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Association of genetic variants in *CDK6* and *XRCC1* with the risk of dysplastic nevi in melanoma-prone families

Xueying Liang[1,2], **Ruth M. Pfeiffer**[1], **Wen-Qing Li**[1], **Myriam Brossard**[3,4,5], **Laura S. Burke**[1], **William Wheeler**[6], **Donato Calista**[7], **Maria Concetta Fargnoli**[8], **Paola Ghiorzo**[9], **Ketty Peris**[8], **Giovanna Bianchi-Scarra'**[9], **Valerie Chaudru**[3,4,10], **Diana Zelenika**[11], **Dennis Maeder**[1], **Laurie Burdette**[1], **Meredith Yeager**[1], **Stephen Chanock**[1], **Maria Teresa Landi**[1], **Florence Demenais**[3,4], **Margaret A. Tucker**[1], **Alisa M. Goldstein**[1], and **Xiaohong R. Yang**[1,*]

[1]Division of Cancer Epidemiology and Genetics, NCI/NIH, Bethesda, MD, USA

[2]Office of In Vitro Diagnostics and Radiological Health, CDRH/FDA, Silver Spring, MD, USA

[3]INSERM, UMR-946, Genetic Variation and Human Diseases Unit, Paris, F-75010, France

[4]Université Paris Diderot, Sorbonne Paris Cité, Institut Universitaire d'Hématologie, Paris, F-75010, France

[5]Université Paris Sud, Le Kremlin Bicêtre, F-94276, France

[6]Information Management Services, Inc., Rockville, MD, USA

[7]Department of Dermatology, M. Bufalini Hospital, Cesena, Italy

[8]Department of Dermatology, University of L'Aquila, L'Aquila, Italy

[9]Department of Internal Medicine, University of Genoa, Genoa, Italy; Laboratory of Genetics of Rare Hereditary Cancers, IRCCS AOU San Martino-IST, Genoa, Italy

[10]Université d'Evry-Val d'Essonne, Evry, F-91025, France

[11]Commissariat à l'Energie Atomique (CEA), Institut de Génomique, Centre National de Génotypage, Evry, F-91000, France

Abstract

Dysplastic nevi (DN) is a strong risk factor for cutaneous malignant melanoma (CMM), and it frequently occurs in melanoma-prone families. To identify genetic variants for DN, we genotyped 677 tagSNPs in 38 melanoma candidate genes that are involved in pigmentation, DNA repair, cell cycle control, and melanocyte proliferation pathways in a total of 504 individuals (310 with DN, 194 without DN) from 53 melanoma-prone families (23 *CDKN2A* mutation positive and 30 negative). Conditional logistic regression, conditioning on families, was used to estimate the association between DN and each SNP separately, adjusted for age, sex, CMM and *CDKN2A*

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Correspondence to: Xiaohong R. Yang, Division of Cancer Epidemiology and Genetics, NCI/NIH, Bethesda, MD, USA. Tel: 301-594-7802; Fax: 301-402-4489; Royang@mail.nih.gov.

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status. *P*-values for SNPs in the same gene were combined to yield gene-specific p-values. Two genes, *CDK6* and *XRCC1*, were significantly associated with DN after Bonferroni correction for multiple testing (*P*=0.0001 and 0.00025, respectively), whereas neither gene was significantly associated with CMM. Associations for *CDK6* SNPs were stronger in *CDKN2A* mutation positive families (rs2079147, $P_{interaction}$=0.0033), whereas *XRCC1* SNPs had similar effects in mutation-positive and negative families. The association for one of the associated SNPs in *XRCC1* (rs25487) was replicated in two independent datasets (random effect meta-analysis: *P*<0.0001). Our findings suggest that some genetic variants may contribute to DN risk independently of their association with CMM in melanoma-prone families.

