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Associations of 9p21 variants with cutaneous malignant melanoma, nevi, and pigmentation phenotypes in melanoma-prone families with and without CDKN2A mutations

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Abstract

Chromosome 9p21 has been implicated in the pathogenesis of cutaneous malignant melanoma (CMM). In addition to CDKN2A, the major known high-risk susceptibility gene for CMM, recent studies suggest that other 9p21 genes may be involved in melanoma/nevi development. To identify 9p21 variants that influence susceptibility to CMM and number of nevi in CMM-prone families with and without CDKN2A mutations, we analyzed 562 individuals (183 CMM) from 53 families (23 CDKN2A+, 30 CDKN2A−) for 233 tagging SNPs in 21 genes at 9p21. Single SNP- and gene-based regression analyses were used to assess the risk of CMM, nevi count, skin complexion, and tanning ability associated with these SNPs and genes. We found that SNP rs7023329 in the MTAP gene was associated with number of nevi (P_{trend}=0.003) confirming a recent finding by a genome-wide association study. In addition, three SNPs in the ACO1 gene, rs7855483 (P_{trend}=0.002), rs17288067 (P_{trend}=0.0009), and rs10813813 (P_{trend}=0.005), showed the strongest associations with CMM risk. None of the examined 9p21 SNPs was associated with skin complexion, whereas two SNPs, rs10964862 in IFNW1 (P_{trend}=0.003), and rs13290968 in TUSC1 (P_{trend}=0.0006), were associated with tanning ability. Gene-based analyses suggested that the ACO1 gene was significantly associated with CMM (P=0.0004); genes IFNW1 (P=0.002) and ACO1 (P=0.0002) were significantly associated with tanning ability. Our findings are consistent with recent proposals that additional 9p21 genes may contribute to CMM susceptibility in CMM-prone families. These genetic variants may, at least partially, exert their effects through nevi and tanning ability.

Keywords

melanoma; nevi; chromosome 9p21; SNP; CDKN2A

Introduction

Cutaneous malignant melanoma (CMM) is an etiologically heterogeneous disease. Established risk factors for CMM include number of nevi, pigmentation phenotype, and sun exposure [1]. Genetic predisposition also plays an important role in melanoma etiology with

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two high-penetrance melanoma susceptibility genes, CDKN2A and CDK4, identified to date. Germline mutations of CDKN2A have been described in ~20% of melanoma families with multiple individuals affected with CMM [2–3]. CDK4 is located on 12q14 and its mutations are very rare. Together, these two genes account for melanoma susceptibility in a small proportion of melanoma-prone families, suggesting the existence of other genetic factors.

Molecular and cytogenetic studies have demonstrated that chromosome 9p21 deletions represent a frequent genetic alteration in melanoma tumors [4]. This region contains two putative tumor suppressor genes for CMM: CDKN2A (the major known high-risk susceptibility gene for CMM encoding two proteins p16 and p14ARF) and CDKN2B. These genes play important roles in cell cycle regulation and cellular senescence through the p53 and Rb signaling pathways. In addition, two independent genome-wide linkage analyses searching for loci for nevus count found evidence of linkage to the CDKN2A region on 9p21[5–6], suggesting that this region may harbor genetic variants for both nevi and CMM. However, CDKN2A may not be the only locus on 9p21 that influences melanoma risk. Numerous melanoma-prone families that showed evidence for linkage to 9p21 did not carry CDKN2A/2B mutations [7–9], suggesting that additional loci at chromosome 9p21 may also be involved in melanoma susceptibility. Anril, an antisense non-coding RNA that is located within the CDKN2A/2B locus, has been suggested to be involved in melanoma-prone families with no identified CDKN2A mutations [10]. Recent genome-wide association studies (GWAS) have shown that variants in Methylthioadenosine phosphorylase (MTAP), a gene in 9p21 next to the CDKN2 complex, were significantly associated with the risk of melanoma and cutaneous nevi [11–12]. In addition, loss of MTAP expression, which exerts a tumor-promoting effect, has been observed in melanoma and a variety of other tumors [13], suggesting that MTAP may function as a tumor suppressor gene. Furthermore, the 9p21 region includes a cluster of type I interferon (IFN) genes. The type I interferons are pleiotropic cytokines that exhibit strong antiviral, antiproliferative and immunomodulatory effects. Indeed, IFN-α is often used for the treatment of metastatic CMM [14]. Given the importance of the 9p21 region in the development of CMM, the goal of this study was to identify variants in genes located on 9p21 that influence susceptibility to CMM and number of nevi in CMM-prone families with and without CDKN2A mutations. Since certain pigmentation phenotypes are well-known risk factors for CMM and most low- to moderate-risk susceptibility genes for CMM identified so far are related to pigmentation, we also evaluated the associations of 9p21 SNPs with pigmentation phenotypes including skin complexion and tanning ability.

