)	0.83
5	*SLC45A2*	rs26722	T/C	0.74 (0.22–2.53)	*Nonestimable*	*Nonestimable*	0.90 (0.19–4.16)	0.99
5	*SLC45A2*	rs13289	G/C	0.97 (0.77–1.22)	0.68 (0.46–0.99)	0.71 (0.43–1.18)	0.79 (0.62–1.01)	0.09
6	*IRF4*	rs12203592	T/C	0.59 (0.43–0.79)	0.65 (0.41–1.03)	1.57 (0.93–2.65)	0.99 (0.75–1.30)	**0.001**
6	*IRF4*	rs872071	A/G	1.04 (0.83–1.31)	1.17 (0.82–1.67)	0.51 (0.30–0.89)	1.03 (0.81–1.32)	0.14
9	*TYRP1*	rs1408799	T/C	1.04 (0.82–1.33)	1.13 (0.78–1.63)	0.84 (0.49–1.46)	0.86 (0.66–1.12)	0.66
9	*TYRP1*	rs2733832	C/T	0.97 (0.77–1.22)	1.10 (0.77–1.56)	0.98 (0.60–1.61)	0.99 (0.77–1.27)	0.98
9	*MTAP*	rs2218220	T/C	0.90 (0.71–1.13)	0.79 (0.55–1.12)	0.87 (0.54–1.43)	0.85 (0.66–1.08)	0.48
9	*MTAP*	rs1335510	G/T	0.87 (0.69–1.10)	0.75 (0.51–1.08)	0.94 (0.58–1.54)	0.86 (0.67–1.11)	0.41
9	*MTAP*	rs7023329	G/A	0.91 (0.72–1.14)	0.95 (0.66–1.35)	1.03 (0.64–1.68)	0.92 (0.72–1.18)	0.90
9	*MTAP*	rs10811629	G/A	0.84 (0.67–1.06)	0.83 (0.58–1.19)	0.88 (0.53–1.44)	1.07 (0.84–1.36)	0.43
11	*CCND1*	rs11604821	G/A	0.92 (0.72–1.17)	0.89 (0.61–1.30)	0.77 (0.45–1.30)	0.95 (0.73–1.23)	0.82
11	*CCND1*	rs1485993	T/C	0.94 (0.75–1.19)	0.91 (0.63–1.31)	0.75 (0.44–1.27)	0.95 (0.74–1.22)	0.83
11	*CCND1*	rs11263498	T/C	0.92 (0.73–1.16)	0.88 (0.61–1.28)	0.65 (0.38–1.13)	0.95 (0.74–1.23)	0.57
11	*TYR*	rs1042602	A/C	1.26 (1.00–1.59)	1.09 (0.76–1.58)	1.61 (0.99–2.62)	1.00 (0.78–1.29)	0.14
11	*TYR*	rs10765198	C/T	0.93 (0.74–1.17)	1.13 (0.79–1.61)	0.91 (0.54–1.53)	1.04 (0.81–1.33)	0.88
11	*TYR*	rs1847142	A/G	0.97 (0.78–1.22)	1.03 (0.72–1.47)	0.83 (0.49–1.39)	0.97 (0.76–1.25)	0.96
11	*TYR*	rs10830253	G/T	1.01 (0.80–1.27)	1.06 (0.74–1.52)	0.83 (0.49–1.39)	1.00 (0.78–1.29)	0.96
11	*ATM*	rs12278954	A/C	0.98 (0.72–1.34)	0.87 (0.53–1.45)	1.03 (0.53–2.00)	0.89 (0.63–1.26)	0.96
15	*OCA2*	rs1800407	A/G	1.03 (0.68–1.55)	0.80 (0.40–1.62)	1.38 (0.65–2.92)	1.36 (0.92–2.00)	0.48
15	*OCA2*	rs1800401	T/C	0.94 (0.54–1.63)	0.73 (0.26–2.01)	0.34 (0.05–2.48)	0.71 (0.36–1.38)	0.68
15	*HERC2*	rs1129038	G/A	0.82 (0.61–1.09)	0.98 (0.64–1.51)	1.35 (0.78–2.34)	0.90 (0.66–1.21)	0.44
15	*HERC2*	rs12913832	A/G	0.80 (0.60–1.07)	1.02 (0.67–1.56)	1.34 (0.78–2.31)	0.84 (0.62–1.14)	0.31
20	*ASIP*	rs17305657	C/T	0.87 (0.60–1.27)	1.34 (0.80–2.24)	0.80 (0.34–1.90)	0.80 (0.52–1.21)	0.49
20	*ASIP*	rs4911414	T/G	1.00 (0.79–1.28)	1.22 (0.85–1.76)	1.45 (0.88–2.38)	1.09 (0.85–1.41)	0.50
20	*PIGU*	rs910873	A/G	0.87 (0.60–1.26)	1.02 (0.60–1.76)	0.73 (0.32–1.64)	1.03 (0.71–1.49)	0.87
20	*PIGU*	rs17305573	C/T	0.84 (0.58–1.24)	1.07 (0.62–1.84)	0.77 (0.34–1.74)	0.98 (0.67–1.44)	0.87
20	*NCOA6*	rs4911442	G/A	0.89 (0.65–1.23)	1.16 (0.73–1.84)	0.60 (0.28–1.28)	0.88 (0.63–1.24)	0.54
20	*MYH7B*	rs1885120	C/G	0.79 (0.53–1.17)	1.07 (0.61–1.87)	0.84 (0.37–1.90)	0.94 (0.64–1.39)	0.78
20	*LOC647979*	rs1204552	A/T	0.91 (0.60–1.37)	0.67 (0.33–1.36)	0.96 (0.43–2.18)	0.75 (0.47–1.17)	0.62
21	*MX2*	rs45430	G/A	0.91 (0.71–1.15)	0.83 (0.56–1.21)	0.41 (0.22–0.78)	0.95 (0.74–1.23)	0.07
22	*PLA2G6*	rs6001027	G/A	1.11 (0.87–1.42)	0.99 (0.67–1.45)	1.91 (1.14–3.20)	0.85 (0.64–1.11)	0.06
22	*PLA2G6*	rs132985	T/C	1.32 (1.05–1.67)	1.12 (0.78–1.60)	1.82 (1.11–2.98)	0.83 (0.64–1.07)	**0.005**
22	*PLA2G6*	rs738322	G/A	1.28 (1.02–1.60)	1.09 (0.76–1.55)	1.66 (1.01–2.72)	0.84 (0.66–1.08)	**0.02**

