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ARTICLE AVAILABLE ONLINE
Twin Research and Human Genetics
Volume 14 ▮ Number 5 ▮ pp. 422–432

Polymorphisms in Nevus-Associated Genes *MTAP*, *PLA2G6*, and *IRF4* and the Risk of Invasive Cutaneous Melanoma

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An evolving hypothesis postulates that melanomas may arise through 'nevus-associated' and 'chronic sun exposure' pathways. We explored this hypothesis by examining associations between nevus-associated loci and melanoma risk across strata of body site and histological subtype. We genotyped 1028 invasive case patients and 1469 controls for variants in methylthioadenosine phosphorylase (*MTAP*), phospholipase A2, group VI (*PLA2G6*), and Interferon regulatory factor 4 (*IRF4*), and compared allelic frequencies globally and by anatomical site and histological subtype of melanoma. Odds-ratios (ORs) and 95% confidence intervals (CIs) were calculated using classical and multinomial logistic regression models. Among controls, *MTAP* rs10757257, *PLA2G6* rs132985 and *IRF4* rs12203592 were the variants most significantly associated with number of nevi. In adjusted models, a significant association was found between *MTAP* rs10757257 and overall melanoma risk (OR = 1.32, 95% CI = 1.14–1.53), with no evidence of heterogeneity across sites ($P_{homogeneity}$ =.52). In contrast, *MTAP* rs10757257 was associated with superficial spreading/nodular melanoma (OR = 1.34, 95% CI = 1.15–1.57), but not with lentigo maligna melanoma (OR = 0.79, 95% CI = 0.46–1.35) ($P_{homogeneity}$ =.06), the subtype associated with chronic sun exposure. Melanoma was significantly inversely associated with rs12203592 in children (OR = 0.35, 95% CI = 0.16–0.77) and adolescents (OR = 0.61, 95% CI = 0.42–0.91), but not in adults ($P_{homogeneity}$ =.0008). Our results suggest that the relationship between *MTAP* and melanoma is subtype-specific, and that the association between *IRF4* and melanoma is more evident for cases with a younger age at onset. These findings lend some support to the 'divergent pathways' hypothesis and may provide at least one candidate gene underlying this model. Further studies are warranted to confirm these findings and improve our understanding of these relationships.

■ **Keywords:** cutaneous melanoma, epidemiology, genes, nevi, polymorphisms

Melanoma develops through complex effects of both environmental and genetic factors (Miller & Mihm, 2006). Its main risk factors include ultraviolet radiation (UVR), pigmentation, and nevus count (MacKie et al., 2009). Childhood UVR exposure is a significant risk factor for immediate development of nevi, and for subsequent melanoma, but this is modulated by host constitution, anatomical site, and adult UVR exposure. The 'divergent pathways' model suggests two potential pathways for melanoma development: in people with high nevus counts, melanomas tend to develop at younger ages and on body sites with high nevus counts, such as the trunk ('nevus pathway'), whereas in people with lesser tendencies for melanocytic proliferation, melanomas tend to arise at later ages, and on body sites with high cumulative UVR exposure, such as the head and neck ('sun exposure pathway'; Whiteman et al., 2003). There is increasing evi-

RECEIVED 01 July, 2011; ACCEPTED 04 July, 2011.

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Kvaskoff, M., Whiteman, D. C., Zhao, Z. Z., Montgomery, G. W., Martin, G. W., Hayward, N. K., & Duffy, D. L. (2011). Polymorphisms in nevus-associated genes *MTAP, PLA2G6*, and *IRF4* and the risk of invasive cutaneous melanoma. *Twin Research and Human Genetics, 14,* 422–432. DOI 10.1375/twin.14.5.422

dence from epidemiological and molecular analyses to support this model of etiological heterogeneity of cutaneous melanomas, with anatomical site being an important source of observed heterogeneity (Broekaert et al., 2010; Curtin et al., 2005; Edlundh-Rose et al., 2006; Lachiewicz et al., 2008; Lang & MacKie, 2005; Maldonado et al., 2003; Thomas et al., 2007; Viros et al., 2008). Given that nevus count is significantly more strongly associated with melanomas arising on the trunk than on the head and neck (Whiteman et al., 2003), and given that nevus burden is strongly heritable (Wachsmuth et al., 2001; Zhu et al., 1999), it is plausible to speculate that the risks of melanoma conferred by nevus-associated genotypes might differ according to the anatomical site of the lesion.

Through genome-wide association studies (GWAS), we (Falchi et al., 2009) and others (Bishop et al., 2009) have recently identified a number of genes for which common variants were shown to predict nevus count. One of these loci, methylthioadenosine phosphorylase (*MTAP;* 9p21), was found to associate strongly with nevus count in Caucasian populations in Australia and the United Kingdom (Bishop et al., 2009; Falchi et al., 2009); the locus was also significantly associated with melanoma risk in these populations. Phospholipase A2, group VI (*PLA2G6*; 22q13) was similarly associated with nevus counts and melanoma risk in the UK study (Falchi et al., 2009), and Interferon regulatory factor 4 (*IRF4*; 6p25-p23), while associated with skin, hair and eye color (Duffy et al., 2010a; Han et al., 2008), only weakly affected melanoma risk (Duffy et al., 2010a). In recent work, we demonstrated that *IRF4* variants have a strong effect on nevus count (Duffy et al., 2010b), suggesting that the gene needs to be more closely examined as a potential melanoma susceptibility locus.

In this study, we assess these three loci to test and further refine the 'divergent pathways' hypothesis for melanoma. We investigate site- and subtype-specific risks of melanoma in relation to genotype of *MTAP*, *PLA2G6* and *IRF4* variants using data from large Australian population-based samples.

Material and Methods

STUDY POPULATION

We conducted a case-control analysis comprising a sample of melanoma patients from the Queensland study of Melanoma: Environmental and Genetic Associations (Q-MEGA) with controls from the Brisbane Twin Nevus Study (BTNS).

The Q-MEGA is described in detail elsewhere (Baxter et al., 2008). Briefly, this study gathered four population-based samples of Queensland residents who were diagnosed with histologically confirmed melanoma over 1987–1995. The largest panel is a collection of adult cases diagnosed over 1982–1990 ($n = 1619$). The other panels of patients comprise children ($n = 50$), adolescents ($n = 142$), and men over 50 years ($n = 71$); melanomas diagnosed before the age of 20 years were thus intentionally oversampled. The participants were followed-up through a computer-assisted telephone interview in 2002–2005, where updated self-reported data on phenotypic risk factors were obtained as well as blood samples.

The BTNS is an ongoing study initiated in 1994 that includes a sample of adolescent twins and their family members (Zhu et al., 2007). For the present study, the parents of the twins served as healthy controls, for whom self-reported phenotype data and blood samples were also collected. These controls were indeed sampled from the same source population (i.e., Queensland residents).

DATA COLLECTION

Q-MEGA participants self-reported their skin color at age 20 (fair/pale, medium, or olive/dark), natural hair color at age 20 (fair/blonde, light brown, red, dark brown, or black), eye color (blue/grey, green/hazel, or brown), freckling during childhood (none, light, moderate, or heavy), and number of nevi (none, < 10, 10–50, or > 50). BTNS twin parents self-reported pigmentary characteristics using virtually identical scales and nevus count was assessed using a 4-point pictorial scale with descriptors of 'none', 'a few', 'moderate' and 'many' nevi. In addition, in both cases and controls, ancestry was measured via questions about the country of birth and ancestry of each of the grandparents of the participants. Grandparental ancestry could be reported as a mixture of origins. Since all subjects were of European origin, we have constructed an ancestry score based on the proportion of grandparents of Northern European (British, Scandinavian, Danish, Dutch, German, French) descent. Values for the score ranged from 0–100% and were categorized as < 50%, 50–74%, 75–99%, or 100%.

GENOTYPING

Participants were genotyped in multiplex assays using the Sequenom MassARRAY Assay Design software (version 3.0) for variants of *MTAP*, *PLA2G6* and *IRF4* genes, as described previously. DNA samples were available for 73.0% of cases and 81.2% of controls. In cases, individuals with available genotype information did not significantly differ from those with no available genotype information with respects to age, sex, pigmentary characteristics and ancestry (see Supplementary Table 1 online). Among controls, a higher proportion of females (i.e., mothers of twins) than males (fathers) gave a blood sample ($P < .0001$), and more genotype data were available for people with fair skin ($P < .0001$) and higher northern European ancestry score ($P = .0002$).