Materials and Methods

Study population

The study population of this family study has been previously described [15–16]. In brief, US families with at least two living first degree relatives with a history of invasive melanoma were ascertained through health care professionals or self referrals. All family members willing to participate in the study underwent a full-body skin examination for extensive phenotypes (type and total number of nevi, extent of freckling, skin complexion, evidence for solar injury, and hair and eye color) and completed risk factor questionnaires for sun-related exposures such as tanning ability. All diagnoses of melanoma were confirmed by histologic review of pathologic material, pathology reports, or death certificates for deceased CMM cases. The current study was based on 53 families (23 families segregating CDKN2A mutations and 30 families without CDKN2A mutations). All CMM cases with DNA available were selected. Two controls were selected for each case and they included unaffected family members and genetically unrelated spouses. Only adult controls were selected to minimize disease misclassification. All study participants were

Caucasians. The study was approved by the National Cancer Institute Clinical Center Institutional Review Board and conducted according to the Declaration of Helsinki. Informed consent was obtained from all participants.

The outcome variables analyzed in this study included CMM, number of nevi, and pigmentation phenotypes that were significantly associated with CMM risk in the families including skin complexion and tanning ability. Hair color and eye color were not strongly associated with CMM risk in the families (Table 1) and therefore were not analyzed in this study. Freckling and solar injury were not analyzed as outcome variables in this study due to missing data (freckling) and confounding from sun exposure (solar injury).

SNP selection

Two hundred and fifty two tag SNPs for 21 genes located at chromosome 9p21 region (19.9–32.8Mb) were selected for this study. The selected genes included IFN gene cluster, CDKN2A, CDKN2B, Anril, and MTAP (see genes and SNPs in Supplement Table 1). For each gene, tag SNPs were genotyped at the NCI Core Genotyping Facility (Advanced Technology Center, Gaithersburg, MD; http://snp500cancer.nci.nih.gov) using a custom-designed iSelect Infinium assay (Illumina, www.illumina.com), which included a total of 27,904 tag SNPs that were selected for a variety of cancers. Tag SNPs were selected using a minimum minor allele frequency (MAF) criterion of MAF≥5% based upon HapMap data for Caucasian (CEU) and Yoruban (YRI) samples using Tagzilla, a software that implements a tagging algorithm based on pairwise linkage disequilibrium[17]. For each original target gene, SNPs within the region spanning 20 kb 5’ of the start of transcription (exon 1) to 10 kb 3’ of the end of the last exon were grouped using a binning threshold of r^2>0.8 to define a gene/region. When there were multiple transcripts available for genes, only the primary transcript was assessed.

Quality assessment

The iSelect panel was validated with all 3 HapMap populations (CEU, YRI, Japan & China) and SNPs with low completion (<90%) and low concordance (<95%) were excluded (n=19 for 9p21 SNPs). None of the remaining 233 9p21 SNPs showed deviation from Hardy–Weinberg equilibrium (HWE) among founders at P<0.001. Only one marker (rs12553951) showed evidence for deviation from HWE at P<0.01. This marker was not excluded from the analysis but was treated with caution. Among 586 genotyped samples, 20 were excluded due to low completion (<90%, n=12) and Mendelian inconsistencies (n=8). Four individuals were further removed from all analyses due to missing CMM status.