Abbreviations: a, minor allele; A, major allele; Chrom, chromosome; CI, confidence interval; GEM, Genes, Environment and Melanoma; OR, odds ratio; SNP, single-nucleotide polymorphism; WT, wild type (*BRAF*−/*NRAS*−).

[1]Limited to individuals of European origin. Bold type indicates *P*-values < 0.05. We used multinomial logistic regression to estimate the ORs and 95% CIs with somatic *BRAF/NRAS* mutational subtypes simultaneously compared with WT melanoma, adjusted for study features: age at diagnosis (continuous), sex, study center, and whether first- or higher-order primary melanoma. The per-allele OR (based on the minor allele) and *P*-values referring to the global test for etiologic heterogeneity are provided.

Table 3. *IRF4* rs12203592 and *PLA2G6* rs132985 associations with melanoma *BRAF/NRAS* subtypes adjusted for study features and then also adjusted for potential phenotypic mediators[1]

	Compared with WT melanoma				
	***BRAF* V600E**	***BRAF* V600K**	***BRAF* other**	***NRAS*+**	
SNP	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	**Global *P*-value**
IRF4 rs12203592*T[2]					
Adjusted for study features[3]	0.61 (0.45–0.83)	0.64 (0.40–1.03)	1.67 (0.98–2.84)	1.01 (0.76–1.34)	**0.002**
Adjusted for study features and nevi	0.62 (0.45–0.85)	0.69 (0.42–1.11)	1.69 (0.98–2.91)	1.06 (0.79–1.41)	**0.003**
Adjusted for study features and hair color	0.65 (0.47–0.90)	0.60 (0.36–0.99)	1.71 (0.99–2.96)	0.97 (0.72–1.30)	**0.006**
Adjusted for study features and freckling	0.63 (0.46-0.87)	0.78 (0.48–1.28)	1.57 (0.90–2.72)	1.01 (0.75–1.36)	**0.02**
Adjusted for study features, nevi, hair color, and freckling[4]	0.68 (0.48–0.96)	0.79 (0.46–1.34)	1.52 (0.84–2.75)	0.99 (0.72–1.35)	0.10
PLA2G6 rs132985*T[5]					
Adjusted for study features[3]	1.31 (1.04–1.66)	1.10 (0.76–1.59)	1.94 (1.17–3.21)	0.83 (0.64–1.07)	**0.004**
Adjusted for study features and nevi	1.32 (1.04–1.67)	1.10 (0.76–1.59)	1.93 (1.17–3.21)	0.84 (0.65–1.08)	**0.006**
Adjusted for study features and hair color	1.32 (1.04–1.67)	1.11 (0.77–1.61)	1.96 (1.19–3.25)	0.83 (0.65–1.08)	**0.004**
Adjusted for study features and freckling	1.31 (1.03–1.66)	1.10 (0.76–1.59)	1.95 (1.17–3.25)	0.83 (0.64–1.07)	**0.005**
Adjusted for study features, nevi, hair color, and freckling[4]	1.32 (1.04–1.67)	1.10 (0.76–1.60)	1.99 (1.20–3.32)	0.84 (0.65–1.09)	**0.005**