Introduction

Cutaneous malignant melanoma (CMM) is an etiologically heterogeneous disease with genetic, host, and environmental factors, and their interactions contributing to its development (Tucker and Goldstein, 2003). Dysplastic nevi (DN) is a strong risk factor for melanoma and occur frequently in melanoma-prone families (Tucker *et al.*, 2002). Previous studies have suggested a genetic component for DN, but to date no candidate genes have been identified. DN occurs with high prevalence in high-risk melanoma-prone families with germline mutations in *CDKN2A*, a major susceptibility gene for CMM, suggesting *CDKN2A* might confer risk for the development of DN. However, there is a poor correlation within families between *CDKN2A* gene-carrier status and DN (Bishop *et al.*, 2000; Cannon-Albright *et al.*, 1994; Hussussian *et al.*, 1994). In addition, DN occurs in melanoma-prone families with and without *CDKN2A* mutations with similar frequency (Tucker *et al.*, 2002). These findings suggest that additional susceptibility genes or other genetic or epigenetic mechanisms involving *CDKN2A* contributing to the development of DN may exist in melanoma-prone families. Identifying genetic variants for DN may enhance our understanding of CMM susceptibility. Therefore, in this study, we explored the associations between DN phenotype and common genetic variants in 38 candidate genes selected based on functional relevance in melanoma-prone families with and without known *CDKN2A* mutations.

Results

A total of 677 tagSNPs in 38 melanoma candidate genes were tested in 310 individuals with DN and 194 DN-unaffected subjects (4 with CMM) from 53 melanoma-prone families with and without *CDKN2A* mutations. The distribution of age, gender, *CDKN2A* status, CMM, *MC1R*, pigmentation phenotype, and sun exposure variables are listed in Table 1. As expected, age, *CDKN2A*, CMM, number of moles, tanning ability, skin type, hair color and *MC1R* were significantly associated with DN in these families.

Age, gender, *CDKN2A* mutation status, and CMM status were adjustment variables in all DN analyses. Table 2 shows the six genes with gene-based *P* value < 0.05 for DN associations. None of these genes were significantly associated with CMM. Two genes, *CDK6* (*P*=0.0001) and *XRCC1* (*P*=0.0003), were significantly associated with DN after Bonferroni correction for multiple testing (Table 2). Among genotyped SNPs in these two

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genes, 4 out of 18 (none in linkage disequilibrium [LD]) SNPs in *CDK6* and 4 out of 13 (2 in LD) SNPs in *XRCC1* had single SNP *P* values <0.05. Odds ratios (ORs) and 95% confidence intervals (CIs) for the most significant SNPs in these two genes are shown in Table 3. Further adjustment by pigmentation phenotypes (eye color, hair color, skin type, freckles), number of moles, solar injury, and *MC1R* variants did not change the results noticeably (data not shown). Analyses comparing DN cases to unaffected family members and unrelated spouses separately generated similar results (data not shown). In addition, since DN may disappear with age, we further restricted our analyses to subjects younger than 60 years old, and the associations were similar (data not shown). To evaluate whether the associations between these SNPs and DN were influenced by CMM status, we restricted the analyses to CMM-unaffected subjects and obtained similar results (Table 3).

To evaluate whether these genetic variants had similar effects in families with and without *CDKN2A* mutations, we analyzed the top four SNPs with *P* values < 0.05 in *CDK6* and *XRCC1*, respectively, in *CDKN2A* mutation positive and negative families separately. The associations between *XRCC1* SNPs and DN were similar in both sets of families (Table 4). In contrast, the associations between *CDK6* SNPs and DN were observed in *CDKN2A* mutation positive families only ($P<0.05$ for all 4 SNPs), but not in mutation negative families ($P>0.2$ for all 4 SNPs, Table 4). However, except for rs2079147, which showed statistically significant interaction with *CDKN2A* mutation status ($P=0.0033$), effects for the other three SNPs (rs1005346, rs2237570, and rs2285332) were similar among *CDKN2A*-mutation carriers and non-carriers in mutation-positive families (data not shown).

