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Genetic variants in DNA repair genes and the risk of cutaneous malignant melanoma in melanoma-prone families with/without CDKN2A mutations

Xueying Liang^{1,§}, Ruth M. Pfeiffer¹, William Wheeler², Dennis Maeder³, Laurie Burdette³, Meredith Yeager³, Stephen Chanock³, Margaret A. Tucker¹, Alisa M. Goldstein¹, and Xiaohong R. Yang^{1,*}

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

² Information Management Services, Inc., Rockville, MD, USA

³ Core Genotyping Facility, SAIC-Frederick, Inc., NCI-Frederick, Frederick, MD, USA

Abstract

Cutaneous malignant melanoma (CMM) is an etiologically heterogeneous disease with genetic, environmental (sun exposure) and host (pigmentation/nevi) factors, and their interactions contributing to risk. Genetic variants in DNA repair genes may be particularly important since their altered function in response to sun exposure-related DNA damage may be related to risk for CMM. However, systematic evaluations of genetic variants in DNA repair genes are limited, particularly in high-risk families.

We comprehensively analyzed DNA repair gene polymorphisms and CMM risk in melanoma-prone families with/without CDKN2A mutations. A total of 586 individuals (183 CMM) from 53 families (23 CDKN2A (+), 30 CDKN2A (–)) were genotyped for 2964 tagSNPs in 131 DNA repair genes. Conditional logistic regression, conditioning on families, was used to estimate trend p-values, odds ratios and 95% confidence intervals for the association between CMM and each SNP separately, adjusted for age and sex. P-values for SNPs in the same gene were combined to yield gene specific p-values. Two genes, POLN and PRKDC, were significantly associated with melanoma after Bonferroni correction for multiple testing ($p=0.0003$ and 0.00035 , respectively). DCLRE1B showed suggestive association ($p=0.0006$). 28–56% of genotyped SNPs in these genes had single SNP $p<0.05$. The most significant SNPs in POLN and PRKDC had similar effects in CDKN2A (+) and CDKN2A (–) families. Our finding suggests that polymorphisms in DNA repair genes, POLN and PRKDC, were associated with increased melanoma risk in melanoma families with and without CDKN2A mutations.

Introduction

It is well known that the etiology of cutaneous malignant melanoma (CMM) is multifactorial involving both genetic and environmental factors. CDKN2A and CDK4 are the two high-risk melanoma susceptibility genes identified to date (1). However, these genes account for melanoma susceptibility in only a small proportion of melanoma-prone families, suggesting that other genetic factors may exist. Melanocortin-1 receptor (MC1R) is identified as a low-risk melanoma susceptibility gene for CMM. Recent genome-wide association studies suggested that variants in MTAP, ASIP, and TYR genes were significantly associated with

*Corresponding author.

§Current position: Division of Epidemiology, Office of Surveillance and Biometrics, Center for Device and Radiological Health, FDA

CMM risk. (2;3) In addition to genetic susceptibility, large numbers of nevi, pigmentation phenotype (red hair color, poor tanning ability, pale/fair skin color, and extensive freckling), and sun exposure (4;5) are strong risk factors for CMM.

Sun exposure is the most important environmental risk factor for melanoma development. The ultraviolet (UV) radiation is the most energetic component of solar radiation and is strongly related to risk of melanoma (6). Variants in genes (such as XPC, XPD, XPF, XPG, and ERCC1) in the nucleotide excision repair (NER) pathway and genes in other DNA repair pathways (such as XRCC1, XRCC3 and GSTT1) have been associated with melanoma risk in previous studies (7). However, the DNA repair pathway is a complex network of >150 genes (8). Only sixteen of them were previously evaluated for associations with CMM risk and the coverage of these genes was limited (7). No comprehensive study has been conducted in high-risk families.

To systematically evaluate the DNA repair pathway, we examined 2964 candidate SNPs in 131 DNA repair genes in 586 individuals from 53 melanoma-prone families using a high-throughput platform of custom-designed Illumina iSelect Infinium assay. To our knowledge, this is the most comprehensive analysis of DNA repair pathway genes in familial melanoma. The primary goal of our study was to identify genes in the DNA repair pathway that are associated with CMM risk in melanoma-prone families.

Material and methods

Study population

American melanoma-prone families with at least two living first degree relatives with a history of invasive melanoma were ascertained through health care professionals or self referrals. Details of our familial melanoma patients were described previously (9). 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. The study was approved by the National Cancer Institute Clinical Center Institutional Review Board and conducted according to the Declaration of Helsinki. All subjects gave informed consent.

Data from the present study came from 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 controls included unaffected family members and unrelated spouses. Only adult controls were selected to minimize disease misclassification. All study participants were Caucasians.

SNP and gene genotyping

Genes in the DNA repair pathway according to Wood et al. (8) were selected if they could be designed for the Illumina platform. A total of 131 genes were selected and grouped into 13 sub-pathways accordingly (Suppl. Table 1). TagSNPs were chosen from the set of available common SNPs using the program Tagzilla (<http://tagzilla.nci.gov>). 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 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). The Infinium included a total of 27,904 tag SNPs, of which our candidate genes represented 2,964 SNPs.