Statistical analysis

Conditional logistic regression models were used to estimate the trend p-value for the association between each dichotomized outcome variable (CMM [no, yes], skin complexion [dark/medium, pale/fair], and tanning ability [tan/little burn, burn/little tan]) and each SNP, using codominant coding for genotypes (0,1,2) with the homozygote of the common allele as the reference group. Odds ratios (ORs) and 95% confidence intervals (CIs) for each heterozygous and homozygous rare genotype were also calculated. Heterozygous and homozygous rare genotypes were combined when the number of subjects with homozygous minor alleles was less than 10. Conditioning on families was used to account for family ascertainment and differences in disease prevalence among families. While this approach ignores residual familial correlations among family members, it gives estimates that are attenuated toward the null and is thus conservative[18]. We categorized numbers of nevi into four groups (0–24, 25–49, 50–99, and 100+) and assessed the associations between number of nevi and genotypes using a generalized estimating equations (GEE) approach to account for correlations among members of the same family[19]. Odds ratios (ORs), 95%

confidence intervals (CIs), and tests for trend were computed using cumulative logistic regression for ordinal outcomes (PROC GENMOD, SAS 9.1). The working correlation matrix was the independent correlation matrix. We also analyzed the number of nevi as a Box-Cox transformed continuous variable (raw nevi count) using linear regression and obtained similar results (data not shown). All analyses were adjusted for age, sex, and CDKN2A mutation status. CMM was adjusted for in all analyses with non-CMM outcome variables. None of the examined SNPs was significant after Bonferroni correction ($P<0.05/233$ [0.00022]). Given that our study is exploratory, we reported SNPs showing suggestive associations ($P_{trend}<0.01$). For SNPs showing suggestive associations, a comprehensive model including number of nevi, solar injury, and MC1R as a surrogate for pigmentation characteristics, was also fit to the data. Most pigmentation phenotypes, such as red hair color, poor tanning ability, pale/fair skin complexion and extensive freckling were previously associated with MC1R variants in the CDKN2A mutation positive families [15]. MC1R variants were coded as 0 = no variant, 1 = single variant, 2 = multiple variants. Associated SNPs were also analyzed separately in CDKN2A-positive and negative families to assess effect modification by CDKN2A status. Interactions between CDKN2A status and SNPs were also formally tested by including an interaction term (Wald statistics). PedGenie, a program designed for genetic association testing in extended pedigrees [20], was also used and similar results were obtained (data not shown).

Gene-based analyses were performed on the 21 genes to assess the significance of the joint effect of multiple SNPs genotyped in each gene on each outcome. P-values were computed using rank-truncated test statistics and a permutation-based sampling procedure (20,000 permutations) in the same regression models (conditional logistic regression for CMM, skin complexion, and tanning ability, and cumulative logistic regression for number of nevi) adjusted for age, gender, CDKN2A, and CMM (for non-CMM outcomes), taking into account the number of SNPs genotyped in each gene and their LD structure[21]. We used a Bonferroni correction to account for the number of genes tested, and thus used $P < 0.05/21$ (0.0024) to define statistical significance. All analyses were performed using SAS software, version 9.1 (SAS Institute, Inc., Cary, NC).

Results

In total, there were 183 genotyped CMM cases and 379 genotyped unaffected individuals included in the analysis (Table 1). The distribution of age, gender, CDKN2A status, pigmentation phenotypes, and MC1R variants by CMM status is shown in Table 1. In summary, CDKN2A, skin complexion, number of nevi, freckles, solar injury, tanning ability, and MC1R variants were significantly associated with CMM risk in these families.

9p21 SNPs associated with CMM

Among 233 9p21 SNPs analyzed, the strongest associations were seen for three SNPs, rs7855483 ($P_{trend}=0.002$), rs17288067 ($P_{trend}=0.0009$), and rs10813813 ($P_{trend}=0.005$), all in the ACO1 gene. Odds ratios (ORs) for risk alleles of these SNPs ranged from 2.7 to 3 (Table 2). SNPs rs7855483 and rs17288067 were highly correlated ($r^2>0.8$). The adjustment for other covariates including number of nevi, solar injury, and MC1R variants (Model 2) decreased ORs for each SNP (Table 2). ORs for the 3 SNPs were similar among CDKN2A carriers and non-carriers (Supplementary Table 2).