Two of the top four SNPs in *CDK6* in *XRCC1* were also genotyped/imputed in the two replication datasets, one consisting of 489 French probands and their relatives in a nevus family-based study and the other consisting of 545 melanoma-unaffected controls from three case-control studies of sporadic melanoma in Italy, but neither was significantly associated with nevus count or DN in either dataset (data not shown). Three of the top four SNPs in *XRCC1* were genotyped/imputed in each of the two replication datasets; among them, two were genotyped/imputed in all three datasets (Table 5). The same allele (allele C) in one SNP, rs25487, was significantly associated with DN in the Italian study ($P=0.005$) and showed a borderline association with nevus count in the French study ($P=0.084$). This SNP showed a significant association with DN/nevi ($P<0.0001$) in the meta-analysis combining data from all three datasets. The most significant SNP in the original dataset, rs1001581 in *XRCC1*, also showed a significant association ($P=0.0034$) in the meta-analysis, but this SNP is in strong LD ($r^2>0.8$) with rs25487.

Discussion

DN is a strong risk factor for CMM and occurs often in CMM high-risk families. Although previous studies suggested a genetic component for DN, no genes for DN have been identified yet. We systematically evaluated 38 candidate genes in several biologically relevant pathways to identify common genetic variants that are associated with DN in melanoma-prone families with and without *CDKN2A* mutations. We found that, two genes, *CDK6* and *XRCC1*, were significantly associated with DN susceptibility in our analyses.

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XRCC1 is directly involved in the DNA base excision and single-break repair pathways. A polymorphism of this gene, rs25487 (Arg399Gln), has been associated with reduced DNA repair capacity (Duell *et al.*, 2000; Lunn *et al.*, 1999). However, its role in cancer susceptibility seems to be complex. The same allele has been associated with both increased and reduced risk depending on disease phenotype and exposure (Karahalil *et al.*, 2012). We observed a reduced risk of DN associated with the T (Gln) allele, which was observed in both replication datasets, and the *P* value was highly significant ($P<0.0001$) in our meta-analysis combining all three datasets. Our results are in line with previous findings that Arg399Gln was associated with a reduced risk of non-melanoma skin cancers (Nelson *et al.*, 2002) and a reduced risk of metastasis in melanoma patients (Figl *et al.*, 2009). Our data suggest that this DNA repair pathway plays an important role in DN development. In addition, we found that variants in *XRCC1* were associated with DN regardless of *CDKN2A* mutation status.

CDK6, cyclin-dependent kinase 6, is a member of the cyclin-dependent protein kinase (*CDK*) family. Together with *CDK4*, *CDK6* plays an important role in G1 phase progression by regulating the activity of the tumor suppressor protein RB1 (Malumbres and Barbacid, 2009). Amplification and overexpression of *CDK6* have been reported in a variety of cancers (Malumbres and Barbacid, 2009). *CDKN2A* is a specific inhibitor for *CDK4* and *CDK6*, and *CDKN2A* residues that participate in contacting *CDK4* or *CDK6* are mutated in tumors, including familial melanoma (Russo *et al.*, 1998). Given its obvious relevance, *CDK6* has been intensively studied as an important candidate high-penetrance gene for familial melanoma, but disease-cosegregating variants in this gene have not been identified. In our study, we found that common genetic variants in *CDK6* were significantly associated with DN, but not with CMM. Interestingly, the association appeared to be restricted to families with *CDKN2A* mutations. Our results are consistent with findings from a previous genome-wide linkage scan for DN in melanoma families segregating p16-Leiden mutations, in which the strongest linkage signal for DN was mapped to chromosome 7q21.3, a region containing *CDK6* (de Snoo *et al.*, 2008). Given that the association was only seen in *CDKN2A* mutation positive families, it is not surprising that we did not replicate the association in other datasets that mostly included subjects negative for *CDKN2A* mutations. However, although the association was driven by mutation-positive families, most SNPs, including the most significant SNP (rs1005346), showed significant associations even among *CDKN2A*-negative subjects with DN in mutation-positive families. Our data suggest that other variants in the cell-cycle control pathway, in addition to *CDKN2A* mutations, may contribute to DN susceptibility in these families.

