



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 ($P_{\text{homogeneity}} = .52$). 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) ($P_{\text{homogeneity}} = .06$), 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 ($P_{\text{homogeneity}} = .0008$). 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-

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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 over-sampled. 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 μ L 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: ^aChi-square tests or t tests were performed in order to compare cases and controls according to the presented characteristics
^bAge 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 ($P_{\text{homogeneity}} = 0.52$). 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 ($P_{\text{homogeneity}} = .0008$). Also, we found significantly positive dose-effect relationships between nevus category and melanoma risk in all ($P_{\text{trend}} < .0001$) but the older men sample ($P_{\text{trend}} = .68$), and results were stronger in the children and the adolescents samples ($P_{\text{homogeneity}} < .0001$).

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 ($P_{\text{homogeneity}} = 0.52$); specifically, we observed no heterogeneity between melanoma of the trunk and melanoma of the head and neck ($P_{\text{homogeneity}} = 0.70$).

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 ($P_{\text{trend}} < .0001$). 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)

| | | 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) |
| <i>MTAP</i> | | | | | | | | | | | | | | |
| 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 |
| <i>PLA2G6</i> | | | | | | | | | | | | | | |
| 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 |
| <i>IRF4</i> | | | | | | | | | | | | | | |
| 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: ^aMelanomas for which site was not specified were not reported in this table (n = 35).

^bIn 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, O-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. | 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) | Prop. | Adjusted OR ^a (95% CI) | |
| MTA | | | | | | | | | | | | | |
| rs10757257 | | | | | | | | | | | | | |
| G (A) | 0.58 | 1.32 (1.14–1.53) | 0.64 | 1.32 (1.14–1.54) | 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 | 1.07 (0.93–1.24) | 0.56 | 1.08 (0.93–1.25) | 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 | 1.01 (0.83–1.22) | 0.20 | 1.01 (0.83–1.27) | 0.21 | 1.04 (0.85–1.27) | 0.10 | 0.35 (0.16–0.77) | 0.16 | 0.61 (0.42–0.91) | 0.22 | 0.90 (0.59–1.37) | 0.0008 |
| Nevus category | | | | | | | | | | | | | |
| None/a few or < 10 | 0.67 | 1.00 (Reference) | 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 | 2.39 (1.92–2.98) | 0.41 | 2.39 (1.78–2.80) | 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 | 3.07 (2.14–4.39) | 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 |
| | | | | | | | | | | | | | $P_{\text{trend}} = .68$ |
| | | | | | | | | | | | | | $P_{\text{trend}} < .0001$ |
| | | | | | | | | | | | | | $P_{\text{trend}} < .0001$ |

Note: ^aAdjusted for ancestry score, age, number of nevi, freckling, skin color and hair color.

^bTest 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, $P = 4 \times 10^{-3}$ 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, $P = 2.5 \times 10^{-5}$) (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) | | | Trunk (n = 371) | | | Head and neck (n = 120) | | | Cases (n = 993) ^a | | | p value | |
|-----------------------------|------------------------|-----------------------|--------------------------------------|---------------------|-----------------------|--------------------------------------|-------------------------|-----------------------|--------------------------------------|------------------------------|-----------------------|--------------------------------------|---------------------|------|
| | Prop. | Upper limbs (n = 236) | | Prop. | Lower limbs (n = 266) | | Prop. | Upper limbs (n = 236) | | Prop. | Lower limbs (n = 266) | | | |
| | | Crude OR (95% CI) | Adjusted OR ^b (95% CI) | | Crude OR (95% CI) | Adjusted OR ^b (95% CI) | | Crude OR (95% CI) | Adjusted OR ^b (95% CI) | | Crude OR (95% CI) | Adjusted OR ^b (95% CI) | | |
| 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 |
| | | | $P_{trend} < .0001$ | $P_{trend} < .0001$ | $P_{trend} = .0002$ | $P_{trend} = .0003$ | $P_{trend} = .0002$ | $P_{trend} < .0001$ | $P_{trend} < .0001$ | $P_{trend} < .0001$ | $P_{trend} < .0001$ | $P_{trend} < .0001$ | $P_{trend} < .0001$ | |