9p21 SNPs associated with number of nevi

SNP rs7023329 in the MTAP gene showed the strongest association with number of nevi ($P_{trend}=0.003$; OR=0.44, 95% confidence interval [CI]=0.25–0.76, comparing [GG] to reference [AA], Table 3). Results were similar with further adjustment of solar injury and

MC1R variants (Table 3). The associations were stronger among CMM cases and CDKN2A carriers compared to non-cases and non-carriers (Supplementary Table 3). Figure 1 shows that subjects with the protective allele (AG or GG) had smaller median numbers of nevi than subjects homozygous for the common allele (AA) in a dose-dependent manner among both CMM cases and non-cases. The decrease in number of nevi comparing subjects with GG to subjects with AA was 42% for CMM cases and 34% for non-cases.

9p21 SNPs associated with pigmentation phenotype and tanning ability

None of the examined 9p21 SNPs showed suggestive associations ($P_{trend}<0.01$) with skin complexion. Two SNPs, rs10964862 in IFNW1 ($P_{trend}=0.003$), and rs13290968 in TUSC1 ($P_{trend}=0.0006$), were associated with tanning ability. SNP rs10964862 in IFNW1 showed an allele-dose-dependent decreased risk of burning/little tan, whereas the minor allele of SNP rs13290968 conferred an increased risk of burning (Table 4). Adjusting for number of nevi, solar injury, and MC1R variants did not change the results. SNP rs10964862 in IFNW1 had a stronger effect among CMM unaffecteds, whereas SNP rs13290968 in TUSC1 had a stronger effect among CMM cases. Both SNPs had similar effects among CDKN2A carriers and non-carriers, although the association for rs10964862 was slightly stronger among non-carriers (Supplementary Table 4).

Gene-based associations

Consistent with single SNP-based analyses, gene-based analyses suggested that the ACO1 gene was significantly associated with CMM (P=0.0004). In addition, genes IFNW1 (P=0.002) and ACO1 ($P_{trend}=0.0002$) were significantly associated with tanning ability (Table 5). No other genes were significantly associated with any other examined outcome at a Bonferroni corrected P<0.0024.

Discussion

In this study, we evaluated the associations of 233 SNPs in 21 genes located on chromosome 9p21 with the risk of CMM, number of nevi, skin complexion, and tanning ability in CMM-prone families with and without CDKN2A mutations. Our results confirm a previous GWAS finding that a SNP (rs7023329) in MTAP was associated with number of nevi [12]. In addition, the findings from our study suggested that variants in the ACO1 gene may be associated with the risk of CMM and tanning ability. Thus, these data further support the hypothesis that 9p21 may harbor susceptibility genes, in addition to the CDKN2A/2B complex, that are important in the development of CMM. Finally, these genetic variants may also influence nevi and pigmentation phenotypes independently of CMM risk or through a common genetic pathway.

A high nevus count is an established risk factor for CMM[22] in the general population as well as in melanoma-prone families. Therefore, identification of genetic factors for the development of benign nevi may facilitate our understanding of the etiology of CMM. In this study, we found that a SNP in the MTAP gene, rs7023329, was associated with nevus count in a dose-dependent manner. Our finding is consistent with the GWAS data by Falchi et al. that showed that multiple MTAP SNPs were significantly associated with the development of nevi and that rs7023329 was among the SNPs with lowest P values identified (combined $P=2.6\times10^{-11}$) [12]. This SNP was also significantly associated with CMM risk in a GWAS published by the GenoMEL consortium analyzing 1,650 CMM cases and 4,336 controls [11]. However, nevus count was not available and therefore not adjusted for in the GenoMel study. In the GWAS by Falchi et al., the MTAP SNPs were no longer significantly associated with CMM risk after adjustment for nevus count, suggesting that the association between CMM risk and the MTAP SNPs was via nevi. In our study, the

association between nevi and the MTAP SNP rs7023329 was seen in both CMM cases and controls, whereas no association was seen for CMM regardless of nevi adjustment, further suggesting that MTAP may be more directly involved in nevi susceptibility. It is not surprising that the effect of this variant was stronger among CMM cases and CDKN2A carriers in our study since nevus count was significantly higher among cases and mutation carriers compared to unaffecteds and non-carriers.