None of the DN-associated SNPs in *XRCC1* and *CDK6* was significantly associated with CMM risk. Although DN is a strong risk factor for CMM, the majority of individuals affected with DN do not develop melanoma. In addition, DN lesions rarely transform to melanoma, suggesting that additional genetic and/or environmental factors may determine the development of CMM from DN. However, it is also possible that these variants might be associated with CMM, but our analyses had limited power in identifying the significance because of the smaller number of CMM cases (N=157) than DN cases (N=310). In addition, we adjusted CMM in all models in the NCI dataset and restricted to CMM-unaffected

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people in the two replication datasets, therefore, our analyses were more likely to identify DN-specific variants. Further, individuals affected with DN, particularly in CMM high-risk families, may be more self-educated in using sun protection which could disrupt the natural history of CMM development. Indeed, although none of the DN-associated SNPs was significantly associated with CMM, the directions of the associations with DN and CMM were the same.

The strengths of our study include a rich collection of genetic, environmental, clinical, and pigmentation data in DN-enriched melanoma-prone families with and without *CDKN2A* mutations. In addition, we used data from two independent datasets to replicate our findings. Our study also has limitations. We included CMM cases in our analyses, which could potentially cause bias in DN association analyses. However, when we restricted the analyses to CMM-unaffected individuals for the most significant SNPs, we observed similar associations with DN. In addition, analyses were restricted to CMM-unaffected people in the two replication datasets. We also conducted sensitivity analyses by analyzing DN-unaffected family members and spouses separately as controls and the results were similar to those using the combined control group. Another limitation was that our families were ascertained primarily through self- or physician-referral, and thus findings may not be generalizable to the general population.

In summary, we found that genetic variants in *XRCC1* were associated with DN susceptibility, a result that was replicated in two independent datasets. Future studies are needed to identify functional variants in *XRCC1* that influence expression levels of the gene and DNA repair efficiency in DN samples. In addition to *XRCC1*, we found that variants in *CDK6* were associated with DN in families with *CDKN2A* mutations, which is consistent with a previous report of linkage in the *CDK6* region for DN in melanoma families with p16-Leiden mutations (de Snoo *et al.*, 2008). Our data suggest that genetic variants may confer DN susceptibility that is independent of CMM risk in melanoma-prone families. Future evaluations of these genes in relation to DN in melanoma families and in the general population are needed to confirm these findings.

Materials and methods

Study population

American melanoma-prone families with at least two living first degree relatives with a history of invasive CMM were ascertained through health care professionals or self referrals. Details of our familial melanoma patients were described previously (Goldstein *et al.*, 2005; Goldstein *et al.*, 2000). Briefly, all family members willing to participate in the study underwent a full-body skin examination and completed risk factor questionnaires for sun-related exposures. All diagnoses of melanoma were confirmed by histological review of pathologic material, pathology reports, or death certificates. To be defined as dysplastic, a nevus had to be 5 mm or larger in at least one dimension, have a flat component, and meet at least two of the following criteria: variable pigmentation, indistinct borders, and irregular outline. The study was approved by the National Cancer Institute Clinical Center Institutional Review Board and was conducted according to the Declaration of Helsinki. All subjects gave written informed consent. Data from the present study came from 53 families

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(23 families with *CDKN2A* mutations and 30 families without known mutations, Table 1). All CMM and DN cases with DNA available were selected. Controls included DN-unaffected family members and unrelated spouses. Only adult controls were selected to minimize disease misclassification. All study participants were Caucasian.