Note: ^aMelanomas for which site was not specified were not reported in this table (n = 35).
^bAdjusted for ancestry score, sex, age, number of nevi, freckling, skin color and hair color.
^cTest 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) | | | SSM/NM (n = 759) | | | Cases (n = 1028) LMM (n = 31) | | | Other ^a (n = 238) | | | |
|------------------------------------|------------------------|----------------------|--------------------------------------|------------------|----------------------|--------------------------------------|----------------------------------|----------------------|--------------------------------------|------------------------------|----------------------|--------------------------------------|---------|
| | 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 |
| <i>MTAP</i> rs10757257 G (A) | 0.58 | 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 |
| <i>PLA2G6</i> rs132985 C (T) | 0.48 | 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 |
| <i>IRF4</i> rs12203592 T (C) | 0.23 | 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 | 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 | 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 | 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: ^aIn 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.

^bAdjusted for ancestry score, sex, age, number of nevi, freckling, skin color and hair color.

^cTest 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.

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SUPPLEMENTARY TABLE 1

Characteristics of the Study Participants According to Availability of Genotype for the Selected Variants, O-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 | 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 |
| Mean (SD) | 43.7 | 44.3 | | 43.7 | 43.8 | | 43.0 | 43.7 | | 43.0 | 43.8 | |
| Median | 496 | 172 | 44.4 | 486 | 182 | 45.2 | 632 | 258 | 75.9 | 633 | 257 | 75.6 |
| Sex | 552 | 215 | 55.6 | 546 | 221 | 54.8 | 838 | 82 | 24.1 | 837 | 83 | 24.4 |
| Male | 858 | 313 | 80.9 | 846 | 325 | 80.7 | 831 | 158 | 46.5 | 831 | 158 | 46.5 |
| Female | 158 | 61 | 15.8 | 154 | 65 | 16.1 | 504 | 127 | 37.3 | 504 | 127 | 37.3 |
| Olive or dark | 32 | 13 | 3.3 | 32 | 13 | 3.2 | 135 | 55 | 16.2 | 135 | 55 | 16.2 |
| Skin color | 250 | 86 | 22.2 | 247 | 89 | 22.1 | 244 | 54 | 15.9 | 243 | 55 | 16.2 |
| Fair/blonde | 415 | 138 | 35.7 | 410 | 143 | 35.5 | 533 | 101 | 29.7 | 533 | 101 | 29.7 |
| Light brown | 135 | 64 | 16.5 | 132 | 67 | 16.6 | 73 | 24 | 7.1 | 73 | 24 | 7.1 |
| Red | 248 | 99 | 25.6 | 243 | 104 | 25.8 | 620 | 161 | 47.3 | 621 | 160 | 47.0 |
| Dark brown/Black | 447 | 158 | 40.8 | 438 | 167 | 41.4 | 597 | 129 | 37.9 | 597 | 129 | 37.9 |
| Eye color | 428 | 152 | 39.3 | 422 | 158 | 39.2 | 541 | 117 | 34.4 | 540 | 118 | 34.7 |
| Blue or grey | 173 | 77 | 19.9 | 172 | 78 | 19.4 | 332 | 94 | 27.7 | 333 | 93 | 27.4 |
| Green or hazel | 204 | 69 | 17.8 | 203 | 70 | 17.4 | 396 | 98 | 28.8 | 396 | 98 | 28.8 |
| Brown | 429 | 157 | 40.6 | 418 | 168 | 41.7 | 446 | 123 | 36.2 | 446 | 123 | 36.2 |
| Freckling | 291 | 106 | 27.4 | 289 | 108 | 26.8 | 354 | 66 | 19.4 | 354 | 66 | 18.4 |
| None | 124 | 55 | 14.2 | 122 | 57 | 14.1 | 274 | 53 | 15.6 | 274 | 53 | 15.6 |
| Light/A few | 91 | 46 | 11.9 | 90 | 47 | 11.7 | 83 | 31 | 9.1 | 83 | 31 | 9.1 |
| Moderate | 400 | 128 | 33.1 | 398 | 130 | 32.3 | 908 | 193 | 56.8 | 908 | 193 | 56.8 |
| Heavy/Many | 418 | 160 | 41.3 | 407 | 171 | 42.4 | 393 | 94 | 27.6 | 393 | 94 | 27.6 |
| Number of nevi | 139 | 53 | 13.7 | 137 | 55 | 13.6 | 86 | 22 | 6.5 | 86 | 22 | 6.5 |
| None | 12 | 5 | 1.3 | 12 | 5 | 1.2 | 77 | 5.2 | 10.3 | 77 | 5.2 | 10.3 |
| < 10/A few | 13 | 6 | 1.6 | 13 | 6 | 1.5 | 32 | 2.2 | 4.7 | 32 | 2.2 | 4.7 |
| 10-50/Moderate | 45 | 16 | 4.1 | 44 | 17 | 4.2 | 50 | 3.4 | 3.5 | 50 | 3.4 | 3.5 |
| > 50/Many | 978 | 360 | 93.0 | 963 | 375 | 93.1 | 1311 | 89.2 | 81.5 | 1311 | 89.2 | 81.5 |
| Ancestry score | | | | | | | | | | | | |
| < 50% | | | | | | | | | | | | |
| 50-74% | | | | | | | | | | | | |
| 75-99% | | | | | | | | | | | | |
| 100% | | | | | | | | | | | | |