MTAP encodes an enzyme that plays a major role in polyamine metabolism and is important for the salvage of both adenine and methionine. MTAP catalyzes the phosphorylation of 5'-dideoxy-5'-methylthioadenosine (MTA), which acts as a potent inhibitor of polyamine aminopropyltransferase and methyltransferases. MTAP is expressed abundantly in normal cells[23] but is deficient in many cancers [24–27]. It has been hypothesized that loss of MTAP in tumors is due to the co-deletion of this gene with the CDKN2A/2B complex[24, 27]. However, there is growing evidence that MTAP may directly impact tumor cell activity independently of CDKN2A/2B. Loss of MTAP in malignant melanoma was shown to result in a higher invasive potential that was mediated by the increased accumulation of MTA [13]. Expression of MTAP was inversely correlated with the progression of melanoma and re-expression of MTAP in melanoma cells significantly reduced the invasive potential [28]. A recent study found that mice heterozygous for germ-line mutations in MTAP died prematurely of T-cell lymphoma [29], further suggesting that MTAP itself may be a tumor-suppressor gene.

In our study, the only 9p21 gene examined that was associated with CMM risk was ACO1, both at the SNP and gene level. ACO1, encoding Aconitase 1 and also known as iron regulatory protein 1 (IRP1), is a key protein in maintaining iron homeostasis. Iron is an essential micronutrient in the human body but can be toxic at either deficiency or excess through increasing oxidative stress, causing DNA damage, and increasing genomic instability [30–32]. Iron overload has been linked to a variety of human cancers [33–37] and iron deficiency has also been associated with gastrointestinal carcinogenesis [32]. In normal cells, iron level is under a tight control mostly by IRPs. In response to cytosolic iron pool, IRP1 controls iron availability through activating/inhibiting the translation of mRNA encoding the cytosolic iron-binding protein, ferritin, and the degradation of transferrin receptor [38]. IRP1, as well as ferritin and transferring receptors, have been associated with the progression of cancers including melanoma [39–43]. Overexpression of IRP1 has been observed to suppress tumor formation in xenografts, suggesting a direct link between IRP1 and cancer [44]. These findings suggested that altered iron regulation might play an important role in melanoma development and progression.

Our analyses also identified SNPs in IFNW1 and TUSC1 genes that were associated with poor tanning ability, which is a strong risk factor for melanoma. IFNW1 belongs to the type I interferon family encoded by a cluster of single-exon genes in a 400-bp region on chromosome 9p21 exhibiting immune-regulatory activities. It has been demonstrated that genetic variants in cytokine genes may influence susceptibility to melanoma [45–47]. Ultraviolet radiation can not only directly cause DNA damage but also depress the immune response in the skin, which can permit the growth of emerging tumors produced by the effects of DNA damage [48]. TUSC1, tumor suppressor candidate 1, is a novel intronless gene and was shown to have reduced expression in lung cancer cells suggesting a possible tumor suppressor role [49]. Together with our data, these findings suggest that multiple genes on 9p21 are functionally important in cancer development, although it is also possible that certain genetic variant(s) in this region may exert effects through altering the expression of CDKN2A/CDKN2B and/or other genes in the region rather than or in addition to their own functional relevance.