Gene and SNP selection and genotyping

A total of 38 candidate genes were selected based on their reported involvement in melanoma and functional relevance (cell cycle control [*CCNA1*, *CCND1*, *CCND2*, *CCND3*, *CCNE1*, *CDK2*, *CDK4*, *CDK6*, *CDKN1A*, *CDKN1B*, *CDKN2A*, *CDKN2B*, *CDKN2C*, *ING1*, *ING2*], pigmentation [*ASIP*, *OCA2*, *SLC24A2*, *SLC24A5*, *TYRP1*, *TYRP2*, *EDNRB*, *EGFR*, *PPARD*, *DRD2*], DNA repair [*ERCC1*, *ERCC4*, *XRCC3*, *XRCC1*, *APEX1*, *ERCC2*, *XPC*, *ERCC5*, *MGMT*, *VDR*], and melanocyte proliferation [*BRAF*, *MITF*, *KITLG*], Supplementary Table 1). TagSNPs were chosen from the set of available common SNPs using the program Tagzilla (http://tagzilla.nci.gov), which has been described elsewhere (Liang *et al.*, 2011). Briefly, for each originally targeted 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. The selected tagSNPs had a minor allele frequency (MAF) greater than 5% and $r^2<0.8$ based on Caucasian (CEU) and Yoruban (YRI) population samples of the HapMap project (Data Release 20/Phase II, NCBI Build 36.1 assembly, dbSNP b126). Genotyping of tagSNPs was conducted 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).

Quality control

The SNPs with low (<90%) genotyping completion rate, low (<90%) concordance rate with the 3 HapMap population (CEU, YRI, Japan and China) samples, or deviation ($P<0.001$) from Hardy–Weinberg equilibrium among founders were excluded. Among 586 genotyped samples, 20 were excluded due to low completion (<90%, n = 12) and Mendelian inconsistencies (n = 8). Sixty two individuals were further removed from DN analyses due to missing CMM status (n=4) or undetermined DN status (n=58).

Replication Datasets

We used two independent datasets to replicate the associations we found in our original dataset. The detailed description of the two replication datasets is presented in Supplementary material. Briefly, the first dataset was derived from a nevus family-based study conducted in France including 220 families ascertained through probands who had a high number of nevi (≥ 50 nevi) and no melanoma at time of recruitment into the study; Genome-wide genotyping was performed for 489 probands and their relatives (siblings for the most part) using Illumina Humancnv370k array and Illumina Human660W-Quad BeadChip. To combine the SNP data generated from these two arrays, genotypic imputations were performed in each of the genotyped datasets separately using the Hapmap3 reference panel and the MACH software (Li *et al.*, 2010). Stringent quality control criteria

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were applied to both genotyped subjects prior to imputations and imputed SNPs (imputation quality score $r^2 \geq 0.80$ and MAF ≥ 0.05). There were 473 subjects who passed QC, and 429 of them had whole body nevi counts available.

The second replication dataset included controls from three case-control studies of sporadic melanoma. Briefly, in the first case-control study (Landi *et al.*, 2001; Landi *et al.*, 2005), cases were incident sporadic melanoma patients diagnosed at the Dermatology Unit of Maurizio Bufalini Hospital in Cesena, Northern Italy. Controls were spouses or friends of the cases, patients treated at the same hospital for minor accidental trauma, or healthy hospital personnel recruited during the same period without a history of melanoma and coming from the same geographical areas as the cases. A single dermatologist (D.C.) performed skin examinations for all study subjects to determine pigmentation characteristics including nevi and DN. A total of 173 controls with blood samples were included in the present study. In the second study (Fargnoli *et al.*, 2006), sporadic melanoma patients were diagnosed at the Departments of Dermatology of the Universities of L'Aquila, Florence, or Modena in central Italy. Subjects treated for diseases unrelated to melanoma at the Surgery and Internal Medicine Departments of the corresponding Universities were recruited as controls for the study. Clinical examination of all subjects was performed by two dermatologists (K.P. and M.C.F.). A total of 165 controls with blood samples were included in the present study. The third case-control study was of sporadic melanoma cases diagnosed at the units of dermatology, medical oncology and plastic surgery of the National Cancer Research Institute and San Martino Hospital, Genoa, in Northern Italy (Ghiorzo *et al.*, 2012). Similarly, subjects without a history of melanoma, and who were older than 18 years of age were recruited at the same hospital during the same period as controls. A total of 207 controls with blood samples were included in the present study.