Single nucleotide polymorphisms (SNPs) were typed using iPLEX™ Gold chemistry on a MALDI-TOF Mass Spectrometer (Sequenom Inc, San Diego). PCR reactions were carried out in 2.5 μL in standard 384-well plates with 10ng genomic DNA, 0.5 unit of *Taq* poly-

merase (HotStarTaq, Qiagen, Valencia, CA), 500 µmol of each dNTP, and 100 nmol of each PCR primer. PCR thermal cycling was 15 min at 94°C, followed by 45 cycles of 20 sec at 94°C, 30 sec at 56°C, 60 sec at 72°C. To the completed PCR reaction, 1 µL containing 0.15 units Shrimp Alkaline Phosphatase was added and the reaction incubated for 30 min at 37°C followed by inactivation for 5 min at 85°C. After adjusting the concentrations of extension primers to equilibrate signal-to-noise ratios, the post-PCR primer extension reaction of the iPLEX assay was performed in a final 5 µL volume extension reaction containing 0.1 µL of termination mix, 0.02 µL of DNA polymerase (Sequenom, San Diego, CA), and 600 nM to 1200 nM extension primers. A two-step 200 short cycles program was used for the iPLEX reaction: initial denaturation was 30 sec at 94°C followed by five cycles of 5 sec at 52°C and 5 sec at 80°C. An additional 40 annealing and extension cycles were then looped back to 5 sec at 94°C, 5 sec at 52°C and 5 sec at 80°C. The final extension was carried out at 72°C for 3 minutes and the sample was cooled to 20°C. The iPLEX reaction products were desalted by diluting samples with 15 µL of water and adding 3 µL of resin, then centrifuged to remove the resin. The products were spotted on a SpectroChip (Sequenom Inc, San Diego), processed and analyzed in a Compact Mass Spectrometer by MassARRAY Workstation (version 3.3) software (Sequenom Inc, San Diego). This assay is extremely accurate and reproducible: for the *IRF4* rs12203592, we repeated the genotyping and encountered 4 inconsistencies out of 1453 (0.3%).

POPULATION FOR ANALYSIS

Among the 1894 cases in Q-MEGA, we excluded tumors with a metastatic ($n = 9$) or unknown ($n = 4$) behavior, *in situ* cases ($n = 297$), and patients for whom genotype data were not available (n = 558). Of the 2302 controls, subjects with missing information on number of nevi ($n = 206$) were excluded, as well as those with no available information on genotype for the studied genes ($n = 627$). The final sample for analysis included 2497 participants, comprising 1028 invasive cases and 1469 controls.

STATISTICAL ANALYSES

We estimated odds-ratios (ORs) and 95% confidence intervals (CIs) using classical and multinomial logistic regression models. For each gene, we first selected for detailed analysis the SNP most significantly associated with nevus category in controls. We explored the relationship between the minor allele for these SNPs and melanoma risk first globally, and then according to anatomical site (trunk, head and neck, upper limbs, or lower limbs). In separate analyses, we assessed subtype-specific risk of melanoma (superficial spreading melanoma (SSM)/ nodular melanoma (NM), lentigo maligna melanoma (LMM), and 'other' melanomas including those not otherwise specified) in relation to genotype for the selected SNPs. In all analyses, we additionally explored the relationships between nevus category and melanoma risk.

We performed chi-square tests to assess deviations in genotype frequencies from Hardy-Weinberg equilibrium (HWE) in all participants. Only *IRF4* rs12203592 deviated from HWE in controls ($P = .02$) (see Supplementary Table 2 online). Given the high reproducibility of our genotyping assays, the HW disequilibrium for this SNP is unlikely to be due to assay problems, but rather to population structure. We adjusted all analyses for degree of northern European ancestry, which was based on the reported ancestry of the participants and represents the proportion of the participants' grandparents reported to derive their ancestry from northern Europe.

We computed allelic ORs adjusted for sex and quartiles of age (age at diagnosis in cases, age at interview for the controls; < 37.8, 37.8–43.0, 43.1–48.9, ≥ 49.0 years). To control for a potential population bias and to ensure that the studied associations are not due to population structure, we further adjusted for northern European ancestry score (< 50%, 50–74%, 75–99%, or 100%), and nevus category, freckling, skin color, eye color, and hair color using forward stepwise regression models. Since results were not substantially modified when models were adjusted for age and sex only, we only present those arising from crude and fully adjusted models. We then assessed site- and subtype-specific melanoma risk in relation to nevus category.

We also performed chi-square tests to assess potential differences in allelic frequencies between cases and controls, as well as homogeneity tests to compare estimates according to anatomical site and histological subtype of melanoma (Hosmer & Lemeshow, 2000). For all adjustment factors, data were missing for fewer than 5% of subjects and missing data were imputed to the modal category. We checked that the results were not modified when missing data were excluded instead of being imputed. Statistical analyses were performed using the SAS statistical package (version 9.2).

Results

Ages of cases and controls were similar (Table 1). Cases were more likely than controls to be male and to have northern European ancestry, light hair, skin and eye color, freckling, and high nevus counts. Table 2 describes risk allele frequencies for all gene variants in controls, and according to site and type of melanoma in cases (full genotype frequencies are described in Supplementary Table 3 online). Among controls, *MTAP* rs10757257, *PLA2G6* rs132985 and *IRF4* rs12203592 were the variants most significantly associated with nevus category ($P = .01$, $P = .02$, and $P < .0001$, respectively) (see Supplementary Table 4 online) and were thus chosen for further analysis.

TABLE 1

Characteristics of the Study Participants, Q-MEGA (1987–2005) (*n* = 2497)

	Cases (*n* = 1028) *n* (%) or mean (*SD*)	Controls (*n* = 1469) *n* (%) or mean (*SD*)	*p* value[a]
Age (years)[b]	41.8 (16.3)	43.6 (9.9)	.11
Sex			
Male	484 (47.1%)	632 (43.0%)	.04
Female	544 (52.9%)	837 (57.0%)	
Skin color			
Fair or pale	841 (81.8%)	830 (56.5%)	< .0001
Medium	155 (15.1%)	504 (34.3%)	
Olive or dark	32 (3.1%)	135 (9.2%)	
Hair color			
Fair/blonde	246 (23.9%)	244 (16.6%)	< .0001
Light brown	407 (39.6%)	533 (36.3%)	
Red	133 (12.9%)	73 (5.0%)	
Dark brown/Black	242 (23.6%)	619 (42.1%)	
Eye color			
Blue or grey	437 (42.5%)	596 (40.6%)	.0008
Green or hazel	421 (41.0%)	541 (36.8%)	
Brown	170 (16.5%)	332 (22.6%)	
Freckling			
None	203 (19.8%)	396 (27.0%)	< .0001
Light/A few	420 (40.9%)	445 (30.3%)	
Moderate	287 (27.9%)	354 (24.1%)	
Heavy/many	117 (11.5%)	274 (18.6%)	
Number of nevi			
None	87 (8.5%)	83 (5.6%)	< .0001
< 10/A few	391 (38.0%)	907 (61.7%)	
10–50/Moderate	412 (40.1%)	393 (26.8%)	
> 50/Many	138 (13.4%)	86 (5.9%)	
Ancestry score			
< 50%	12 (1.2%)	77 (5.2%)	< .0001
50–74%	13 (1.2%)	32 (2.2%)	
75–99%	45 (4.4%)	50 (3.4%)	
100%	958 (93.2%)	1310 (89.2%)	

Note: [a]Chi-square tests or t tests were performed in order to compare cases and controls according to the presented characteristics
[b]Age at diagnosis in cases, age at interview in controls.

ASSOCIATION BETWEEN GENE VARIANTS AND MELANOMA

Global Melanoma Risk

In all models, associations in the adult sample were very close to those observed in the whole study sample (Table 3). In adjusted models, we found a significantly positive association between *MTAP* rs10757257*G and melanoma risk in the adult sample (OR = 1.32, 95% CI = 1.14–1.54) and a marginally significant positive association in the older men sample (OR = 1.41, 95% CI = 0.99–2.02). Associations between *MTAP* rs10757257*G and melanoma risk were positive in the children and adolescents sample. These were not statistically significant (children: OR = 1.27, 95% CI = 0.79–2.05; adolescents: OR = 1.22, 95% CI = 0.91–1.63), but we detected no significant heterogeneity of risk factors across the four case groups ($P_{homogeneity} = 0.52$). There was no significant association between *PLA2G6* rs132985*C and melanoma risk. Regarding *IRF4* rs12203592*T, while there was no evidence of an association between this polymorphism and melanoma in the adult and the older men samples, this allele was inversely associated with melanoma in the children and adolescents (children: OR = 0.35, 95% CI = 0.16–0.77; adolescents: OR = 0.61, 95% CI = 0.42–0.91). These differences in effect between the younger and older cases were statistically significant ($P_{homogeneity} = .0008$). Also, we found significantly positive dose-effect relationships between nevus category and melanoma risk in all ($P_{trend} < .0001$) but the older men sample ($P_{trend} = .68$), and results were stronger in the children and the adolescents samples ($P_{homogeneity} < .0001$).