Our study is limited by the small sample size and therefore should be viewed as an exploratory study. In addition, the families were ascertained primarily through self- or physician-referral, which might affect the generalizability of the results. However, the families, including those segregating CDKN2A mutations and those who do not have known mutations, with their rich collection of exposure and pigmentation data, represent a unique resource to study genes located on 9p21 and their relationships with CMM, nevi, and important pigmentation phenotypes. We used conditional logistic regression to account for family ascertainment, which is known to result in estimates that are attenuated towards the null and is thus conservative. In addition, our careful selection of genes in the entire 9p21 region and a gene-based permutation analysis allowed us to comprehensively evaluate 9p21 genes. In summary, we identified genetic variants in several 9p21 genes that were related to the risk of CMM, nevi, and tanning ability in CMM-prone families with and without CDKN2A mutations. Future studies of familial and sporadic melanoma with large sample sizes are needed to better characterize the relationship between genetic variants in this region and melanoma risk.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Abbreviations

CMM	Cutaneous malignant melanoma
SNP	Single nucleotide polymorphism
GWAS	Genome-wide association study
MAF	Minimum minor allele frequency
OR	Odds ratio
95% CI	95% confidence interval
GEE	Generalized estimating equations

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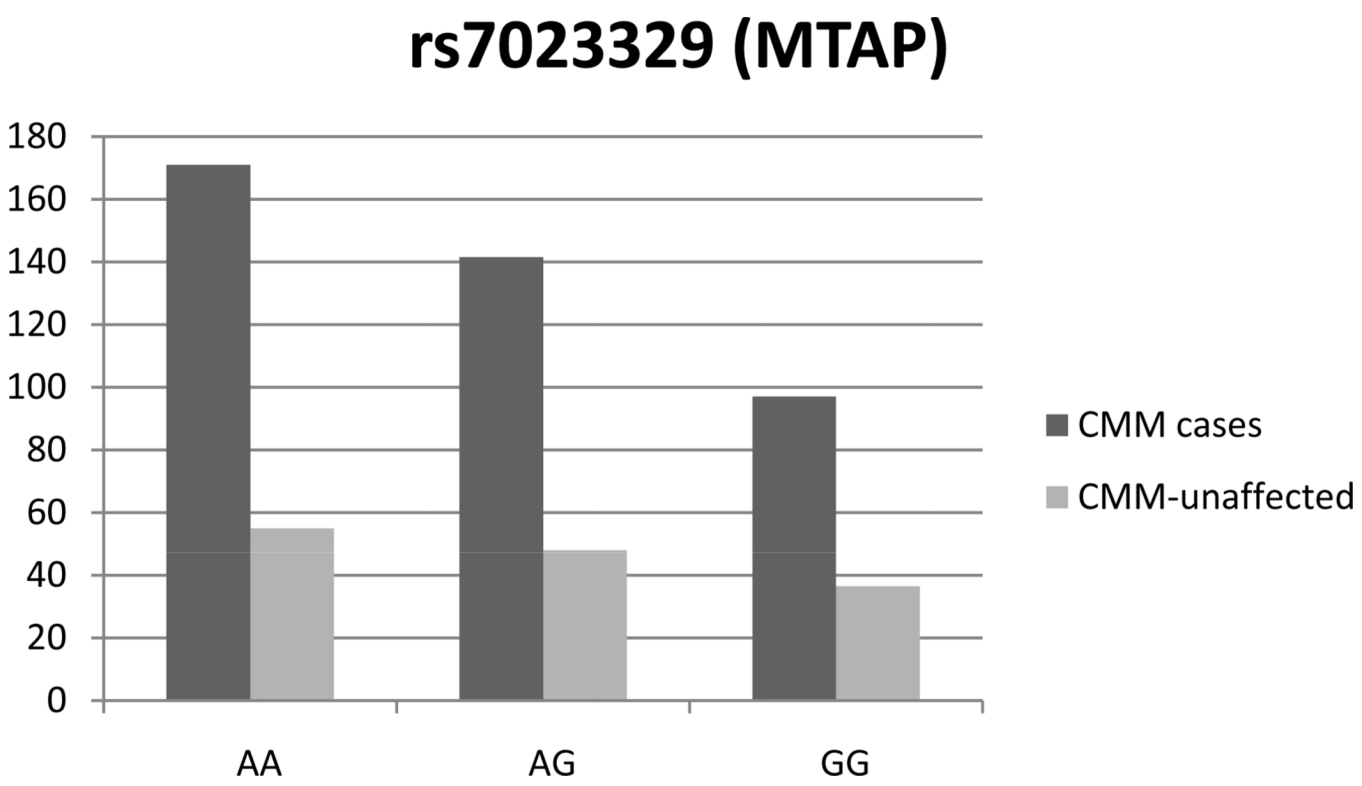

Figure 1.
Distribution of median number of nevi by MTAP SNP genotype among CMM cases and CMM-unaffected people.

