Inherited Genetic Variants Associated with Occurrence of Multiple Primary Melanoma

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Abstract

Recent studies, including genome-wide association studies, have identified several putative low-penetrance susceptibility loci for melanoma. We sought to determine their generalizability to genetic predisposition for multiple primary melanoma in the international population-based Genes, Environment, and Melanoma (GEM) Study. GEM is a case–control study of 1,206 incident cases of multiple primary melanoma and 2,469 incident first primary melanoma patients as the control group. We investigated the odds of developing multiple primary melanoma for 47 SNPs from 21 distinct genetic regions previously reported to be associated with melanoma. ORs and 95% confidence intervals were determined using logistic regression models adjusted for baseline features (age, sex, age by sex interaction, and study center). We investigated univariable models and built multivariable models to assess independent effects of SNPs. Eleven SNPs in 6 gene neighborhoods (TERT/CLPTM1L, TYRP1, MTAP, TYR, NCOA6, and MX2) and a PARP1 haplotype were associated with multiple primary melanoma. In a multivariable model that included only the most statistically significant findings from univariable modeling and adjusted for pigmentation phenotype, back nevi, and baseline features, we found TERT/CLPTM1L rs401681 (P = 0.004), TYRP1 rs2733832 (P = 0.006), MTAP rs1335510 (P = 0.0005), TYR rs10830253 (P = 0.003), and MX2 rs45430 (P = 0.008) to be significantly associated with multiple primary melanoma, while NCOA6 rs4911442 approached significance (P = 0.06). The GEM Study provides additional evidence for the relevance of these genetic variants to melanoma risk and estimates the magnitude of the observed genetic effect on development of subsequent primary melanoma. Cancer Epidemiol Biomarkers Prev; 24(6); 992–7. © 2015 AACR.

Introduction

Clinically, melanoma is one of a small group of cancers where patients are at increased risk of potentially life-threatening subse-

quent primaries (1, 2), but the underlying genetic predispositions to multiple primaries are relatively unexplored. Recent genome-wide association studies (GWAS) and candidate pathways studies have identified several low-penetrant genetic variants associated with cutaneous melanoma (3, 4). The majority of these variants are in gene regions associated with fair pigmentation, such as TYRP1, TYR, HERC2/OCA2, SLCA3A2, and ASIP; nevi, such as PLAG266, MTAP, and NID1; or both, such as IRF4 (4–12). More recent GWAS have identified melanoma risk–associated variants in genes, including ATM, MX2, PARP1, ARNT, and CASP8, which may not be associated with phenotypic risk (8, 13). However, the risk associated with these low-penetrant genetic variants in relation to multiple primary melanomas (MPM) has rarely been evaluated.

We studied these variants in the Genes, Environment and Melanoma (GEM) Study, a large, international population-based case–control study, in which the “cases” are patients with MPM and the “controls” are patients with single primary melanoma (SPM; refs. 14, 15). Participants’ germline DNAs were genotyped for 47 polymorphisms from 21 distinct genomic regions. We compared the odds of carrying the genotypes and haplotypes in MPM relative to SPM patients in univariable and multivariable analyses and assessed effect modification.

Materials and Methods

Study population

The GEM Study is a population-based case–control study that enrolled 1,206 cases diagnosed with MPM (a second o
higher-order invasive or in situ primary melanoma) between 1998 and 2003, and 2,469 controls diagnosed with invasive SPM in 2000. In situ melanomas were eligible as MPM in order to take into account surveillance when the patient had a previous invasive melanoma. Patients were selected from eight population-based cancer registries in the United States (New Jersey, North Carolina, California), Australia (New South Wales, Tasmania), Canada (Ontario, British Columbia), and Italy (Turin), and one hospital center in Michigan. GEM recruitment procedures and data collection have been described (14, 15). The Institutional Review Boards of all participating institutions approved the protocol; informed consent was obtained from each participant.

Demographic and melanoma risk factors, including hair and eye color, ability to tan, and number of back nevi, were collected from telephone interview and self-administered questionnaire. Using a glossy-colored guide to aid in differentiating between nevi and other skin lesions, subjects had the nevi on their backs counted by a family member or friend; and back nevi counts were categorized as 0 to 10 or >10 for this article. Back nevus counts were significantly correlated with whole-body nevus diagrams in GEM (data not shown). A phenotypic index variable was derived from additively combining: hair color (black or dark brown = 0; light brown or blond = 1; red = 2), eye color (black or brown = 0; green, gray, or hazel = 1; blue = 2), and ability to tan in response to sun exposure (deeply or moderately = 0; occasionally or none = 1). Those with index scores of 0 or 1 were defined as very low/low, 2 as moderate low, 3 as moderate high, and 4 or 5 as high/very high risk.