Abbreviations: CI, confidence interval; GEM, Genes, Environment and Melanoma; OR, odds ratio; WT, wild type (*BRAF*−/*NRAS*−).

[1]Bold type indicates *P*-values < 0.05. We used multinomial logistic regression to estimate the ORs and 95% CIs with *BRAF/NRAS* mutational subtypes simultaneously compared with WT melanoma. The per-allele OR (based on the minor allele) and *P*-values referring to the global test for etiologic heterogeneity are provided.

[2]Limited to 1,136 individuals of European origin who had no missing data for the *IRF4* rs12203592 genotype or the phenotypes. The distribution of the melanoma subtypes was WT (n = 682), *BRAF* V600E (n = 199), *BRAF* V600K (n = 64), *BRAF* other (n = 33), and *NRAS*+ (n = 158). Participants with one or more data points missing for rs12203592 genotype (n = 24), number of back nevi (n = 20), hair color (n = 3), or freckling (n = 49) were excluded.

[3]Adjusted for study features: age at diagnosis (continuous), sex, study center, and whether first- or higher-order primary melanoma.

[4]Adjusted for study features and phenotype: number of back nevi (0–4, 5–10, 11–25, >25), hair color (dark brown/black, blonde/light brown, red), and freckling (none, few, many).

[5]Limited to 1,139 individuals of European origin who had no missing data for the *PLA2G6* rs132985 genotype or the phenotypes. The distribution of the melanoma subtypes was WT (n = 683), *BRAF* V600E (n = 203), *BRAF* V600K (n = 63), *BRAF* other (n = 33), and *NRAS*+ (n = 157). Participants with one or more data points missing for rs132985 genotype (n = 21), number of back nevi (n = 20), hair color (n = 3), or freckling (n = 49) were excluded.

(Supplementary Table S2 online). Evaluation of the haplotype blocks and linkage disequilibrium patterns for *IRF4* using both the GEM data and the Hapmap CEU population revealed that the two *IRF4* SNPs were in different haplotype blocks; therefore, haplotype analysis did not apply to *IRF4*.

We had previously found that a number of back nevi, hair color, and freckling were associated with *BRAF/NRAS* subtype (Thomas et al., 2017), indicating that these phenotypes could be mediators of genotype associations with *BRAF/NRAS* subtype. To further explore this possibility, we examined, in participants with tumor *BRAF/NRAS* subtype analyzed, whether *IRF4* rs12203592 or *PLA2G6* rs132985 genotypes were associated with these phenotypes in models adjusted for study features (Supplementary Tables S3 online). rs12203592*T was significantly associated with fewer back nevi, darker hair color, and less freckling (all $P_{global} < 0.001$). rs132985 was not significantly associated with back nevi, hair color, or freckling.

Next, we built multivariable models for each SNP that included these three phenotypes, first examining the association of each SNP with *BRAF/NRAS* subtype adjusted for study features, and then adding back nevi, hair color, and freckling, separately and then together to the models (Table 3). In the models for *IRF4* rs12203592, the ORs for all three *BRAF* subtypes were attenuated as the phenotypes were progressively added to the model; in the model that included all factors, the global test for etiologic heterogeneity was not significant ($P_{global} = 0.10$). When examining the associations of *PLA2G6* rs132985 with *BRAF/NRAS* subtypes, the OR of rs132985*T for *BRAF* V600E remained the same or similar in all the models. The OR of rs132985*T for *BRAF* other exon 15 mutations increased from 1.94 in the model adjusted for study features only to 1.99 in the fully adjusted model. The global tests for etiologic heterogeneity remained nominally significant.