Table 1

Distribution of age, gender, CDKN2A status, pigmentation phenotype, and sun exposure variables in the 53 families by CMM status.

Variables	CMM cases (N=183)		Controls (N=379)		*P*
	N	%	N	%	
Age					
≤30	26	14.2	77	20.3	
30–40	36	19.7	82	21.6	
40–50	55	30.1	97	25.6	
50–60	30	16.4	70	18.5	
60+	36	19.7	53	14.0	0.17
Gender					
Female	92	50.3	218	57.5	
Male	91	49.7	161	42.5	0.11
CDKN2A					
Negative	94	51.9	319	85.8	
Positive	87	48.1	53	14.2	<0.0001
Skin type					
Dark/Medium	24	14.4	115	32.0	
Pale/Fair	143	85.6	244	68.0	<0.0001
Eye color					
Black/Brown	32	18.9	90	25.1	
Hazel	40	23.7	85	23.7	
Green/Gray	25	14.8	35	9.7	
Blue	72	42.6	149	41.5	0.21
Hair color					
Black/Brown	70	41.4	168	47.2	
Blonde brown/Light brown	49	29.0	103	28.9	
Blonde	24	14.2	51	14.3	
Red	26	15.4	34	9.6	0.24
Moles					
0–24	9	5.4	122	33.5	
25–49	21	12.5	66	18.1	

Variables	CMM cases (N=183)		Controls (N=379)		*P*
	N	%	N	%	
50–99	27	16.1	81	22.3	
100+	111	66.1	95	26.1	<0.0001
Freckles					
None/Few	28	20.0	120	40.0	
Moderate	36	25.7	75	25.0	
Many	76	54.3	105	35.0	<0.0001
Solar injury					
None/Mild	79	47.3	243	66.8	
Moderate	53	31.7	73	20.1	
Severe	35	21.0	48	13.2	0.0001
Tanning ability					
Tan/Little burn	65	42.8	194	57.4	
Burn/Little tan	87	57.2	144	42.6	0.003
MC1R					
Wild type	15	8.2	70	19.4	
1 nonsynonymous variant	88	48.1	182	50.4	
2 nonsynonymous variants	80	43.7	109	30.2	0.0003

P values were obtained by comparing CMM cases to unaffected people using χ^2 test.

Table 2

Associations of SNPs in the 9p21 region with CMM in 53 melanoma families[1].

SNP	Controls		Cases		Model1[2]				Model2[3]			
	N	%	N	%	OR	95% CI		P	OR	95% CI		P
ACO1												
rs7855483[4]												
CC	255	67.3	106	57.9	Ref				Ref			
CT+TT	124	32.7	77	42.1	2.65	1.52	4.64	0.0006	2.08	1.07	4.04	0.03
P trend								0.002				0.08
rs17288067[4]												
GG	257	68.4	105	58.0	Ref				Ref			
GA+AA	119	31.6	76	42.0	2.87	1.64	5.02	0.0002	2.14	1.10	4.16	0.02
P trend								0.0009				0.09
rs10813813												
AA	147	38.8	66	36.1	Ref				Ref			
AT	178	47.0	90	49.2	2.03	1.15	3.57	0.01	1.68	0.87	3.23	0.12
TT	54	14.2	27	14.7	3.00	1.34	6.72	0.008	2.53	1.02	6.28	0.05
P trend								0.005				0.04

1. ORs and P values are obtained from likelihood ratio test in conditional logistic regression with CMM as the outcome variable.

2. Model 1: age, gender, and CDKN2A adjustment.

3. Model 2: age, gender, CDKN2A, number of nevi, solar injury, and MC1R adjustment.

4. SNPs rs7855483 and rs17288067 were highly correlated (r^2>0.8).

Table 3

Associations of rs7023329 in MTAP with the number of moles in 53 melanoma families[1].