Quality control (QC)

TagSNPs that failed manufacturing (ordered but did not convert), failed validation (no amplification or clustering) and assays that had less than 90% completion or <90% concordance with the 3 HapMap population (CEU, YRI, Japan and China) samples used for validation were excluded. SNPs with Hardy-Weinberg Equilibrium (HWE) $p < 10^{-5}$ among founders (Bonferroni correction for 2964 SNPs at $p=0.05$) were excluded. Among 586 genotyped samples, 20 subjects were excluded due to low completion rate (<90%, $n=12$) or Mendelian inconsistencies ($n=8$). Four additional individuals were removed from all analyses due to uncertain CMM status.

Statistical Analysis

The primary goal of our study was to identify genes in the DNA repair pathway that are associated with CMM risk. Therefore, we focused more on gene-based rather than SNP-based analyses. SNP-based analyses were performed to obtain gene-based statistics and to evaluate effect modification by CDKN2A. We calculated the P_{trend} based on the three-level ordinal variable (0, 1, 2) of homozygote common allele, heterozygote, and homozygote minor allele in conditional logistic regression models for the association between CMM and each SNP, conditioning on family to account for the ascertainment. Although this approach ignores residual familial correlations among family members, it gives estimates that are attenuated toward the null and is thus conservative (10). We calculated odds ratios (OR) and 95% confidence intervals (95% CI) 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 and gender. For most significant SNPs in gene-based analyses, we further adjusted for CDKN2A status, number of nevi (including dysplastic nevi), solar injury, and MC1R. Number of nevi was categorized into four groups (0-24, 25-49, 50-99, and 100+). Solar injury was categorized as 0=absent/mild, 1=moderate, 2=severe/very severe. We also included adjustment for MC1R variants in the models 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 (11). MC1R variants were coded as 0=no variant, 1=single variant, 2=multiple variants. We also analyzed the most significant SNPs in CDKN2A positive and negative families separately to assess effects modified by CDKN2A status. We performed separate analyses using spouses only or unaffected family members only as controls. The results were similar (data not shown) so we therefore present only the analysis with the two groups combined.

We assessed gene-based summary of associations using the adaptive combination of p values (12;13). P-values for trend were computed using rank-truncated test statistics and a permutation-based sampling procedure (20,000 permutations) in the conditional logistic regression model, taking into account the number of SNPs genotyped in each gene and their LD structure. Age and gender were included in all models. To account for multiple comparisons, we applied the Bonferroni correction method. Therefore, gene-based significance level was set at $p < 0.05/131$ (0.00038).

All analyses were conducted using SAS version 9.1 (SAS Institute, Inc., Cary, NC).

Results

In total, 183 CMM cases and 379 controls were analyzed for 2964 tagSNPs in 131 DNA repair genes in melanoma-prone families with and without CDKN2A mutations. The distribution of age, gender, CDKN2A status, number of nevi, solar injury and MC1R are listed in table 1. As expected, CDKN2A, number of nevi, solar injury and MC1R were significantly associated with CMM in these families.

Figure 1 shows gene-based p-values of the 131 DNA repair genes for associations with CMM. Two genes, POLN ($p=0.0003$) and PRKDC ($p=0.00035$), were significantly associated with melanoma after Bonferroni correction for multiple testing. Among genotyped SNPs in these 2 genes, 56.4% SNPs in POLN and 28.6% SNPs in PRKDC had single SNP p value < 0.05 (table 2 and suppl. table 2). DCLRE1B showed suggestive association with CMM ($p=0.0006$) and 36.4% genotyped SNPs in this gene had single SNP $p < 0.05$ (table 2). Single SNP association p-values for all genotyped SNPs in these 3 genes are listed in Suppl. Table 2.

The correlations between significant SNPs within each gene were not extensive at cutoff of $r^2 > 0.8$ (data available upon request). We also evaluated the most significant SNP in each of these genes in a comprehensive model that included adjustment for number of nevi, solar injury and MC1R and found similar results (suppl. Table 3). We then analyzed these SNPs in these genes separately in CDKN2A (+) and CDKN2A (-) families. As shown in Table 3, the most significant SNPs, rs17132382 in POLN and rs8178158 in PRKDC had similar effects in CDKN2A (+) and CDKN2A (-) families. SNP rs12046289 in DCLRE1B appeared to have stronger effect in CDKN2A (+) families, although the test for interaction between this SNP and CDKN2A was not significant ($p=0.1$) possibly due to limited power.

Discussion

Our study systematically and comprehensively evaluated 131 DNA repair genes with risk of CMM in melanoma-prone families with and without CDKN2A mutations. We found that two genes were significantly associated with CMM susceptibility.