For completeness, we also analyzed the available SNPs that were not originally selected for further study, and the results were similar to those presented for the selected SNPs (see Supplementary Table 5 online). In addition, we examined the linkage disequilibrium patterns between SNPs in each of the studied loci in the control sample (see Supplementary Table 6 online). Correlation coefficients (r^2) were above 0.9 between rs4636294 and rs2218220, rs1335510 and rs1341866, rs1335510 and rs10757257, and rs1341866 and rs10757257 for *MTAP*; between rs2284063 and rs6001027, and rs132985 and rs738322 for *PLA2G6*; and above 0.8 between rs2292383 and rs17825664 for *IRF4*.

Site-Specific Risk of Melanoma

Within the whole study sample, we found significant associations between *MTAP* rs10757257*G and risk of melanoma of the trunk (OR = 1.26, 95% CI = 1.04–1.53), melanoma of the upper limbs (OR = 1.35, 95% CI = 1.08–1.69), and melanoma of the lower limbs (OR = 1.38, 95% CI = 1.11–1.71) (Table 4). There was no significant difference across sites ($P_{homogeneity} = 0.52$); specifically, we observed no heterogeneity between melanoma of the trunk and melanoma of the head and neck ($P_{homogeneity} = 0.70$).

Overall, no association was found between the rs132985*C allele and site-specific melanoma risk. However, in crude models, there was a significant association between *PLA2G6* rs132985*C and melanoma on the upper limbs (OR = 1.26, 95% CI = 1.04–1.54) which became non-significant after adjustment.

In crude models, patients with melanoma on the trunk were significantly less likely to carry the *IRF4* rs12203592*T allele compared with controls (OR = 0.73, 95% CI = 0.60–0.90). However, these associations were no longer statistically significant after adjustment, and no other associations were found between rs12203592 and site-specific melanoma risk.

We found significantly positive dose-response relationships between nevus propensity and risk of melanoma on the trunk, and lower and upper limbs ($P_{trend} < .0001$). For melanoma on the head and neck, risks were significantly elevated with a moderate number of nevi, although somewhat attenuated for the highest nevus category (OR = 1.44, 95% CI = 0.61–3.38). The overall trend for head and neck melanoma remained strongly significant, however

TABLE 2

Risk Allele Frequencies for *MTAP*, *PLA2G6* and *IRF4* Variants in Cases and Controls, Q-MEGA (1987–2005) (*n* = 2462)

			Cases (*n* = 993)[a]											
			Trunk (*n* = 371)			Head and neck (*n* = 120)			Upper limbs (*n* = 236)			Lower limbs (*n* = 266)		
		Controls (*n* = 1469)	SSM/NM (*n* = 277)	LMM (*n* = 5)	Other[b] (*n* = 89)	SSM/NM (*n* = 74)	LMM (*n* = 14)	Other[b] (*n* = 32)	SSM/NM (*n* = 165)	LMM (*n* = 8)	Other[b] (*n* = 63)	SSM/NM (*n* = 215)	LMM (*n* = 3)	Other[b] (*n* = 48)
MTAP														
rs4636294	A	0.48	0.53	0.30	0.53	0.56	0.50	0.59	0.55	0.50	0.55	0.56	0.50	0.51
rs2218220	C	0.48	0.52	0.30	0.53	0.56	0.50	0.59	0.55	0.50	0.55	0.56	0.50	0.51
rs7023329	A	0.49	0.53	0.10	0.52	0.56	0.54	0.59	0.57	0.50	0.52	0.55	0.50	0.52
rs10757257	G	0.58	0.63	0.30	0.63	0.66	0.57	0.61	0.64	0.56	0.64	0.65	0.50	0.63
rs751173	G	0.46	0.50	0.30	0.49	0.55	0.61	0.61	0.52	0.56	0.49	0.51	0.50	0.46
rs1335510	T	0.58	0.62	0.40	0.61	0.66	0.54	0.64	0.63	0.56	0.64	0.65	0.50	0.63
rs1341866	A	0.58	0.62	0.40	0.62	0.66	0.57	0.61	0.63	0.56	0.63	0.64	0.50	0.63
rs10811629	A	0.56	0.61	0.38	0.61	0.64	0.50	0.56	0.60	0.50	0.60	0.63	0.50	0.61
PLA2G6														
rs2284063	A	0.64	0.64	0.70	0.69	0.67	0.72	0.63	0.68	0.68	0.69	0.63	0.67	0.64
rs6001027	A	0.64	0.64	0.70	0.69	0.67	0.61	0.63	0.68	0.56	0.69	0.63	0.67	0.64
rs132985	C	0.52	0.54	0.70	0.58	0.58	0.50	0.55	0.59	0.44	0.57	0.53	0.67	0.52
rs738322	A	0.52	0.54	0.70	0.57	0.58	0.50	0.47	0.58	0.44	0.57	0.53	0.67	0.50
IRF4														
rs2797307	G	0.97	0.98	1.00	0.97	0.97	1.00	0.95	0.97	1.00	0.98	0.96	1.00	0.98
rs12203592	T	0.23	0.18	0.20	0.17	0.23	0.25	0.19	0.21	0.38	0.24	0.17	0.17	0.29
rs2671422	G	0.89	0.89	0.80	0.91	0.86	0.82	0.91	0.90	0.94	0.89	0.89	0.83	0.91
rs2292383	C	0.06	0.07	0.20	0.08	0.10	0.11	0.03	0.06	0.00	0.07	0.06	0.50	0.10
rs17825664	C	0.05	0.05	0.30	0.06	0.09	0.11	0.03	0.04	0.00	0.08	0.06	0.17	0.06

Note: [a]Melanomas for which site was not specified were not reported in this table (n = 35).
[b]In the total population of invasive cases (n = 1028), the 238 melanomas from this category included 234 melanomas not otherwise specified, 2 amelanotic melanomas, and 2 desmoplastic melanomas.

(P_{trend} = .0002). Results in the highest nevus category differed significantly between melanoma on the trunk and melanoma on the head and neck ($P_{homogeneity}$ =.04).

Subtype-Specific Risk of Melanoma

There were significantly positive associations between *MTAP* rs10757257*G and superficial spreading melanoma (SSM)/nodular melanoma (NM) (OR = 1.34, 95% CI = 1.15–1.57) and 'other' types (OR = 1.33, 95% CI = 1.06–1.66), but not lentigo maligna melanoma (LMM) (OR = 0.79, 95% CI = 0.46–1.35) ($P_{homogeneity}$ = .06) (Table 5).

In crude models, SSM/NM and 'other' melanoma patients were more likely to be *PLA2G6* rs132985*C carriers than controls (OR = 1.14, 95% CI = 1.01–1.30; OR = 1.21, 95% CI=0.99–1.47; respectively). However, in adjusted models, we found no significant association between rs132985 and melanoma risk by subtype.

While a significant inverse relationship was found between *IRF4* rs12203592*T and SSM/NM in unadjusted models (OR = 0.80, 95% CI = 0.68–0.93), this result was no longer significant in adjusted models, and no other significant association was found.

As expected, we found significantly positive dose-response relationships between nevus category and melanoma in SSM/NM and 'other' melanomas. However, there was no significant or consistent trend between nevus category and LMM risk (P_{trend} = .24), with marginally significant elevation in risk with the moderate category of nevus, but not the highest category (OR = 0.92, 95% CI = 0.12–7.33).

Discussion

Within a large population-based sample of melanoma patients from Australia, we confirm significant associations between variants of *MTAP*, *PLA2G6* and *IRF4* with the propensity to develop nevi, as well as a significant association between *MTAP* rs10757257 and melanoma risk.

Importantly, while we found no evidence that the relationship between *MTAP* rs10757257 and melanoma varied according to anatomical site of the tumor, we did observe marginally significant differences in the magnitude of association by histological subtype. Specifically, risk alleles of *MTAP* rs10757257 were more common among patients with SSM/NM subtypes than among controls, whereas patients with LMM, the subtype associated with chronic sun exposure (Duncan, 2009), were no more likely than controls to harbor these alleles.

Although some crude associations were found for the selected *PLA2G6* and *IRF4* variants with melanoma of the upper limbs and of the trunk, respectively, and with the SSM/NM subtype, adjusted models showed no significant associations between these variants and melanoma risk, globally or by anatomical site or histological subtype. However, we found that children and adolescents were significantly less likely than controls to harbor the *IRF4* rs12203592*T allele.