DISCUSSION

Passing false discovery, *IRF4* rs12203592*T was inversely associated with melanoma carrying *BRAF* V600E and V600K somatic mutations relative to WT melanoma. We, like others (Duffy et al., 2010a, 2010b; Han et al., 2008; Zhang et al., 2013), found rs12203592*T to be associated with fewer nevi, darker hair color, and increased freckles. Previously, we reported that increased nevi were associated with *BRAF* V600E and V600K; lighter hair color with *BRAF* V600E; and decreased freckling with *BRAF* V600K compared with WT melanoma (Thomas et al., 2017). Hacker et al. (2016) also found increased nevi to be associated with *BRAF* V600E and V600K vs. WT melanoma. Thus, these associations are in the directions expected for potential mediation by these phenotypes of the associations of rs12203592 with *BRAF* subtypes. The *IRF4* rs12203592 associations with *BRAF* subtypes were attenuated after adjustment for these three phenotypes, and these results suggest that a substantial portion of the impact of rs12203592 is mediated through these phenotypes or their underlying genotypes.

We report here positive associations of *PLA2G6* rs132985*T with somatic *BRAF* V600E and *BRAF* other somatic mutations relative to WT melanoma. The literature supports that rs132985*C is positively associated with both nevus counts and melanoma risk (Duffy et al., 2017; Falchi et al., 2009; Fang et al., 2013; Kvaskoff et al., 2011). Falchi et al. (2009) explored whether nevus count mediated the association between rs132985 and melanoma and reported attenuation of the melanoma OR when adding nevus count to their model. Because of these findings and *BRAF* V600's known association with increased nevi (Hacker et al., 2016; Thomas et al., 2007, 2017), it might have been expected that *PLA2G6* rs132985*C would have been positively associated with *BRAF* subtypes. However, instead, we found a positive association with rs132985*T. Also, the associations of rs132985 with *BRAF* subtypes reported here in GEM were overall independent of the number of nevi, hair color, and freckling. Thus, the evidence provided here indicates that the associations of rs132985 with *BRAF* subtypes are not mediated by phenotypes. Possibly, the associations could instead be mediated by the reported apoptotic effects of the *PLA2G6* gene (Akiba and Sato, 2004).

Our study's strengths are its population-based design, large sample size, and rigorous mutational analysis of *BRAF* and *NRAS* mutations. The study limitations include low numbers of *BRAF* V600K and *BRAF* other subtypes, limiting statistical power. It is possible that the power of the study was insufficient to detect the associations of some of the other SNPs tested with *BRAF/NRAS* subtypes. Also, we investigated a limited number of melanoma-risk–associated genotypes and others remain to be tested in relationship to *BRAF/NRAS* subtypes (Duffy et al., 2017; Iles et al., 2013; Law et al., 2015). Our findings remain to be replicated.

Our results provide a link between the genetics of the person and somatic genetic data in melanoma to gain an understanding of how the genetics of both the person and the tumor interact. Our findings suggest roles for inherited *IRF4* and *PLA2G6* polymorphisms in the development of *BRAF/NRAS* melanoma subtypes and that these roles have different underlying mechanisms. Although our work indicates that *IRF4*'s associations are mediated by specific phenotypes, further investigation of factors underlying *PLA2G6*'s associations with subtype is needed. Larger studies or pooled analyses and studies including more inherited melanoma-risk variants may provide additional insight into the development of melanoma molecular subtypes, further defining their risk factors and providing information that may lead toward improved prevention of this complex disease.