SNP	Number of nevi								Model1[2]				Model2[3]			
	0–24		25–49		50–99		100+									
	N	%	N	%	N	%	N	%	OR	95% CI		P	OR	95% CI		P
MTAP rs7023329																
AA	23	17.4	25	28.7	28	25.9	66	31.6	Ref				Ref			
AG	67	50.8	38	43.7	53	49.1	104	49.8	0.69	0.43	1.11	0.12	0.77	0.48	1.24	0.28
GG	42	31.8	24	27.6	27	25.0	39	18.7	0.44	0.25	0.76	0.004	0.53	0.31	0.93	0.03
P trend												0.003				0.03

1. ORs and P values estimated by cumulative logistic regression with number of moles as outcome variable (as an ordinal variable with 4 categories); independent variables; familial correlation adjusted by generalised estimating equations.

2. Model1: age, gender, CDKN2A, and CMM adjustment.

3. Model2: age, gender, CDKN2A, CMM, MC1R, and solar injury adjustment.

Table 4

Associations of 9p21 SNPs with tanning ability in 53 melanoma families[1].

SNP	Tan/Little burn		Burn/Little tan		Model1[2]				Model2[3]			
	N	%	N	%	OR	95% CI		P	OR	95% CI		P
IFNW1												
rs10964862												
TT	100	38.5	118	50.4	Ref				Ref			
TG	129	49.6	97	41.5	0.59	0.38	0.92	0.02	0.60	0.38	0.94	0.03
GG	31	11.9	19	8.1	0.35	0.16	0.78	0.01	0.32	0.14	0.74	0.007
P trend								0.003				0.002
TUSC1												
rs13290968												
GG	180	78.6	133	64.3	Ref				Ref			
GC+CC	49	21.4	74	35.7	2.31	1.38	3.89	0.002	2.25	1.31	3.84	0.003
P trend								0.0006				0.002

1. ORs and P values are obtained from likelihood ratio test in conditional logistic regression with tanning ability (comparing burn/little tan to tan/little burn) as the outcome variable.

2. Model 1: age, gender, CMM, and CDKN2A adjustment.

3. Model 2: age, gender, CDKN2A, CMM, number of nevi, solar injury, and MC1R adjustment.

Fam Cancer. Author manuscript; available in PMC 2011 December 8.

Table 5

Trend p-values for associations between genes located on 9p21 and CMM, number of nevi, skin complexion, and tanning ability using gene-based analysis[1].

Gene	#SNP	CMM	Nevi	Skin complexion	Tanning
IFNB1	23	0.79	0.97	0.25	0.02
IFNW1	12	0.97	0.50	0.49	0.002
IFNA21	4	0.51	0.06	0.48	0.27
IFNA10	2	0.20	0.07	0.32	0.41
IFNA16	2	0.55	0.09	0.59	0.75
IFNA17	1	0.14	0.12	0.60	0.43
IFNA14	3	0.24	0.39	0.52	0.32
IFNA5	3	0.29	0.18	0.66	0.50
KLHL9	2	0.40	0.71	0.20	0.63
IFNA6	4	0.21	0.62	0.55	0.94
IFNA2	7	0.36	0.72	0.28	0.72
IFNA8	11	0.19	0.68	0.14	0.54
IFNA1	7	0.96	0.38	0.95	0.34
IFNE1	5	0.33	0.92	0.84	0.79
MTAP	17	0.85	0.06	0.26	1.00
CDKN2A	25	0.79	0.01	0.90	0.81
CDKN2B	17	0.99	0.72	0.30	0.03
ANRIL	15	0.68	0.24	0.12	0.04
TUSC1	12	0.02	0.44	0.50	0.62
PLAA	14	0.99	0.89	0.05	0.91
IFNK	9	0.38	0.45	0.95	0.15
ACO1	35	0.0004	0.90	0.56	0.0002

1. p-values were computed using rank-truncated test statistics and a permutation-based sampling procedure (20,000 permutations).