A recent GWAS performed in a sample of UK and Australian patients showed a significant association between *MTAP* and *PLA2G6* variants and nevi, with lead SNPs (rs4636294 and rs2284063) that were different from

TABLE 3

Odds-Ratios and 95% Confidence Intervals for Risk of Cutaneous Melanoma in Relation to Type of Allele for Selected Gene Variants and Nevus Category According to Subsample, Q-MEGA (1987–2005) (n = 2497)

	Controls (n = 1469)	All cases (n = 1028)		Adults (n = 819)		Children (n = 36)		Adolescents (n = 102)		Men over 50 years (n = 69)		p value[b]
	Prop.	Prop.	Adjusted OR[a] (95% CI)	Prop	Adjusted OR_a, (95% CI)	Prop.	Adjusted OR[a] (95% CI)	Prop.	Adjusted OR[a] (95% CI)	Prop.	Adjusted OR[a] (95% CI)	
MTA rs10757257 G (A)	0.58	0.64	1.32 (1.14–1.53)	0.63	1.32 (1.14–1.54)	0.64	1.27 (0.79–2.05)	0.63	1.22 (0.91–1.63)	0.66	1.41 (0.99–2.02)	0.52
PLA2G6 rs132985 C (T)	0.52	0.56	1.07 (0.93–1.24)	0.56	1.08 (0.93–1.25)	0.61	1.42 (0.87–2.32)	0.52	1.00 (0.75–1.34)	0.54	1.05 (0.74–1.49)	0.23
IRF4 rs12203592 T (C)	0.23	0.20	1.01 (0.83–1.22)	0.21	1.04 (0.85–1.27)	0.10	0.35 (0.16–0.77)	0.16	0.41 (0.42–0.91)	0.22	0.90 (0.59–1.37)	0.0008
Nevus category												
None/a few or < 10	0.67	0.46	1.00 (Reference)	0.49	1.00 (Reference)	0.19	1.00 (Reference)	0.22	1.00 (Reference)	0.65	1.00 (Reference)	
Moderate or 10–50	0.27	0.41	2.39 (1.92–2.98)	0.39	2.23 (1.78–2.80)	0.53	6.55 (2.73–15.73)	0.55	6.34 (3.82–10.55)	0.28	1.06 (0.61–1.83)	< .0001
Many or > 50	0.06	0.13	3.07 (2.14–4.39)	0.12	2.79 (1.92–4.05)	0.28	15.47 (5.70–41.97)	0.23	12.30 (6.60–22.93)	0.07	1.27 (0.49–3.27)	< .0001
			P_{trend} < .0001		P_{trend} < .0001		P_{trend} <.0001		P_{trend} < .0001		P_{trend} = .68	

Note: [a]Adjusted for ancestry score, age, number of nevi, freckling, skin color and hair color.
[b]Test for homogeneity in estimates across subsamples.
CI: Confidence Interval; OR: Odds-Ratio.

those most significantly associated with nevi in controls in our study (Falchi et al., 2009). The authors also showed a significant association between *MTAP* rs10757257 and *PLA2G6* rs132985 and melanoma risk (OR = 1.23, 95% CI = 1.15–1.30). These associations have been confirmed in a separate GWAS conducted by the GenoMEL Consortium, where the ancestral alleles *MTAP* rs10757257*A and *PLA2G6* rs2284063*G were significantly associated with melanoma risk (OR = 0.83, 95% CI = 0.76–0.91) (Bishop et al., 2009). Our findings confirm an association between melanoma risk and *MTAP*, however we found no significant association with *PLA2G6* variants.

IRF4 has recently been identified as a novel locus controlling nevus count (Duffy et al., 2010b), as well as skin, hair and eye color (Duffy et al., 2010a; Han et al., 2008). In the present analyses, *IRF4* was not shown to be a strong predictor of melanoma risk in adults, either overall, or by melanoma site or subtype, but showed a significant association in the children and adolescents samples. In a multicentre analysis involving our sample, combination of multiple datasets was necessary to achieve statistical significance in adults (OR = 1.15, $P = 4x10^{-3}$ for the C allele). The C allele was associated with higher nevus count in adults from that study, a finding that we confirm in the present analysis. Interestingly, it was also demonstrated that the effects of *IRF4* genotype on nevus count differed substantially in children (where the rs12203592*T allele increased total nevus count) compared with the effect in adults (Duffy et al., 2010b). This may parallel our current finding that the effects of *IRF4* genotype on melanoma risk were more obvious in cases with an onset in childhood. Moreover, the rs12203592*C allele was significantly associated with trunk melanoma in the multicentre analysis (OR = 1.33, $P = 2.5x10^{-5}$) (Duffy et al., 2010b), consistent with our crude estimate showing a significant inverse association between rs12203592*T and trunk melanoma, although the adjusted estimate did not reach statistical significance. Finally, in our study, crude models showed that patients with SSM/NM were more likely to carry the rs12203592*C allele than were controls. Consistently, a significant association was found between the C allele and higher nevus count in this sample (see Supplementary Table 2 online), and this finding has recently been replicated in a UK sample (Duffy et al., 2010b).

A recent study performed in the United Kingdom confirmed an association between nevi and variants of *MTAP* (rs7023329), *PLA2G6* (rs2284063) and *IRF4* (rs12203592) (Newton-Bishop et al., 2010). Number of nevi was significantly associated with the *MTAP* and *PLA2G6* SNPs but not with the *IRF4* SNP, whereas number of large nevi was associated with all three SNPs. While we found no significant association between melanoma risk and our selected *PLA2G6* variant in fully adjusted models, the authors of the UK study reported significant inverse relationships

TABLE 4

Odds-Ratios and 95% Confidence Intervals for Site-Specific Risk of Cutaneous Melanoma in Relation to Type of Allele for Selected Gene Variants and Nevus Category, Q-MEGA (1987–2005) (*n* = 2462)

	Controls (*n* = 1469)	Cases (*n* = 993)[a]												
		Trunk (*n* = 371)			Head and neck (*n* = 120)			Upper limbs (*n* = 236)			Lower limbs (*n* = 266)			
	Prop.	Prop.	Crude OR (95% CI)	Adjusted OR[b] (95% CI)	Prop.	Crude OR (95% CI)	Adjusted OR[b] (95% CI)	Prop.	Crude OR (95% CI)	Adjusted OR[b] (95% CI)	Prop.	Crude OR (95% CI)	Adjusted OR[b] (95% CI)	*p* value
MTAP rs10757257														
G (A)	0.58	0.63	1.24 (1.05–1.47)	1.26 (1.04–1.53)	0.64	1.29 (0.98–1.69)	1.35 (1.01–1.81)	0.64	1.30 (1.06–1.59)	1.35 (1.08–1.69)	0.64	1.32 (1.09–1.60)	1.38 (1.11–1.71)	0.70
PLA2G6 rs132985														
C (T)	0.52	0.56	1.15 (0.97–1.35)	1.06 (0.88–1.29)	0.56	1.18 (0.90–1.55)	1.10 (0.82–1.46)	0.58	1.26 (1.04–1.54)	1.17 (0.94–1.45)	0.53	1.04 (0.86–1.26)	0.96 (0.77–1.18)	0.87
IRF4 rs12203592														
T (C)	0.23	0.18	0.73 (0.60–0.90)	0.88 (0.68–1.14)	0.22	0.96 (0.70–1.31)	1.16 (0.80–1.68)	0.22	0.96 (0.76–1.21)	1.10 (0.83–1.46)	0.19	0.81 (0.64–1.02)	1.04 (0.78–1.38)	0.24
Nevus category														
None/a few or < 10	0.67	0.41	1.00 (Reference)	1.00 (Reference)	0.47	1.00 (Reference)	1.00 (Reference)	0.49	1.00 (Reference)	1.00 (Reference)	0.50	1.00 (Reference)	1.00 (Reference)	—
Moderate or 10–50	0.27	0.42	2.64 (2.05–3.39)	2.85 (2.13–3.81)	0.47	2.56 (1.74–3.78)	3.03 (1.98–4.63)	0.36	1.82 (1.35–2.47)	2.04 (1.46–2.85)	0.38	1.90 (1.43–2.52)	2.11 (1.53–2.91)	0.81
Many or > 50	0.06	0.17	4.73 (3.27–6.83)	3.96 (2.57–6.11)	0.06	1.44 (0.64–3.25)	1.44 (0.61–3.38)	0.15	3.57 (2.32–5.51)	3.41 (2.10–5.53)	0.12	2.66 (1.70–4.17)	2.61 (1.57–4.32)	0.04
			$P_{trend} < .0001$	$P_{trend} < .0001$		$P_{trend} = .0003$	$P_{trend} = .0002$		$P_{trend} < .0001$	$P_{trend} < .0001$		$P_{trend} < .0001$	$P_{trend} < .0001$	

Note: [a]Melanomas for which site was not specified were not reported in this table (*n* = 35).
[b]Adjusted for ancestry score, sex, age, number of nevi, freckling, skin color and hair color.
[c]Test for homogeneity in estimates between trunk melanoma and head and neck melanoma.
CI: Confidence Interval; OR: Odds-Ratio.

