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Polymorphisms in naevus-associated genes *MTAP*, *PLA2G6*, and *IRF4* and the risk of invasive cutaneous melanoma

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Abstract

An evolving hypothesis postulates that melanomas may arise through "naevus-associated" and "chronic sun exposure" pathways. We explored this hypothesis by examining associations between naevus-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 *MTAP*, *PLA2G6*, and *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 naevi. 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 ($P_{homogeneity}$=0.52). 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) ($P_{homogeneity}$=0.06), 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 ($P_{homogeneity}$=0.0008). 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; naevi; polymorphisms

Melanoma develops through complex effects of both environmental and genetic factors (Miller and Mihm, 2006). Its main risk factors include ultraviolet radiation (UVR), pigmentation, and naevus count (MacKie et al., 2009). Childhood UVR exposure is a significant risk factor for immediate development of naevi, and for subsequent melanoma, but this is modulated by host constitution, anatomical site, and adult UVR exposure. The

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"divergent pathways" model suggests two potential pathways for melanoma development: in people with high naevus counts, melanomas tend to develop at younger ages and on body sites with high naevus counts, such as the trunk ("naevus 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 evidence from epidemiological and molecular analyses to support this model of aetiological 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 and MacKie, 2005; Maldonado et al., 2003; Thomas et al., 2007; Viros et al., 2008). Given that naevus 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 naevus burden is strongly heritable (Wachsmuth et al., 2001; Zhu et al., 1999), it is plausible to speculate that the risks of melanoma conferred by naevus-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 naevus count. One of these loci, *MTAP* (9p21), was found to associate strongly with naevus count in Caucasian populations in Australia and the UK (Bishop et al., 2009; Falchi et al., 2009); the locus was also significantly associated with melanoma risk in these populations. *PLA2G6* (22q13) was similarly associated with naevus counts and melanoma risk in the UK study (Falchi et al., 2009), and *IRF4* (6p25-p23), while associated with skin, hair and eye colour (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 naevus 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 & 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 Naevus 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 which 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 colour at age 20 (fair/pale, medium, or olive/dark), natural hair colour at age 20 (fair/blonde, light brown, red, dark brown, or black), eye colour (blue/grey, green/hazel, or brown), freckling during childhood (none, light, moderate, or heavy), and number of naevi (none, <10, 10–50, or >50). BTNS twin parents self-reported pigmentary characteristics using virtually identical scales and naevus count was assessed using a four-point pictorial scale with descriptors of “none”, “a few”, “moderate” and “many” naevi. 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 categorised 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 (Table S1). Among controls, a higher proportion of females (i.e. mothers of twins) than males (fathers) gave a blood sample ($P<0.0001$), and more genotype data were available for people with fair skin ($P<0.0001$) and higher northern European ancestry score ($P=0.0002$).

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* polymerase (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 programme was used for the iPLEX reaction: initial denaturation was 30 sec at 94°C followed by 5 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 three 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 analysed 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 tumours with a metastatic (n=9) or unknown (n=4) behaviour, *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 naevi (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 naevus 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 naevus 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=0.02) (Table S2). 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 naevus category, freckling, skin colour, eye colour, and hair colour 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 naevus 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 and 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 colour, freckling, and high naevus 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 Table S3). Among controls, *MTAP* rs10757257, *PLA2G6* rs132985 and *IRF4* rs12203592 were the variants most significantly associated with naevus category (P=0.01, P=0.02, and P<0.0001, respectively) (Table S4) and were thus chosen for further analysis.

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 ($P_{homogeneity}$=0.0008). Also, we found significantly positive dose-effect relationships between naevus category and melanoma risk in all (P_{trend}<0.0001) but the older men sample (P_{trend}=0.68), and results were stronger in the children and the adolescents samples ($P_{homogeneity}$<0.0001).

For completeness, we also analysed the available SNPs that were not originally selected for further study, and the results were similar to those presented for the selected SNPs (Table S5). In addition, we examined the linkage disequilibrium patterns between SNPs in each of the studied loci in the control sample (Table S6). 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 naevus propensity and risk of melanoma on the trunk, and lower and upper limbs (P_{trend}<0.0001). For melanoma

on the head and neck, risks were significantly elevated with a moderate number of naevi, although somewhat attenuated for the highest naevus category (OR=1.44, 95% CI=0.61–3.38). The overall trend for head and neck melanoma remained strongly significant, however (P_{trend}=0.0002). Results in the highest naevus category differed significantly between melanoma on the trunk and melanoma on the head and neck ($P_{homogeneity}$=0.04).

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) ($P_{homogeneity}$=0.06) (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 naevus category and melanoma in SSM/NM and "other" melanomas. However, there was no significant or consistent trend between naevus category and LMM risk (P_{trend}=0.24), with marginally significant elevation in risk with the moderate category of naevus, 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 naevi, 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 tumour, 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 naevi, with lead SNPs (rs4636294 and rs2284063) that were different from those most significantly associated with naevi 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 naevus count (Duffy et al., 2010b), as well as skin, hair and eye colour (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, $P=4\times10^{-3}$ for the C allele). The C allele was associated with higher naevus 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 naevus count differed substantially in children (where the rs12203592*T allele increased total naevus 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, $P=2.5\times10^{-5}$) (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 naevus count in this sample (Table S2), and this finding has recently been replicated in a UK sample (Duffy et al., 2010b).

A recent study performed in the UK confirmed an association between naevi and variants of *MTAP* (rs7023329), *PLA2G6* (rs2284063) and *IRF4* (rs12203592) (Newton-Bishop et al., 2010). Number of naevi was significantly associated with the *MTAP* and *PLA2G6* SNPs but not with the *IRF4* SNP, whereas number of large naevi 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 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 naevi has also been confirmed in a recent familial case-control study on melanoma (Yang et al., 2010).