The PRKDC and DCLRE1B genes belong to the non-homologous end-joining (NHEJ) sub-pathway. The NHEJ pathway is the main mechanism for repair of double strand breaks (DSB) in mammalian cells, especially in the G1 phase of the cell cycle. An inability to respond properly to DSBs leads to genomic instability and promotes carcinogenesis. PRKDC encodes the catalytic subunit of the DNA-dependent protein kinase (DNA-PK). It functions with the Ku70/Ku80 heterodimer protein in DNA DSB repair and recombination. It was suggested to be important in the processing of complex DNA damage and be potentially associated with breast cancer development (14). The most significant SNP in PRKDC increased CMM risk 3.58-fold (rs8178158, 95% CI 1.50-8.53, $p=0.004$, table 2). Three other SNPs in PRKDC were also significant at $p<0.05$ (suppl. Table 2). DCLRE1B encodes DNA cross-link repair 1B protein and is one of several evolutionarily conserved genes involved in repair of interstrand cross-links. We found 4/11 SNPs in DCLRE1B associated with CMM with $p<0.05$. The most significant SNP increased CMM risk 2-fold (rs12046289: OR=2.11, 95% CI=1.22-3.65, $p=0.01$). This SNP showed a stronger effect in CDKN2A (+) families. DCLRE1B has not been studied in melanoma, but has been shown to be required for the prophase cell cycle checkpoint in response to DNA interstrand cross-links (15).

A DNA polymerase gene, POLN, was also significant in our study with gene-based p values of 0.0003. POLN (DNA polymerase nu) is a recently discovered enzyme. There are only few gene function studies and it has not been studied in any disease. But functional data

suggested that POLN participates in cross-link DNA repair through homologous recombination and it is required for repair of DNA cross-links in mammalian cells (16).

Our study is exploratory due to the limited number of melanoma cases. Another limitation is that families were ascertained primarily through self- or physician-referral, and thus findings may not be generalizable to other familial melanoma sample sets and particularly with distinctly different ethnic origins or to sporadic melanoma patients. However, our families have a rich collection of exposure and pigmentation data, and are heavily genetically loaded. Furthermore, we have the most comprehensive coverage of DNA repair genes, allowing a systematic investigation of the role of DNA repair genes in familial melanoma.

In conclusion, polymorphisms in two DNA repair genes (POLN and PRKDC) were significantly associated with the susceptibility of developing melanoma in high risk melanoma-prone families. These findings warrant replication and further investigation of the role of DNA repair genes in melanoma development. In addition, a larger dataset is needed to examine the effect of interaction between environmental/host factors and DNA repair genes on melanoma susceptibility. Moreover, studies in other familial melanoma and sporadic melanoma populations are needed to examine whether our findings are generalizable.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Associations of genetic variants in 131 DNA repair genes with CMM risk in 53 melanomaprone families with and without CDKN2A mutations. Single SNP associations were shown in dots. Two lines of $p=0.05$ and $p=0.001$ indicated significance levels for single SNP association. Gene-based p values were shown for the genes that were significantly associated with CMM risk at gene level.

Table 1

Distribution of age, gender, CDKN2A status, number of nevi, solar injury, and MCIR variables in cases and controls from 53 families

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
Nevi					
0-24	9	5.4	122	33.5	
25-49	21	12.5	66	18.1	
50-99	27	16.1	81	22.3	
100+	111	66.1	95	26.1	<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
MCIR					
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 for differences in frequencies.

Gene-based p values for genes significantly associated with CMM and ORs for the most significant SNP in each gene

Table 2

Gene	location	gene.p	# SNPs/gene in our study	#SNPs with p<0.05 (%)	Most significant SNP	OR for most significant SNP*		
						Genotype	OR	95% CI
POLN	4p16.3	0.00030	39	22 (56.4%)	rs17132382	CC TC+TT	ref 0.31	0.13-0.75
PRKDC	8q11	0.00035	14	4 (28.6%)	rs8178158	CC CG+GG	ref 3.58	1.50-8.53
DCLRE1B	1p13.2	0.00060	11	4 (36.4%)	rs12046289	GG AG+AA	ref 2.11	1.22-3.65

* ORs and P values were obtained from likelihood ratio test in conditional logistic regression and were adjusted by age, sex and CDKN2A status. Homozygote of common allele was the reference; heterozygote and homozygote of rare allele were combined due to low genotype frequency

Table 3
Most significant SNP in genes associated with CMM in CDKN2A (+) and CDKN2A (-) families

SNP	Gene	chr	Location (bp)	Genotype	CDKN2A (+) families (n=314)			CDKN2A (-) families (n=249)		
					p *	OR *	95% CI	p *	OR *	95% CI
rs17132382	POLN	4	2205997	CC TC+TT	ref 0.30	0.39	0.07-2.29	ref <i>0.02</i>	0.30	0.10-0.86
rs8178158	PRKDC	8	48930071	CC CG+GG	ref 0.05	3.24	0.98-10.7	ref <i>0.03</i>	4.02	1.11-14.55
rs12046289	DCLRE1B	1	114252909	GG AG+AA	ref <i>0.01</i>	3.41	1.44-8.09	ref 0.23	1.56	0.75-3.25

* ORs and p values were obtained from likelihood ratio test in conditional logistic regression and were adjusted by age and sex. Homozygote of common allele was the reference; heterozygote and homozygote of rare allele were combined due to low genotype frequency