TABLE 5

Odds-Ratios and 95% Confidence Intervals for Subtype-Specific Risk of Cutaneous Melanoma in Relation to Type of Allele for Selected Gene Variants and Nevus Category (n = 2497)

	Controls (n = 1469)	Cases (n = 1028) SSM/NM (n = 759)			LMM (n = 31)			Other [a] (n = 238)			
	Prop.	Prop.	Crude OR (95% CI)	Adjusted OR[b] (95% CI)	Prop.	Crude OR (95% CI)	Adjusted OR[b] (95% CI)	Prop.	Crude OR (95% CI)	Adjusted OR[b] (95% CI)	p value
MTAP rs10757257											
G (A)	0.58	0.64	1.30 (1.14–1.48)	1.34 (1.15–1.57)	0.52	0.78 (0.47–1.29)	0.79 (0.46–1.35)	0.64	1.29 (1.06–1.58)	1.33 (1.06–1.66)	0.06
PLA2G6 rs132985											
C (T)	0.48	0.45	1.14 (1.01–1.30)	1.06 (0.91–1.23)	0.47	1.04 (0.63–1.74)	1.00 (0.58–1.71)	0.43	1.21 (0.99–1.47)	1.12 (0.90–1.40)	0.84
IRF4 rs12203592											
T (C)	0.23	0.19	0.80 (0.68–0.93)	0.94 (0.76–1.15)	0.26	1.16 (0.66–2.05)	0.99 (0.50–1.98)	0.22	0.93 (0.74–1.17)	1.27 (0.95–1.70)	0.87
Nevus category											
None/a few or < 10	0.67	0.47	1.00 (Reference)	1.00 (Reference)	0.64	1.00 (Reference)	1.00 (Reference)	0.42	1.00 (Reference)	1.00 (Reference)	—
Moderate or 10–50	0.27	0.41	2.17 (1.80–2.63)	2.40 (1.90–3.03)	0.33	1.26 (0.58–2.72)	2.20 (0.97–4.99)	0.39	2.34 (1.73–3.18)	2.38 (1.70–3.35)	0.84
Many or > 50	0.06	0.12	2.96 (2.15–4.07)	2.74 (1.88–4.01)	0.03	0.58 (0.08–4.34)	0.92 (0.12–7.33)	0.19	5.18 (3.42–7.85)	4.24 (2.64–6.82)	0.31
			P_{trend} < .0001	P_{trend} < .0001		P_{trend} =.98	P_{trend} = .24		P_{trend} < .0001	P_{trend} < .0001	

Note: [a]In the total population of invasive cases (n = 1028), the 238 melanomas from this category included 234 melanomas not otherwise specified, 2 amelanotic melanomas, and 2 desmoplastic melanomas.
[b]Adjusted for ancestry score, sex, age, number of nevi, freckling, skin color and hair color.
[c]Test for homogeneity in estimates between SSM/NM and LMM.

between rarer alleles of their three selected SNPs and melanoma risk at all body sites (Newton-Bishop et al., 2010). An association between *MTAP* rs7023329 and number of nevi has also been confirmed in a recent familial case-control study on melanoma (Yang et al., 2010).

After adjustment for nevi in our analyses, estimates were somewhat reduced for *MTAP* but remained statistically significant, and the findings remained unchanged, consistent with results from the two GWAS reports (Bishop et al., 2009; Falchi et al., 2009). Regarding *PLA2G6* and *IRF4*, however, adjustment for nevi resulted in loss of statistical significance and reduction of the associations towards unity. This indicates that the association between nevi and melanoma is not fully explained by *MTAP* genotype, and that the associations with nevi and *MTAP* are probably independent, possibly synergistic, while those observed in crude models for *PLA2G6* and *IRF4* are mainly driven through number of nevi. An alternative explanation for *MTAP* is that measurement error in the nevus counts is confounding the true magnitude of the relationship. The recent UK study reported reduced and marginally significant associations in all three SNPs after adjustment for nevus phenotype (Newton-Bishop et al., 2010).

The 'divergent pathways' hypothesis would predict that nevus loci should exert their strongest effects on risk of SSM/NM, and at non-sun-exposed sites, whereas they should have little effect on the LMM type and at chronically sun-exposed sites.

Here, there were significantly differential associations between nevus category and melanoma site and type, which lend support to the hypothesis. Specifically, individuals with a high nevus propensity were significantly more likely to develop trunk melanoma (i.e., non-chronically sun-exposed site) than melanoma on the head and neck (i.e., chronically sun-exposed site), although the findings here were less striking than in earlier reports (Whiteman et al., 2006). Such individuals were also more likely to develop SSM/NM (i.e., associated with intermittent sun exposure) than the LMM type (i.e., associated with chronic sun exposure).

While we found no evidence that the association between *MTAP* and melanoma risk differed by anatomical site, we observed stronger associations with SSM/NM compared with LMM. The heterogeneity in estimates was only marginally statistically significant, however. Taken together, these findings are consistent with the 'divergent pathways' hypothesis and may provide at least one potential candidate gene to explain this model.

In the case of the *IRF4* SNP, the interpretation is more difficult; first, in that a recent investigation suggested that the effects of *IRF4* variants on nevus count differed by age (Duffy et al., 2010b); and second, that their effect through skin color was opposite to that observed with nevus count: the rs12203592*C increased both adult nevus count and skin pigmentation in that study (Duffy et al., 2010b). As noted above, an effect of *IRF4* on trunk melanoma was detected in the anticipated direction, but not on tumor subtype. Our finding of a protective effect of the *IRF4* rs12203592*T allele on melanoma in children and adolescents is consistent with the recently reported associations between this allele and nevi in adolescents (Viros et al., 2008).

Key strengths were the large sample size and the ability to examine site- and subtype-specific invasive melanoma risk in relation to the selected gene variants. However, several limitations should be considered. First, cases and controls were interviewed at different periods, and the instruments used to assess phenotype were very similar, but not identical. Nevus category was recorded using a semi-quantitative scale for cases, and a qualitative scale for controls, and semi-quantitative items for nevi and freckling showed moderate correlations with qualitative items in the Q-MEGA, ranging from 0.36 to 0.55 for nevi and from 0.30 to 0.53 for freckling (Baxter et al., 2008). Second, phenotypic factors were self-reported in cases and controls, which could have induced a recall bias. However, key findings were similar regardless of adjusting factors, suggesting that phenotypic factors were unlikely to strongly confound these associations. Another limitation is that sun exposure data were not available for controls, and thus adjustment for this factor was not possible. However, although the role of sun exposure in melanoma risk has been largely established in ecological studies (IARC, 1992; Lens & Dawes, 2004), this factor has generally shown modest associations in epidemiological investigations (Gandini et al., 2005; Nelemans et al., 1995). Indeed, historic sun exposure is difficult to measure accurately and has only a moderate reliability (Oliveria et al., 2006; Veierod et al., 2008). It can thus be speculated that our lack of adjustment for this factor would have little effect on the findings. Finally, no correction was made for multiple testing, and given the multiple tests performed, we cannot exclude the possibility that our results may have occurred by chance. However, our results corroborate those reported by the GWAS regarding *MTAP*, although our study did not confirm the association with *PLA2G6* in adjusted models.

In conclusion, these results suggest an association between *MTAP*, *PLA2G6* and *IRF4* variants and nevus count. They also confirm an association between *MTAP* and melanoma, and raise the prospect that the relationship is subtype-specific. The *MTAP* gene is located on chromosome band 9p21, adjacent to *CDKN2A*, which region has been found to be strongly associated with nevus count (Zhu et al., 2007). Because it is not yet clear whether *MTAP* variants are tagged or independent to those in *CDKN2A*, more research will be needed to determine whether the observed associations can be attributed to *MTAP* independently of *CDKN2A*. These findings also

suggest that the association between *IRF4* and melanoma is more evident in cases with an onset early in life.

Acknowledgments

We thank Dixie Statham, Amanda Baxter, Monica de Nooyer, Isabel Gardner, and Barbara Haddon for project management, David Smyth and Harry Beeby for data management, and Jane Palmer and Judy Symmons for ascertainment of clinical records. We also thank the numerous interviewers who collected the CATI data, and Nirmala Pandeya for her help in statistics. Most of all we thank the melanoma patients and their families for their co-operation.

Grant Support

The Cancer Council Queensland; the US National Cancer Institute (CA88363); the Cooperative Research Centre for Discovery of Genes for Common Diseases (project support); the National Health and Medical Research Council of Australia (Research Fellowships to D.W., D.D., G.M., and N.H.). The Cancer Council Queensland; the Foundation of France; the French Région Ile-de-France; the L'Oréal Foundation; the UNESCO (PhD Scholarships and Research Fellowships to MK).