MATERIALS AND METHODS

Study population

Details concerning the GEM study population, genotyping, and *BRAF/NRAS* mutational subtyping have been published previously (Begg et al., 2005; Gibbs et al., 2015, 2016, 2017; Thomas et al., 2015, 2017; Vernali et al., 2017). Patient characteristics were collected via phone interviews and self-completed questionnaires (Kricker et al., 2007; Thomas et al., 2007, 2010b). We collected patients' self-reported number of back nevi counted by a family member or friend, a measure that has been reported in other studies as predictive of total body nevus counts (Autier et al., 2001; English and Armstrong, 1994; English et al., 1988). GEM's 3,579 participants had first- or higher-order primary melanoma diagnosed between 1998 and 2003 in Australia, Canada, Italy, and the United States (Begg et al., 2004, 2006; Millikan et al., 2006; Murali et al., 2012; Orlow et al., 2007). The institutional review board at each participating site approved the study protocol. Study participants provided written informed consent. Diagnostic slides were reviewed centrally for histopathologic criteria (Thomas et al., 2010b, 2013, 2014, 2015). We sought tissue sections from 2,116 participants' first- or higher-order incident invasive primary melanomas diagnosed in New South Wales (Australia), California, North Carolina, and Michigan. Of these 2,116 GEM participants, 1,227 (58%) had formalin-fixed, paraffin-embedded melanoma tissues obtained and analyzed for *BRAF* exon 15 (including codon 600) and *NRAS* exon 2 and 3 (including codons 61, 12, and 13) mutations using single-strand conformational polymorphism analysis and radiolabeled sequencing of single-strand conformational polymorphism-positive samples (Thomas et al., 2007, 2015). Of these 1,227 patients, we limited the analyses presented here to the 1,223 participants of European origin. We previously reported the associations of *BRAF/NRAS* subtypes with age, sex, tumor characteristics, and survival for 912 GEM first primaries (Thomas et al., 2015), with phenotype and *MC1R* for 1,227 participants (Thomas et al., 2017), and with age, sex, and phenotype in 214 GEM first primaries from North Carolina (Thomas et al., 2007) and 88 from Michigan (Poynter et al., 2006).

Genotyping

We selected 47 SNPs from 21 loci based on evidence that they were low-penetrant risk variants for melanoma in other studies (Amos et al., 2011; Barrett et al., 2011; Bishop et al., 2009; Fernandez et al., 2008; Gudbjartsson et al., 2008; Han et al., 2008; Jannot et al., 2005; Law et al., 2012; Macgregor et al., 2011; Nan et al., 2011; Zhang et al., 2012). DNA was extracted from buccal swab kits (Begg et al., 2005). SNPs were genotyped using the MassArray iPLEX platform (Agena Bioscience, San Diego, CA) with quality control measures described previously (Orlow et al., 2012). *CASP8* rs10931936 and *ATM* rs1801516 (Barrett et al., 2011) were not compatible with the platform design, and proxy SNPs rs6735656 and rs12278954, respectively ($r^2 > 0.95$), were chosen (1000 Genomes, CEU population; Proxy SNP; Broad Institute).

Statistical analysis

The melanomas were grouped as *BRAF* V600E, *BRAF* V600K, *BRAF* other (exon 15 mutations besides V600E and V600K), *NRAS*+ (exon 2 or 3 mutation), or WT (wild-type negative for these mutations). Assuming an additive model of inheritance of the minor allele for each SNP, multinomial logistic regression models were used to estimate simultaneously the OR and 95% CI with *BRAF* V600E, *BRAF* V600K, *BRAF* other, and *NRAS*+, compared with WT (*BRAF*–/*NRAS*–) melanoma adjusted for study features: age at diagnosis (continuous), sex, study center, whether first or higher order primary. Some analyses were also adjusted for phenotypes. Statistical significance was assessed using Wald tests. The false discovery threshold adjusted for multiple comparisons was computed using a resampling method that takes into account the linkage disequilibrium information among SNPs evaluated and is less conservative than the classical Bonferroni procedure (He et al., 2013; Lin, 2005).

For the genes with at least two SNPs genotyped, we first determined their haplotype blocks using the Haploview software algorithm, as previously described (Gibbs et al., 2015). Each haplotype or grouped rare haplotypes were then compared with the most

common haplotypes in our study population. Some associations could not be examined because of low genotype minor allele frequencies or infrequent haplotypes in some subtype categories and noted as nonestimable in the tables. For the two nominally significantly associated SNPs in the *PLA2G6* locus, we applied stepwise logistic regression to determine the SNP with the stronger association keeping study features fixed.