All studies in the replication datasets were approved by the local and NCI Institutional Review Boards, and all subjects signed an informed consent form.

Statistical analysis

For each SNP we calculated the P_{trend} based on the three-level ordinal genotype variable (0, 1, 2) that counted the number of minor alleles in conditional logistic regression models for the association with DN, conditioning on family to account for the ascertainment. Although this approach ignores residual correlation among family members, it gives estimates that are attenuated toward the null and thus is considered conservative (Pfeiffer, 2001). We calculated odds ratios (ORs) and 95% confidence intervals (95% CIs) for each genotype using the homozygous common allele genotype as the referent group. When the number of subjects with homozygous minor alleles was less than 5, heterozygote and homozygote minor allele genotypes were combined. All regression models were adjusted for age, gender, CMM status, and individual *CDKN2A* mutation status. For the most significant SNPs in *XRCC1* and *CDK6*, we further adjusted for solar injury and *MC1R* as a surrogate for pigmentation characteristics. Most pigmentation risk phenotypes, such as red hair color, poor tanning ability, pale/fair skin color and extensive freckling, were previously associated with *MC1R* variants in our *CDKN2A* mutation positive families (Goldstein *et al.*, 2005). *MC1R* variants were coded as 0=no variant, 1=single variant, 2=multiple variants. We also

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analyzed the most significant SNPs in *CDKN2A* mutation positive and negative families separately to assess effects modified by mutation status in families.

The primary goal of our study was to identify candidate genes associated with DN risk. Gene-based *P* values for association were obtained by combining SNP-based *P* values for trend using rank-truncated test statistics (Dudbridge and Koeleman, 2003) and a permutation-based sampling procedure (20,000 permutations), which takes into account the number of SNPs genotyped in each gene and their LD structure. Age, gender, CMM status, and *CDKN2A* mutation status were included in all conditional regression models. To adjust for multiple gene comparisons, we applied the Bonferroni correction method and considered gene based *P* values < 0.0013 (0.05/38) to be statistically significant. All analyses were conducted using SAS version 9.3 (SAS Institute, Inc., Cary, NC).

Analyses for both replication datasets were restricted to CMM-unaffected individuals. In the French dataset, the nevus density (nevus count divided by body surface) was used because DN were not recorded with sufficient accuracy. In addition, DN and nevus count are known to be strongly correlated (Tucker *et al.*, 1997). The association analyses were conducted by comparing those with extremely high values of the sex and age-adjusted log-transformed nevus density (above the 75th percentile, n=108) to those with extremely low values of nevus density (lower than the 25th percentile, n=107). The analysis used unconditional logistic regression and the allele dosage to take into account the uncertainty of imputed genotypes with robust sandwich estimation of the variance as implemented in the Stata™ logit function to model clustering of family genotypes (Williams, 2000); the type of genotyping chip was included as a covariate in the model. The analysis was also repeated by using the age and sex-adjusted log-nevus density as a quantitative phenotype, and similar results were obtained. In the Italian study, unconditional logistic regression models were used to analyze DN as a binary variable (presence vs. absence), adjusted for age and gender. We used a random-effect meta-analysis to combine SNP effects across different datasets.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviation list

CMM cutaneous malignant melanoma

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DN	dysplastic nevi
SNP	single nucleotide polymorphism
LD	linkage disequilibrium
OR	odds ratio
95% CI	95% confidence interval