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SUPPLEMENTARY TABLE 1

Characteristics of the Study Participants According to Availability of Genotype for the Selected Variants, Q-MEGA (1987–2005) (*n* = 3245)

	Cases (*n* = 1435)									Controls (*n* = 1810)								
	MTAP rs10757257 / *PLA2G6* rs132985					*IRF4* rs12203592				*MTAP* rs10757257 / *PLA2G6* rs132985					*IRF4* rs12203592			
	Available (*n* = 1048)	%	Not available (*n* = 387)	%	*p* value	Available (*n* = 1032)	%	Not available (*n* = 403)	%	*p* value	Available (*n* = 1470)	%	Not available (*n* = 340)	%	*p* value	Available (*n* = 1470)	%	Not available (*n* = 340)	%	*p* value
Age																			
Mean (*SD*)	42.7 (16.3)	—	44.4 (15.8)	—	.08	42.7 (16.3)	—	44.3 (15.7)	—	.09	43.6 (9.9)	—	43.9 (5.4)	—	.65	43.6 (9.9)	—	43.9 (5.4)	—	.63
Median	43.7	—	44.3	—		43.7	—	43.8	—		43.0	—	43.7	—		43.0	—	43.8	—	
Sex																			
Male	496	47.3	172	44.4	.33	486	47.1	182	45.2	.51	632	43.0	258	75.9	< .0001	633	43.1	257	75.6	< .0001
Female	552	52.7	215	55.6		546	52.9	221	54.8		838	57.0	82	24.1		837	56.9	83	24.4	
Skin color																			
Fair or pale	858	81.9	313	80.9	.90	846	82.0	325	80.7	.84	831	56.5	158	46.5	< .0001	831	56.5	158	46.5	< .0001
Medium	158	15.1	61	15.8		154	14.9	65	16.1		504	34.3	127	37.3		504	34.3	127	37.3	
Olive or dark	32	3.0	13	3.3		32	3.1	13	3.2		135	9.2	55	16.2		135	9.2	55	16.2	
Hair color																			
Fair/blonde	250	23.8	86	22.2	.20	247	23.9	89	22.1	.14	244	16.6	54	15.9	.06	243	16.5	55	16.2	.07
Light brown	415	39.6	138	35.7		410	39.7	143	35.5		533	36.2	101	29.7		533	36.3	101	29.7	
Red	135	12.9	64	16.5		132	12.8	67	16.6		73	5.0	24	7.1		73	5.0	24	7.1	
Dark brown/Black	248	23.7	99	25.6		243	23.5	104	25.8		620	42.2	161	47.3		621	42.2	160	47.0	
Eye color																			
Blue or grey	447	42.7	158	40.8	.32	438	42.4	167	41.4	.48	597	40.6	129	37.9	.14	597	40.6	129	37.9	.18
Green or hazel	428	40.8	152	39.3		422	40.9	158	39.2		541	36.8	117	34.4		540	36.7	118	34.7	
Brown	173	16.5	77	19.9		172	16.7	78	19.4		332	22.6	94	27.7		333	22.7	93	27.4	
Freckling																			
None	204	19.5	69	17.8	.64	203	19.7	70	17.4	.51	396	26.9	98	28.8	.06	396	26.9	98	28.8	.06
Light/A few	429	40.9	157	40.6		418	40.5	168	41.7		446	30.4	123	36.2		446	30.3	123	36.2	
Moderate	291	27.8	106	27.4		289	28.0	108	26.8		354	24.1	66	19.4		354	24.1	66	18.4	
Heavy/Many	124	11.8	55	14.2		122	11.8	57	14.1		274	18.6	53	15.6		274	18.6	53	15.6	
Number of nevi																			
None	91	8.7	46	11.9	.16	90	8.7	47	11.7	.09	83	5.7	31	9.1	.08	83	5.7	31	9.1	.08
< 10/A few	400	38.2	128	33.1		398	38.6	130	32.3		908	61.8	193	56.8		908	61.8	193	56.8	
10-50/Moderate	418	39.9	160	41.3		407	39.4	171	42.4		393	26.7	94	27.6		393	26.7	94	27.6	
> 50/Many	139	13.2	53	13.7		137	13.3	55	13.6		86	5.8	22	6.5		86	5.8	22	6.5	
Ancestry score																			
< 50%	12	1.2	5	1.3	.96	12	1.2	5	1.2	.99	77	5.2	35	10.3	.0002	77	5.2	35	10.3	.0002
50–74%	13	1.2	6	1.6		13	1.3	6	1.5		32	2.2	16	4.7		32	2.2	16	4.7	
75–99%	45	4.3	16	4.1		44	4.2	17	4.2		50	3.4	12	3.5		50	3.4	12	3.5	
100%	978	93.3	360	93.0		963	93.3	375	93.1		1311	89.2	277	81.5		1311	89.2	277	81.5	

SUPPLEMENTARY TABLE 2

Chi-Square Tests for Deviation from Hardy-Weinberg Equilibrium in Cases and Controls, Q-MEGA (1987–2005) (*n* = 2497)

	Controls (*n* = 1469)		*p* value	Cases (*n* = 1028)		*p* value
	n	%		*n*	%	
MTAP						
rs4636294						
A/A	350	23.8	.31	293	28.5	.33
A/G	714	48.6		526	51.2	
G/G	405	27.6		209	20.3	
rs2218220						
C/C	351	23.9	.33	293	28.5	.44
C/T	715	48.7		523	50.9	
T/T	403	27.4		212	20.6	
rs7023329						
A/A	363	24.7	.47	277	28.1	.28
A/G	720	49.1		507	51.4	
G/G	385	26.2		202	20.5	
rs10757257						
A/A	274	18.7	.24	129	12.5	.32
A/G	695	47.3		491	47.8	
G/G	500	34.0		408	39.7	
rs751173						
A/A	426	29.0	.95	240	23.3	.52
A/G	729	49.6		524	51.0	
G/G	314	21.4		264	25.7	
rs1335510						
G/G	275	18.7	.25	135	13.2	.36
G/T	696	47.4		493	48.0	
T/T	498	33.9		398	38.8	
rs1341866						
A/A	500	34.0	.19	398	38.7	.47
A/G	693	47.2		492	47.9	
G/G	276	18.8		138	13.4	
rs10811629						
A/A	471	32.0	.69	382	37.1	.98
A/G	715	48.7		489	47.6	
G/G	283	19.3		157	15.3	
PLA2G6						
rs2284063						
A/A	593	40.4	.18	439	42.6	.76
A/G	699	47.6		469	45.7	
G/G	177	12.0		120	11.7	
rs6001027						
A/A	592	40.3	.17	439	42.7	.78
A/G	700	47.7		468	45.6	
G/G	177	12.0		120	11.7	
rs132985						
C/C	386	26.3	.15	318	31.0	.94
C/T	762	51.9		508	49.5	
T/T	321	21.8		201	19.6	
rs738322						
A/A	379	25.8	.12	315	30.7	.94
A/G	763	51.9		507	49.3	
G/G	327	22.3		206	20.0	
IRF4						
rs2797307						
G/G	1335	93.8	.17	949	94.2	.35
G/T	85	6.0		58	5.8	
T/T	3	0.2		0	0.0	
rs12203592						
C/C	871	59.3	.86	654	64.8	.13
C/T	518	35.3		307	30.4	
T/T	79	5.4		48	4.8	
rs2671422						
A/A	24	1.7	.15	10	01.0	.54
A/G	276	19.5		199	19.7	
G/G	1117	78.8		800	79.3	
rs2292383						
C/C	6	0.4	.54	2	0.2	.18
C/T	152	10.8		133	13.3	
T/T	1256	88.8		869	86.5	
rs17825664						
C/C	5	0.3	.60	2	0.2	.42
C/T	142	10.1		112	11.2	
T/T	1265	89.6		889	88.6	

SUPPLEMENTARY TABLE 3

Genotype Frequencies (%) for *MTAP*, *PLA2G6* and *IRF4* Variants in Cases and Controls, Q-MEGA (1987–2005) (*n* = 2462)