After adjustment for naevi 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 naevi resulted in loss of statistical significance and reduction of the associations towards unity. This indicates that the association between naevi and melanoma is not fully explained by *MTAP* genotype, and that the associations with naevi and *MTAP* are probably independent, possibly synergistic, while those observed in crude models for *PLA2G6* and *IRF4* are mainly driven through number of naevi. An alternative explanation for *MTAP* is that measurement error in the naevus 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 naevus phenotype (Newton-Bishop et al., 2010).

The “divergent pathways” hypothesis would predict that naevus 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 naevus category and melanoma site and type, which lend support to the hypothesis. Specifically, individuals with a high naevus 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 naevus count differed by age (Duffy et al., 2010b), and secondly that their effect through skin colour was opposite to that observed with naevus count: the rs12203592*C increased both adult naevus 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 tumour 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 naevi 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. Firstly, cases and controls were interviewed at different periods, and the instruments used to assess phenotype were very similar, but not identical. Naevus category was recorded using a semi-quantitative scale for cases, and a qualitative scale for controls, and semi-quantitative items for naevi and freckling showed moderate correlations with qualitative items in the Q-MEGA, ranging from 0.36 to 0.55 for naevi and from 0.30 to 0.53 for freckling (Baxter et al., 2008). Secondly, 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 and 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 naevus 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 naevus 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ABBREVIATIONS

BTNS	Brisbane Twin Naevus Study
CI	Confidence Interval
DNA	Deoxyribonucleic Acid
GWAS	Genome-Wide Association Study
HWE	Hardy-Weinberg Equilibrium
IRF4	Interferon Regulatory Factor 4
LMM	Lentigo Maligna Melanoma
MC1R	Melanocortin-1-Receptor
MTAP	Methylthioadenosine Phosphorylase
NM	Nodular Melanoma
NOS	Melanoma Not Otherwise Specified
OR	Odds-Ratio
PLA2G6	Phospholipase A2, Group VI
Q-MEGA	Queensland study of Melanoma: Environmental and Genetic Associations
SNP	Single Nucleotide Polymorphism
SSM	Superficial Spreading Melanoma
UVR	Ultraviolet Radiation

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Table 1

Characteristics of the study participants, Q-MEGA (1987–2005) (n=2497)

	Cases (n=1028)	Controls (n=1469)	p-value[a]
	n (%) or mean (SD)	n (%) or mean (SD)	
Age (years)[b]			
	41.8 (16.3)	43.6 (9.9)	0.11
Sex			
Male	484 (47.1%)	632 (43.0%)	0.04
Female	544 (52.9%)	837 (57.0%)	
Skin colour			
Fair or pale	841 (81.8%)	830 (56.5%)	<0.0001
Medium	155 (15.1%)	504 (34.3%)	
Olive or dark	32 (3.1%)	135 (9.2%)	
Hair colour			
Fair/blonde	246 (23.9%)	244 (16.6%)	<0.0001
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 colour			
Blue or grey	437 (42.5%)	596 (40.6%)	0.0008
Green or hazel	421 (41.0%)	541 (36.8%)	
Brown	170 (16.5%)	332 (22.6%)	
Freckling			
None	203 (19.8%)	396 (27.0%)	<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 naevi			
None	87 (8.5%)	83 (5.6%)	<0.0001
<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%)	<0.0001
50–74%	13 (1.2%)	32 (2.2%)	
75–99%	45 (4.4%)	50 (3.4%)	
100%	958 (93.2%)	1310 (89.2%)	

[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

Table 2

Risk allele frequencies for *MTAP*, *PLA2G6* and *RF4* variants in cases and controls, Q-MEGA (1987–2005) (n=2462)

			Cases (n=993)[a]											
		Controls (n=1469)	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														
	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														

			Cases (n=993)[a]											
		Controls (n=1469)	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)
	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

[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

Table 3

Odds-ratios and 95% confidence intervals for risk of cutaneous melanoma in relation to type of allele for selected gene variants and naevus 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)		
	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)	p-value[b]
MTAP												
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	0.61 (0.42–0.91)	0.22	0.90 (0.59–1.37)	0.0008
Naevus 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)	<0.0001
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)	<0.0001
			P_{trend}<0.0001		P_{trend}<0.0001		P_{trend}<0.0001		P_{trend}<0.0001		P_{trend}=0.68	

[a] Adjusted for ancestry score, age, number of naevi, freckling, skin colour and hair colour

[b] Test for homogeneity in estimates across subsamples

CI: Confidence Interval; OR: Odds-Ratio

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 naevus 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[†] (95% CI)	Prop.	Crude OR (95% CI)	Adjusted OR[b] (95% CI)	p-value[c]
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
Naevus 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}<0.0001	P_{trend}<0.0001		P_{trend}=0.0003	P_{trend}=0.0002		P_{trend}<0.0001	P_{trend}<0.0001		P_{trend}<0.0001	P_{trend}<0.0001	

Melanomas for which site was not specified were not reported in this table (n=35)

Adjusted for ancestry score, sex, age, number of naevi, freckling, skin colour and hair colour

Test for homogeneity in estimates between trunk melanoma and head and neck melanoma

I: 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 naevus 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* (95% CI)	Prop.	Crude OR (95% CI)	Adjusted OR[b] (95% CI)	Prop.	Crude OR (95% CI)	Adjusted OR[b] (95% CI)	p-value[c]
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
Naevus 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}<0.0001	P_{trend}<0.0001		P_{trend}=0.98	P_{trend}=0.24		P_{trend}<0.0001	P_{trend}<0.0001	

[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 naevi, freckling, skin colour and hair colour

[c] Test for homogeneity in estimates between SSM/NM and LMM