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Table 1

Distribution of age, gender, CMM status, *CDKN2A* status, *MC1R*, pigmentation phenotype, and sun exposure variables in individuals with and without DN from 53 families

	Individuals with DN (N=310)		Individuals without DN (N=194)		*P*
	N	%	N	%	
Age					
≤30	88	28.4	12	6.2	
30–40	75	24.2	38	19.6	
40–50	72	23.2	71	36.6	
50–60	43	13.9	53	27.3	
60+	32	10.3	20	10.3	<.0001
Gender					
Female	172	55.5	108	55.7	
Male	138	44.5	86	44.3	0.97
CDKN2A					
Non-Carrier	196	64.3	172	90.5	
Carrier	109	35.7	18	9.5	<.0001
CMM					
Unaffected	157	50.6	190	97.9	
Case	153	49.4	4	2.1	<.0001
Moles					
0–24	15	4.9	94	49.7	
25–99	101	33.1	80	42.3	
100+	189	62.0	15	7.9	<.0001
Solar injury					
None/mild	188	62.0	120	63.8	
Moderate	74	24.4	40	21.3	
Severe	41	13.5	28	14.9	0.69
Tanning ability					
Tan/Little burn	128	41.3	109	61.9	
Burn/Little tan	142	45.8	67	38.1	0.01

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	Individuals with DN (N=310)		Individuals without DN (N=194)		*P*
	N	%	N	%	
Skin type					
Dark/medium	63	20.9	66	35.7	
Pale/fair	239	79.1	119	64.3	0.0002
Eye color					
Black/brown	60	19.8	56	30.3	
Hazel	76	25.1	41	22.2	
Green/gray	40	13.2	17	9.2	
Blue	127	41.9	71	38.4	0.08
Hair color					
Black/brown	120	39.6	97	53.0	
Blonde brown/light brown	98	32.3	51	27.9	
Blonde	44	14.5	23	12.6	
Red	41	13.5	12	11.8	0.004
MC1R					
Wild type	23	9.5	37	27.2	
1 nonsynonymous variant	132	54.3	53	39.0	
2 nonsynonymous variants	88	36.2	46	33.8	<.0001

P values were obtained by comparing individuals with DN to unaffected individuals using a generalized estimating equation and adjusting for familial correlation in the variance.

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Table 2

Genes associated with DN in melanoma families with $P<0.05$.

			Gene-based *P* values	
Gene	**Chr**	**#SNP**	**DN**	**CMM**
CDK6	7	18	**0.0001**	0.58
XRCC1	19	13	**0.0003**	0.61
EGFR	7	91	0.0086	0.09
CDKN1B	12	4	0.039	0.62
CCND3	6	1	0.045	0.7
APEX1	14	3	0.049	0.51

P values that remained significant after Bonferroni correction for multiple testing are bolded.

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Table 3

ORs for the most significant SNPs in *CDK6* and *XRCC1*.

SNP	DN unaffected		DN affected		Model1[2]				Model2[3]				Model3[4]			
	N	%	N	%	OR[1]	95% CI[1]		P[1]	OR	95% CI		P	OR	95% CI		P
CDK6																
rs1005346																
CC	88	45.1	176	56.2	Ref				Ref				Ref			
CT	82	42.1	122	39.0	0.44	0.24	0.80	0.0075	0.48	0.25	0.91	0.024	0.31	0.16	0.61	0.0007
TT	25	12.8	15	4.8	0.13	0.04	0.41	0.0006	0.14	0.04	0.47	0.0015	0.09	0.02	0.36	0.0007
P trend								0.0002				0.0007				<0.0001
XRCC1																
rs1001581																
CC	62	31.8	126	40.3	Ref				Ref				Ref			
CT	99	50.8	149	47.6	0.49	0.26	0.90	0.021	0.51	0.27	0.97	0.039	0.44	0.23	0.85	0.015
TT	34	17.4	38	12.1	0.35	0.15	0.82	0.016	0.33	0.013	0.82	0.017	0.41	0.17	1.01	0.053
P trend								0.0088				0.0094				0.027

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

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

[3] Model 2: age, gender, CMM, *CDKN2A*, solar injury, and *MC1R* adjustment.