	Controls (*n* = 1469)	Cases (*n* = 993)[a]											
		Trunk (*n* = 371)			Head and neck (*n* = 120)			Upper limbs (*n* = 236)			Lower limbs (*n* = 266)		
		SSM/NM (n = 277)	LMM (*n* = 5)	Other[b] (*n* = 89)	SSM/NM (*n* = 74)	LMM (*n* = 14)	Other[b] (*n* = 32)	SSM/NM (*n* = 165)	LMM (*n* = 8)	Other[b] (*n* = 63)	SSM/NM (*n* = 215)	LMM (*n* = 3)	Other[b] (*n* = 48)
MTAP													
rs4636294													
G/G	27.6	23.8	40.0	23.6	16.2	21.4	12.5	17.0	12.5	23.8	16.7	33.3	22.9
A/G	48.6	47.3	60.0	46.1	55.4	57.2	56.3	56.4	75.0	42.9	54.0	33.3	52.1
A/A	23.8	28.9	0.0	30.3	28.4	21.4	31.2	26.6	12.5	33.3	29.3	33.3	25.0
rs2218220													
C/C	23.9	28.9	0.0	30.3	28.4	21.4	31.3	26.7	12.5	33.3	29.3	33.3	25.0
C/T	48.7	46.9	60.0	44.9	55.4	57.2	56.2	55.8	75.0	42.9	54.0	33.3	52.1
T/T	27.4	24.2	40.0	24.7	16.2	21.4	12.5	17.6	12.5	23.8	16.7	33.3	22.9
rs7023329													
G/G	26.2	22.0	80.0	22.7	15.3	21.4	15.6	16.1	12.5	26.2	19.1	33.3	21.7
A/G	49.1	50.0	20.0	50.0	56.9	50.0	50.0	54.2	75.0	44.3	52.8	33.3	52.2
A/A	24.7	28.0	0.0	27.3	27.8	28.6	34.4	29.7	12.5	29.5	28.1	33.3	26.1
rs10757257													
G/G	34.0	39.7	0.0	42.7	41.9	28.6	37.5	39.4	25.0	41.3	39.5	33.3	41.7
A/G	47.3	47.3	60.0	41.6	48.6	57.1	46.9	49.7	62.5	46.0	50.7	33.3	41.7
A/A	18.7	13.0	40.0	15.7	9.5	14.3	15.6	10.9	12.5	12.7	9.8	33.3	16.6
rs751173													
G/G	21.4	26.4	0.0	22.5	28.4	35.7	34.4	25.5	12.5	25.4	24.2	33.3	25.0
A/G	49.6	47.3	60.0	53.9	52.7	50.0	53.1	53.9	87.5	47.6	53.5	33.3	41.7
A/A	29.0	26.3	40.0	23.6	18.9	14.3	12.5	20.6	0.0	27.0	22.3	33.3	33.3
rs1335510													
T/T	33.9	38.8	0.0	41.6	40.5	21.4	37.5	37.8	25.0	41.3	39.6	33.3	41.7
G/T	47.4	46.4	80.0	39.3	51.4	64.3	53.1	51.2	62.5	46.0	50.2	33.3	41.7
G/G	18.7	14.8	20.0	19.1	8.1	14.3	9.4	11.0	12.5	12.7	10.2	33.3	16.6
rs1341866													
A/A	34.0	39.0	0.0	41.6	40.5	28.6	37.5	37.6	25.0	39.7	39.1	33.3	41.7
A/G	47.2	45.9	80.0	40.4	51.4	57.1	46.9	50.9	62.5	47.6	50.7	33.3	41.7
G/G	18.8	15.1	20.0	18.0	8.1	14.3	15.6	11.5	12.5	12.7	10.2	33.3	16.6
rs10811629													
A/A	32.0	37.6	20.0	40.4	41.9	21.4	31.3	35.2	25.0	33.3	38.6	33.3	39.6
A/G	48.7	47.6	40.0	41.6	43.2	57.2	50.0	50.3	50.0	52.4	49.8	33.3	43.7
G/G	19.3	14.8	40.0	18.0	14.9	21.4	18.7	14.5	25.0	14.3	11.6	33.3	16.7
PLA2G6													
rs2284063													
G/G	12.0	12.6	0.0	13.5	10.8	21.4	15.6	9.2	12.5	6.4	12.1	0.0	12.5
A/G	47.6	45.9	60.0	36.0	44.6	35.7	43.8	45.1	62.5	49.2	48.8	66.7	47.9
A/A	40.4	41.5	40.0	50.5	44.6	42.9	40.6	45.7	25.0	44.4	39.1	33.3	39.6
rs6001027													
G/G	12.0	12.6	0.0	13.5	10.8	21.4	15.6	9.2	12.5	6.4	12.1	0.0	12.5
A/G	47.7	45.5	60.0	36.0	44.6	35.7	43.8	45.1	62.5	49.2	48.8	66.7	47.9
A/A	40.3	41.9	40.0	50.5	44.6	42.9	40.6	45.7	25.0	44.4	39.1	33.3	39.6
rs132985													
T/T	21.8	21.3	0.0	22.5	13.5	35.7	28.1	17.7	25.0	14.3	20.0	0.0	20.8
C/T	51.9	48.7	60.0	38.2	56.8	28.6	34.4	47.0	62.5	57.1	53.5	66.7	54.2
C/C	26.3	30.0	40.0	39.3	29.7	35.7	37.5	35.3	12.5	28.6	26.5	33.3	25.0
rs738322													
G/G	22.3	22.7	0.0	23.6	13.5	35.7	28.1	17.6	25.0	14.3	19.5	0.0	22.9
A/G	51.9	46.9	60.0	38.2	56.8	28.6	37.5	47.9	62.5	57.1	54.0	66.7	54.2
A/A	25.8	30.3	40.0	38.2	29.7	35.7	38.4	34.5	12.5	28.6	26.5	33.3	22.9
IRF4													
rs2797307													
T/T	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
G/T	6.0	4.9	0.0	6.7	6.8	0.0	9.7	6.2	0.0	3.2	7.6	0.0	4.3
G/G	93.8	95.1	100.0	93.3	93.2	100.0	90.3	93.8	100.0	96.8	92.4	100.0	95.7
rs12203592													
C/C	59.3	67.1	60.0	68.2	56.8	57.2	67.7	61.6	37.5	61.9	70.0	66.7	57.4
C/T	35.3	29.5	40.0	29.5	40.5	35.7	25.8	34.8	50.0	28.6	25.2	33.3	27.7
T/T	5.4	3.4	0.0	2.3	2.7	7.1	6.5	3.6	12.5	9.5	4.8	0.0	14.9
rs2671422													
A/A	1.7	1.5	0.0	0.0	1.4	7.2	0.0	0.0	0.0	0.0	1.4	0.0	2.1
A/G	19.5	19.5	40.0	18.0	24.3	21.4	18.8	19.8	12.5	22.2	18.6	33.3	12.8
G/G	78.8	79.0	60.0	82.0	74.3	71.4	81.2	80.2	87.5	77.8	80.0	66.7	85.1
rs2292383													
T/T	88.8	86.8	60.0	84.3	79.5	78.6	93.5	88.2	100.0	85.7	89.0	66.7	87.2
C/T	10.8	13.2	40.0	15.7	20.5	21.4	6.5	11.8	0.0	14.3	10.1	33.3	12.8
C/C	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.9	0.0	0.0
rs17825664													
T/T	89.6	90.2	40.0	88.6	82.2	78.6	93.3	91.3	100.0	84.1	89.5	66.7	87.2
C/T	10.1	9.8	60.0	11.4	17.8	21.4	6.7	8.7	0.0	15.9	9.5	33.3	12.8
C/C	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.9	0.0	0.0

Note: [a]Melanomas for which site was not specified were not reported in this table (*n* = 35).

[b]In the total population of invasive cases (*n* = 1028), the 238 melanomas from this category included 234 melanomas not otherwise specified, 2 amelanotic melanomas, and 2 desmoplastic melanomas.

SUPPLEMENTARY TABLE 4

Nevus Category in Relation to Genotypes for *MTAP*, *PLA2G6* and *IRF4* Variants in Cases and Controls, Q-MEGA (1987–2005) (*n* = 2497)