We examined the relationship between the significantly associated genotypes and *BRAF/NRAS* subtype for potential mediation by phenotypes (back nevi, hair color, and freckling) associated in GEM with *BRAF/NRAS* subtype (Thomas et al., 2017). Using multinomial logistic regression adjusted for study features, we estimated the associations of the genotypes with these phenotypes limited to participants of European origin who had no missing data for the genotype, *BRAF/NRAS* subtype, or these phenotypes. Next, we used multivariable models for each SNP to examine its associations with *BRAF/NRAS* subtypes, adding the phenotypes separately and then together to models. All analytic models were adjusted for study features. All tests were two-sided with $P < 0.05$ considered statistically significant. All data were analyzed using SAS 9.4 (Cary, NC) or R (http://www.r-project.org/) programs.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2018.04.025.

REFERENCES

Akiba S, Sato T. Cellular function of calcium-independent phospholipase A2. Biol Pharm Bull 2004;27:1174–8.

Amos CI, Wang LE, Lee JE, Gershenwald JE, Chen WV, Fang S, et al. Genome-wide association study identifies novel loci predisposing to cutaneous melanoma. Hum Mol Genet 2011;20:5012–23.

Autier P, Boniol M, Severi G, Giles G, Cattaruzza MS, Luther H, et al. The body site distribution of melanocytic naevi in 6-7 year old European children. Melanoma Res 2001;11:123–31.

Barrett JH, Iles MM, Harland M, Taylor JC, Aitken JF, Andresen PA, et al. Genome-wide association study identifies three new melanoma susceptibility loci. Nat Genet 2011;43:1108–13.

Begg CB, Hummer A, Mujumdar U, Armstrong BK, Kricker A, Marrett LD, et al. Familial aggregation of melanoma risks in a large population-based sample of melanoma cases. Cancer Causes Control 2004;15:957–65.

Begg CB, Hummer AJ, Mujumdar U, Armstrong BK, Kricker A, Marrett LD, et al. A design for cancer case-control studies using only incident cases: experience with the GEM study of melanoma. Int J Epidemiol 2006;35:756–64.

Begg CB, Orlow I, Hummer AJ, Armstrong BK, Kricker A, Marrett LD, et al. Lifetime risk of melanoma in CDKN2A mutation carriers in a population-based sample. J Natl Cancer Inst 2005;97:1507–15.

Bishop DT, Demenais F, Iles MM, Harland M, Taylor JC, Corda E, et al. Genome-wide association study identifies three loci associated with melanoma risk. Nat Genet 2009;41:920–5.

Duffy D, Zhu G, Li X, Sanna M, Iles M, Jacobs LC, et al. Novel pleiotropic risk loci for melanoma and nevus density implicate multiple biological pathways. bioRxiv 2017. https://doi.org/10.1101/173112. Accessed 25 May 2018.

Duffy DL, Iles MM, Glass D, Zhu G, Barrett JH, Hoiom V, et al. IRF4 variants have age-specific effects on nevus count and predispose to melanoma. Am J Hum Genet 2010a;87:6–16.

Duffy DL, Zhao ZZ, Sturm RA, Hayward NK, Martin NG, Montgomery GW. Multiple pigmentation gene polymorphisms account for a substantial proportion of risk of cutaneous malignant melanoma. J Invest Dermatol 2010b;130:520–8.

English DR, Armstrong BK. Melanocytic nevi in children. I. Anatomic sites and demographic and host factors. Am J Epidemiol 1994;139:390–401.

English JS, Swerdlow AJ, Mackie RM, O'Doherty CJ, Hunter JA, Clark J, et al. Site-specific melanocytic naevus counts as predictors of whole body naevi. Br J Dermatol 1988;118:641–4.

Falchi M, Bataille V, Hayward NK, Duffy DL, Bishop JA, Pastinen T, et al. Genome-wide association study identifies variants at 9p21 and 22q13 associated with development of cutaneous nevi. Nat Genet 2009;41:915–9.

Fang S, Han J, Zhang M, Wang LE, Wei Q, Amos CI, et al. Joint effect of multiple common SNPs predicts melanoma susceptibility. PLoS One 2013;8:e85642.

Fargnoli MC, Pike K, Pfeiffer RM, Tsang S, Rozenblum E, Munroe DJ, et al. MC1R variants increase risk of melanomas harboring BRAF mutations. J Invest Dermatol 2008;128:2485–90.