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 (<i>n</i> = 1469) | Cases (<i>n</i> = 993) ^a | | | | | | | | | | | |
|---------------|--------------------------------|--------------------------------------|------------------------|--|---------------------------------|-------------------------|--|-------------------------------|------------------------|--|-------------------------------|------------------------|--|
| | | Trunk (<i>n</i> = 371) | | | Head and neck (<i>n</i> = 120) | | | Upper limbs (<i>n</i> = 236) | | | Lower limbs (<i>n</i> = 266) | | |
| | | SSM/NM (<i>n</i> = 277) | LMM (<i>n</i> = 5) | Other ^b (<i>n</i> = 89) | SSM/NM (<i>n</i> = 74) | LMM (<i>n</i> = 14) | Other ^b (<i>n</i> = 32) | SSM/NM (<i>n</i> = 165) | LMM (<i>n</i> = 8) | Other ^b (<i>n</i> = 63) | SSM/NM (<i>n</i> = 215) | LMM (<i>n</i> = 3) | Other ^b (<i>n</i> = 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: ^aMelanomas for which site was not specified were not reported in this table (*n* = 35).^bIn 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 5Odds-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) |
| <i>MTAP</i> | | | | |
| 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) |
| <i>PLA2G6</i> | | | | |
| 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) |
| <i>IRF4</i> | | | | |
| 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: ^aAdjusted for ancestry score, age, number of nevi, freckling, skin color and hair color.
CI: Confidence Interval; OR: Odds-Ratio.

SUPPLEMENTARY TABLE 6Linkage Disequilibrium (r^2) between the Assessed SNPs in Each of the Studied Loci *MTAP*, *PLA2G6* and *IRF4*, Q-MEGA (1987–2005)

| | | | | | | | | | |
|---------------|-------|-------|-------|-------|-------|-------|-------|---|--|
| <i>MTAP</i> | | | | | | | | | |
| 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 | |
| <i>PLA2G6</i> | | | | | | | | | |
| rs2284063 | 1 | | | | | | | | |
| rs6001027 | 0.997 | 1 | | | | | | | |
| rs132985 | 0.625 | 0.627 | 1 | | | | | | |
| rs738322 | 0.610 | 0.612 | 0.973 | 1 | | | | | |
| <i>IRF4</i> | | | | | | | | | |
| 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 | | | | |