	Cases (*n* = 1028) Nevus category									Controls (*n* = 1469) Nevus category								
	None		<10		10–50		>50		Chi-square	None		A few		Moderate		Many		Chi-square
	n	%	*n*	%	*n*	%	*n*	%		*n*	%	*n*	%	*n*	%	*n*	%	
MTAP																		
rs4636294																		
G/G	21	24.1	83	21.2	78	18.9	27	19.6	0.13	21	25.3	268	29.5	99	25.2	17	19.8	0.18
A/G	45	51.8	201	51.4	222	53.9	58	42.0		43	51.8	434	47.9	197	50.1	40	46.5	
A/A	21	24.1	107	27.4	112	27.2	53	38.4		19	22.9	205	22.6	97	24.7	29	33.7	
rs2218220																		
C/C	21	24.1	107	27.4	112	27.2	53	38.4	0.15	19	22.9	205	22.6	97	24.7	30	34.9	0.12
C/T	45	51.8	200	51.1	220	53.4	58	42.0		43	51.8	435	48.0	198	50.4	39	45.3	
T/T	21	24.1	84	21.5	80	19.4	27	19.6		21	25.3	267	29.4	98	24.9	17	19.8	
rs7023329																		
G/G	19	23.2	83	22.3	74	18.7	26	19.3	0.06	19	22.9	259	28.6	91	23.2	16	18.6	0.10
A/G	43	52.4	190	50.9	218	55.0	56	41.5		42	50.6	436	48.0	202	51.5	40	46.5	
A/A	20	24.4	100	26.8	104	26.3	53	39.2		22	26.5	212	23.4	99	25.3	30	34.9	
rs10757257																		
G/G	31	35.6	146	37.4	162	39.3	69	50.0	0.18	32	38.5	291	32.1	134	34.1	43	50.0	0.01
A/G	44	50.6	196	50.1	200	48.6	51	37.0		37	44.6	429	47.3	198	50.4	31	36.0	
A/A	12	13.8	49	12.5	50	12.1	18	13.0		14	16.9	187	20.6	61	15.5	12	14.0	
rs751173																		
G/G	22	25.3	93	23.8	106	25.7	43	31.2	0.76	20	24.1	186	20.5	86	21.9	22	25.6	0.25
A/G	43	49.4	202	51.7	212	51.5	67	48.5		45	54.2	438	48.3	200	50.9	46	53.5	
A/A	22	25.3	96	24.5	94	22.8	28	20.3		18	21.7	283	31.2	107	27.2	18	20.9	
rs1335510																		
T/T	31	36.0	143	36.6	156	38.0	68	49.3	0.09	31	37.4	292	32.2	135	34.3	40	46.5	0.05
G/T	41	47.7	201	51.4	201	48.9	50	36.2		37	44.6	429	47.3	196	49.9	34	39.5	
G/G	14	16.3	47	12.0	54	13.1	20	14.5		15	18.0	186	20.5	62	15.8	12	14.0	
rs1341866																		
A/A	31	35.6	142	36.3	157	38.1	68	49.3	0.11	31	37.4	292	32.2	137	34.9	40	46.5	0.08
A/G	42	48.3	199	50.9	201	48.8	50	36.2		37	44.6	428	47.2	194	49.3	34	39.5	
G/G	14	16.1	50	12.8	54	13.1	20	14.5		15	18.0	187	20.6	62	15.8	12	14.0	
rs10811629																		
A/A	32	36.8	133	34.0	153	37.1	64	46.4	0.02	30	36.1	273	30.1	132	33.6	36	41.9	0.10
A/G	36	41.4	206	52.7	198	48.1	49	35.5		39	47.0	441	48.6	195	49.6	40	46.5	
G/G	19	21.8	52	13.3	61	14.8	25	18.1		14	16.9	193	21.3	66	16.8	10	11.6	
PLA2G6																		
rs2284063																		
G/G	15	17.3	43	11.0	49	11.9	13	9.4	0.30	15	18.1	112	12.4	44	11.2	6	7.0	0.21
A/G	41	47.1	182	46.6	175	42.6	71	51.5		41	49.4	439	48.4	179	45.5	40	46.5	
A/A	31	35.6	166	42.4	187	45.5	54	39.1		27	32.5	356	39.3	170	43.3	40	46.5	
rs6001027																		
G/G	15	17.3	43	11.0	49	11.9	13	9.4	0.30	15	18.1	112	12.4	44	11.2	6	7.0	0.21
A/G	41	47.1	181	46.3	175	42.6	71	51.5		41	49.4	440	48.5	179	45.5	40	46.5	
A/A	31	35.6	167	42.7	187	45.5	54	39.1		27	32.5	355	39.1	170	43.3	40	46.5	
rs132985																		
T/T	24	27.6	83	21.2	71	17.2	23	16.7	0.06	26	31.3	204	22.5	82	20.9	9	10.5	0.02
C/T	36	41.4	203	51.9	195	47.5	74	53.6		44	53.0	470	51.8	197	50.1	51	59.3	
C/C	27	31.0	105	26.9	145	35.3	41	29.7		13	15.7	233	25.7	114	29.0	26	30.2	
rs738322																		
G/G	24	27.6	84	21.5	72	17.5	26	18.8	0.08	25	30.1	211	23.3	81	20.6	10	11.6	0.03
A/G	36	41.4	204	52.2	196	47.6	71	51.5		45	54.2	468	51.6	199	50.6	51	59.3	
A/A	27	31.0	103	26.3	144	34.9	41	29.7		13	15.7	228	25.1	113	28.8	25	29.1	
IRF4																		
rs2797307																		
T/T	0	0.0	0	0.0	0	0.0	0	0.0	0.64	0	0.0	2	0.2	0	0.0	1	1.2	0.54
G/T	3	3.5	23	6.0	26	6.5	6	4.4		3	3.7	55	6.3	22	5.7	5	6.3	
G/G	83	96.5	361	94.0	375	93.5	130	95.6		79	96.3	818	93.5	364	94.3	74	92.5	
rs12203592																		
C/C	38	44.2	236	60.8	280	70.2	100	73.5	<0.0001	35	42.2	525	57.9	262	66.7	49	57.0	<0.0001
C/T	34	39.5	131	33.8	108	27.1	34	25.0		35	42.2	337	37.2	116	29.5	30	34.9	
T/T	14	16.3	21	5.4	11	2.7	2	1.5		13	15.6	44	4.9	15	3.8	7	8.1	
rs2671422																		
A/A	0	0.0	3	0.8	6	1.5	1	0.7	0.56	2	2.5	15	1.7	4	1.0	3	3.8	0.32
A/G	12	14.0	83	21.5	77	19.2	27	19.9		12	14.8	176	20.2	78	20.3	10	12.5	
G/G	74	86.0	300	77.7	318	79.3	108	79.4		67	82.7	680	78.1	303	78.7	67	83.7	
rs2292383																		
T/T	78	91.8	332	86.5	341	85.5	118	86.8	0.83	73	91.2	769	88.3	342	89.1	72	91.1	0.32
C/T	7	8.2	51	13.3	57	14.3	18	13.2		6	7.5	98	11.2	42	10.9	6	7.6	
C/C	0	0.0	1	0.2	1	0.2	0	0.0		1	1.3	4	0.5	0	0.0	1	1.3	
rs17825664																		
T/T	80	93.0	334	87.2	352	88.4	123	90.4	0.78	73	91.3	775	89.2	345	90.1	72	90.0	0.44
C/T	6	7.0	48	12.5	45	11.3	13	9.6		6	7.5	91	10.5	38	9.9	7	8.7	
C/C	0	0.0	1	0.3	1	0.3	0	0.0		1	1.3	3	0.3	0	0.0	1	1.3	

SUPPLEMENTARY TABLE 5

Odds-Ratios and 95% Confidence Intervals for Risk of Cutaneous Melanoma in Relation to Type of Allele for Unselected Gene Variants, Q-MEGA (1987–2005) (*n* = 2497)

	Controls (*n* = 1469)	Cases (*n* = 1028)		
	Prop.	Prop.	Unadjusted OR (95% CI)	Adjusted OR[a] (95% CI)
MTAP				
rs4636294				
A	0.48	0.54	1.27 (1.13–1.42)	1.31 (1.13–1.51)
rs2218220				
C	0.48	0.54	1.26 (1.12–1.41)	1.30 (1.12–1.50)
rs7023329				
A	0.49	0.54	1.20 (1.07–1.35)	1.23 (1.07–1.43)
rs751173				
G	0.46	0.51	1.22 (1.09–1.37)	1.25 (1.09–1.45)
rs1335510				
T	0.58	0.63	1.24 (1.11–1.39)	1.31 (1.13–1.52)
rs1341866				
A	0.58	0.63	1.23 (1.10–1.38)	1.30 (1.12–1.50)
rs10811629				
A	0.56	0.61	1.21 (1.07–1.35)	1.25 (1.08–1.45)
PLA2G6				
rs2284063				
A	0.64	0.66	1.06 (0.94–1.20)	1.01 (0.87–1.18)
rs6001027				
A	0.64	0.66	1.07 (0.95–1.20)	1.01 (0.87–1.18)
rs738322				
A	0.52	0.55	1.16 (1.03–1.30)	1.06 (0.92–1.23)
IRF4				
rs2797307				
G	0.97	0.97	1.11 (0.80–1.55)	1.10 (0.72–1.70)
rs2671422				
G	0.89	0.89	1.06 (0.88–1.27)	0.97 (0.77–1.23)
rs2292383				
C	0.06	0.07	1.19 (0.94–1.51)	1.20 (0.89–1.62)
rs17825664				
C	0.05	0.06	1.08 (0.84–1.39)	1.14 (0.83–1.56)

Note: [a]Adjusted for ancestry score, age, number of nevi, freckling, skin color and hair color.
CI: Confidence Interval; OR: Odds-Ratio.

SUPPLEMENTARY TABLE 6

Linkage Disequilibrium (r^2) between the Assessed SNPs in Each of the Studied Loci *MTAP*, *PLA2G6* and *IRF4*, Q-MEGA (1987–2005)

MTAP								
rs751173	1							
rs4636294	0.614	1						
rs2218220	0.615	0.996	1					
rs1335510	0.389	0.685	0.687	1				
rs1341866	0.387	0.679	0.680	0.986	1			
rs10757257	0.395	0.624	0.626	0.916	0.917	1		
rs7023329	0.581	0.705	0.706	0.620	0.620	0.678	1	
rs10811629	0.249	0.428	0.429	0.681	0.675	0.728	0.482	1
PLA2G6								
rs2284063	1							
rs6001027	0.997	1						
rs132985	0.625	0.627	1					
rs738322	0.610	0.612	0.973	1				
IRF4								
rs2797307	1							
rs12203592	0.009	1						
rs2671422	0.095	0.036	1					
rs2292383	0.002	0.018	0.432	1				
rs17825664	0.002	0.016	0.426	0.848	1			