Fernandez LP, Milne RL, Pita G, Aviles JA, Lazaro P, Benitez J, et al. SLC45A2: a novel malignant melanoma-associated gene. Hum Mutat 2008;29:1161–7.

Gibbs DC, Orlow I, Bramson JI, Kanetsky PA, Luo L, Kricker A, et al. Association of interferon regulatory factor-4 polymorphism rs12203592 with divergent melanoma pathways. J Natl Cancer Inst 2016;108:djw004.

Gibbs DC, Orlow I, Kanetsky PA, Luo L, Kricker A, Armstrong BK, et al. Inherited genetic variants associated with occurrence of multiple primary melanoma. Cancer Epidemiol Biomarkers Prev 2015;24:992–7.

Gibbs DC, Ward SV, Orlow I, Cadby G, Kanetsky PA, Luo L, et al. Functional melanoma-risk variant IRF4 rs12203592 associated with Breslow thickness: a pooled international study of primary melanomas. Br J Dermatol 2017;177:e180–2.

Gudbjartsson DF, Sulem P, Stacey SN, Goldstein AM, Rafnar T, Sigurgeirsson B, et al. ASIP and TYR pigmentation variants associate with

cutaneous melanoma and basal cell carcinoma. Nat Genet 2008;40:886–91.

Hacker E, Hayward NK, Dumenil T, James MR, Whiteman DC. The association between MC1R genotype and BRAF mutation status in cutaneous melanoma: findings from an Australian population. J Invest Dermatol 2010;130:241–8.

Hacker E, Nagore E, Cerroni L, Woods SL, Hayward NK, Chapman B, et al. NRAS and BRAF mutations in cutaneous melanoma and the association with MC1R genotype: findings from Spanish and Austrian populations. J Invest Dermatol 2013;133:1027–33.

Hacker E, Olsen CM, Kvaskoff M, Pandeya N, Yeo A, Green AC, et al. Histologic and Phenotypic Factors and MC1R Status Associated with BRAF(V600E), BRAF(V600K), and NRAS Mutations in a Community-Based Sample of 414 Cutaneous Melanomas. J Invest Dermatol 2016;136:829–37.

Han J, Kraft P, Nan H, Guo Q, Chen C, Qureshi A, et al. A genome-wide association study identifies novel alleles associated with hair color and skin pigmentation. PLoS Genet 2008;4:e1000074.

He Q, Avery CL, Lin DY. A general framework for association tests with multivariate traits in large-scale genomics studies. Genet Epidemiol 2013;37:759–67.

Iles MM, Law MH, Stacey SN, Han J, Fang S, Pfeiffer R, et al. A variant in FTO shows association with melanoma risk not due to BMI. Nat Genet 2013;45:428–32, 32e1.

Jannot AS, Meziani R, Bertrand G, Gerard B, Descamps V, Archimbaud A, et al. Allele variations in the OCA2 gene (pink-eyed-dilution locus) are associated with genetic susceptibility to melanoma. Eur J Hum Genet 2005;13:913–20.

Kricker A, Armstrong BK, Goumas C, Litchfield M, Begg CB, Hummer AJ, et al. Ambient UV, personal sun exposure and risk of multiple primary melanomas. Cancer Causes Control 2007;18:295–304.

Kvaskoff M, Whiteman DC, Zhao ZZ, Montgomery GW, Martin NG, Hayward NK, et al. Polymorphisms in nevus-associated genes MTAP, PLA2G6, and IRF4 and the risk of invasive cutaneous melanoma. Twin Res Hum Genet 2011;14:422–32.

Landi MT, Bauer J, Pfeiffer RM, Elder DE, Hulley B, Minghetti P, et al. MC1R germline variants confer risk for BRAF-mutant melanoma. Science 2006;313:521–2.

Law MH, Bishop DT, Lee JE, Brossard M, Martin NG, Moses EK, et al. Genome-wide meta-analysis identifies five new susceptibility loci for cutaneous malignant melanoma. Nat Genet 2015;47:987–95.

Law MH, Montgomery GW, Brown KM, Martin NG, Mann GJ, Hayward NK, et al. Meta-analysis combining new and existing data sets confirms that the TERT-CLPTM1L locus influences melanoma risk. J Invest Dermatol 2012;132:485–7.

Lin DY. An efficient Monte Carlo approach to assessing statistical significance in genomic studies. Bioinformatics 2005;21:781–7.