[4] Model 3: restricted to CMM-unaffected subjects with age, gender, *CDKN2A* adjustment.

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Table 4

Associations of the most significant SNPs in *CDK6* and *XRCC1* with DN in *CDKN2A*-positive and negative families.

			***CDKN2A*+ families**			***CDKN2A*− families**		
SNP	**Ref**	**Var**	**OR***	**95% CI***	***P****	**OR***	**95% CI***	***P****
CDK6								
rs1005346	C	T	0.36	0.20, 0.63	0.0004	0.65	0.30, 1.42	0.28
rs2079147	G	A	0.52	0.32, 0.87	0.0013	0.91	0.46, 1.77	0.77
rs2237570	A	T	0.24	0.08, 0.71	0.0095	0.91	0.27, 3.04	0.88
rs2285332**	G	C	0.46	0.25, 0.85	0.014	1.17	0.57, 2.42	0.67
XRCC1								
rs1001581[a]	C	T	0.64	0.39, 1.06	0.086	0.43	0.22, 0.84	0.0013
rs25487[a]	C	T	0.62	0.37, 1.03	0.064	0.46	0.24, 0.89	0.022
rs2023614[b]	C	G	2.48	1.04, 5.92	0.041	2.97	0.95, 9.32	0.062
rs939461[b]	A	C	2.59	1.09, 6.13	0.031	2.43	0.85, 6.93	0.098

*ORs and *P* values are obtained from likelihood ratio test in conditional logistic regression with DN as the outcome variable and genotype analyzed as three-level ordinal variable.

**This SNP showed significant interaction with *CDKN2A* mutation status (*P*=0.0033).

[a,b]SNPs in LD (r^2>0.8).

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Table 5

XRCC1 SNPs in three independent datasets.

SNP	NCI test[1]				Italian[2]				French[3]				Combined[4]			
	OR	**lower**	**upper**	***P***	**OR**	**lower**	**upper**	***P***	**OR**	**lower**	**upper**	***P***	**OR**	**lower**	**upper**	***P***
rs25487	0.57	0.37	0.87	0.0093	0.51	0.32	0.81	0.005	0.69	0.45	1.05	0.084	**0.59**	**0.46**	**0.76**	**<0.0001**
rs2023614	2.74	1.32	5.67	0.0067	0.91	0.47	1.79	0.79					1.56	0.53	4.61	0.42[5]
rs1001581	0.57	0.37	0.87	0.0088					0.70	0.45	1.08	0.10	**0.63**	**0.46**	**0.86**	**0.0034**
rs939461	2.53	1.25	5.11	0.0095	0.94	0.45	1.97	0.87	1.06	0.57	1.98	0.84	1.35	0.75	2.46	0.32

[1] ORs and *P* values are obtained from likelihood ratio test in conditional logistic regression with DN as the outcome variable and genotype analyzed as three-level ordinal variable, with the adjustment of age, gender, CMM, *CDKN2A* status.

[2] ORs and *P* values are obtained from unconditional logistic regression models with DN as a binary variable (presence vs. absence) and the adjustment of age and gender. The analysis was restricted to CMM-unaffected subjects.

[3] ORs and *P* values are obtained by comparing the extremes of the distribution of the sex and age-adjusted log-transformed nevus density. The analysis used unconditional logistic regression and the allele dosage to take into account the uncertainty of imputed genotypes with robust sandwich estimation of the variance as implemented in the Stata™ logit function to model clustering of family genotypes. The analysis was restricted to CMM-unaffected subjects.

[4] Meta-analysis was conducted using random-effect model to combine SNP effects across different datasets.

[5] *P* for heterogeneity was significant for this SNP (*P*=0.03).