Macgregor S, Montgomery GW, Liu JZ, Zhao ZZ, Henders AK, Stark M, et al. Genome-wide association study identifies a new melanoma susceptibility locus at 1q21.3. Nat Genet 2011;43:1114–8.

Millikan RC, Hummer A, Begg C, Player J, de Cotret AR, Winkel S, et al. Polymorphisms in nucleotide excision repair genes and risk of multiple primary melanoma: the Genes Environment and Melanoma Study. Carcinogenesis 2006;27:610–8.

Murali R, Goumas C, Kricker A, From L, Busam KJ, Begg CB, et al. Clinicopathologic features of incident and subsequent tumors in patients with multiple primary cutaneous melanomas. Ann Surg Oncol 2012;19:1024–33.

Nan H, Xu M, Zhang J, Zhang M, Kraft P, Qureshi AA, et al. Genome-wide association study identifies nidogen 1 (NID1) as a susceptibility locus to cutaneous nevi and melanoma risk. Hum Mol Genet 2011;20:2673–9.

Orlow I, Begg CB, Cotignola J, Roy P, Hummer AJ, Clas BA, et al. CDKN2A germline mutations in individuals with cutaneous malignant melanoma. J Invest Dermatol 2007;127:1234–43.

Orlow I, Roy P, Reiner AS, Yoo S, Patel H, Paine S, et al. Vitamin D receptor polymorphisms in patients with cutaneous melanoma. Int J Cancer 2012;130:405–18.

Poynter JN, Elder JT, Fullen DR, Nair RP, Soengas MS, Johnson TM, et al. BRAF and NRAS mutations in melanoma and melanocytic nevi. Melanoma Res 2006;16:267–73.

Thomas NE, Busam KJ, From L, Kricker A, Armstrong BK, Anton-Culver H, et al. Tumor-infiltrating lymphocyte grade in primary melanomas is independently associated with melanoma-specific survival in the population-based genes, environment and melanoma study. J Clin Oncol 2013;31:4252–9.

Thomas NE, Edmiston SN, Alexander A, Groben PA, Parrish E, Kricker A, et al. Association between NRAS and BRAF mutational status and melanoma-specific survival among patients with higher-risk primary melanoma. JAMA Oncol 2015;1:359–68.

Thomas NE, Edmiston SN, Alexander A, Millikan RC, Groben PA, Hao H, et al. Number of nevi and early-life ambient UV exposure are associated with BRAF-mutant melanoma. Cancer Epidemiol Biomarkers Prev 2007;16:991–7.

Thomas NE, Edmiston SN, Kanetsky PA, Busam KJ, Kricker A, Armstrong BK, et al. Associations of MC1R genotype and patient phenotypes with BRAF and NRAS mutations in melanoma. J Invest Dermatol 2017;137:2588–98.

Thomas NE, Kanetsky PA, Edmiston SN, Alexander A, Begg CB, Groben PA, et al. Relationship between germline MC1R variants and BRAF-mutant melanoma in a North Carolina population-based study. J Invest Dermatol 2010a;130:1463–5.

Thomas NE, Kricker A, From L, Busam K, Millikan RC, Ritchey ME, et al. Associations of cumulative sun exposure and phenotypic characteristics with histologic solar elastosis. Cancer Epidemiol Biomarkers Prev 2010b;19:2932–41.

Thomas NE, Kricker A, Waxweiler WT, Dillon PM, Busam KJ, From L, et al. Comparison of clinicopathologic features and survival of histopathologically amelanotic and pigmented melanomas: a population-based study. JAMA Dermatol 2014;150:1306–14.

Vernali S, Waxweiler WT, Dillon PM, Kanetsky PA, Orlow I, Luo L, et al. Association of incident amelanotic melanoma with phenotypic characteristics, MC1R status, and prior amelanotic melanoma. JAMA Dermatol 2017;153:1026–31.

Zhang M, Song F, Liang L, Nan H, Zhang J, Liu H, et al. Genome-wide association studies identify several new loci associated with pigmentation traits and skin cancer risk in European Americans. Hum Mol Genet 2013;22:2948–59.

Zhang W, Chen Y, Yang X, Fan J, Mi X, Wang J, et al. Functional haplotypes of the hTERT gene, leukocyte telomere length shortening, and the risk of peripheral arterial disease. PLoS One 2012;7:e47029.