Genotyping
DNA was collected from buccal swab kits. SNPs were genotyped using the MassArray iPLEX platform (Sequenom Inc.) with quality control measures as previously reported (16). Two SNPs of interest were genotyped. SNP locations, minor allele frequencies, and proxy SNPs (r² > 0.95) were chosen (1000 Genomes, CEU population; Proxy SNP; Broad Institute).

Statistical analyses
Logistic regression models were used to estimate the OR and 95% confidence interval (95% CI) for each SNP assuming an additive model of inheritance of the variant allele. All models were adjusted for baseline features: age, sex, an age by sex interaction, and study center. For each locus with multiple associated SNPs, we applied stepwise logistic regression to determine the SNP with the strongest association from among the significantly associated SNPs, keeping baseline variables fixed.

For the genes with at least two SNPs genotyped, we determined their haplotype blocks using the Haploview software algorithm (17) based on the pair-wise linkage disequilibrium information of the GEM population in combination with the HapMap CEU population (18). Within each haplotype block, we inferred the haplotypes in terms of probabilities for each individual from the SNP genotype input data using the PHASE algorithm, a Bayesian method in which the prior was chosen to approximate the coalescent process (19). For each haplotype block, the haplotype associations with MPM were assessed through haplotype trend regressions (HTR; ref. 20). The HTR method effectively took into account the haplotype phase uncertainty and reduced bias by incorporating the inferred individual haplotype probabilities as the predictor variables in the regression models. Haplotypes with low estimated frequencies (<0.01) were grouped together, reducing the number of haplotype categories and increasing the efficiency and power of haplotype analysis. Each haplotype or group of rare haplotypes were then compared with the most common haplotypes in our study population.

Genotype and haplotype associations with phenotypic index were estimated using multinomial models and with back nevi using logistic regression models. In subsequent analyses, we limited the participants to those with no missing data for phenotypic index, back nevus counts, genotypes, and haplotypes of interest. Baseline models for genotypes and haplotypes were adjusted for baseline features; we then also adjusted the models for phenotypic index and back nevi, and finally also included all genotypes and haplotypes of interest in a multi-variable model. Further, in exploratory stratified analyses, we assessed effect measure modification by phenotypic index and back nevus counts. The likelihood ratio test was used to test interactions, comparing models with main effects to models with main effects and interaction terms. All statistical tests were two-sided with P < 0.05 considered statistically significant. All data were analyzed using R (http://www.r-project.org/) or SAS 9.3 programs.

Results
The SPM and MPM patients’ age, sex, race, number of back nevi, phenotypic index, and tumors’ Breslow thicknesses are in Supplementary Table S1. Twelve non-Caucasian patients were excluded from analyses. Forty-seven SNPs within 21 genetic loci previously reported to be in association with melanoma were genotyped. SNP locations, minor allele frequencies, numbers of cases and controls genotyped, and literature references are in Supplementary Table S2. Proxy SNPs rs6735656 and rs12278954 were used, respectively, for CASP8 rs10931936 and ATM rs1801516 identified by Barrett and colleagues (8).

Eleven SNPs in 6 gene neighborhoods (TERT/CLPTM1L, TYRP1, MTAP, TYR, NCOA6, and MX2) were significantly (P < 0.05) associated with MPM compared with SPM using additive models adjusted for baseline features (Table 1). The MTAP and TYR loci each had more than one significantly associated SNP. MTAP rs1335510 and TYR rs10830253 were brought forward for subsequent analyses because they each were the only SNP that remained in the stepwise logistic regression model for their locus.

Of the haplotypes examined (Table 2 and Supplementary Table S3), a PARP1 haplotype (rs3219090, rs2695238) was significantly (P = 0.03) associated with MPM, as were haplotypes in MTAP and TYR, when adjusting for baseline features. However, the statistical significance of the MTAP and TYR haplotype associations with MPM was weaker than the respective single MTAP rs1335510 and TYR rs10830253 SNP associations (Supplementary Table S3). Thus, only the PARP1 haplotype was selected for further analysis.

Several of the MPM-associated genotypes were associated with phenotypic index or back nevus counts (Supplementary Table S4), indicating that these SNPs are correlated with these phenotypes and may increase risk of MPM via these phenotypes, which are known melanoma risk factors.

When limiting the dataset to patients with no missing data for genotypes, haplotype, or traits of interest (Table 3), the TERT/CLPTM1L, TYRP1, MTAP, TYR, NCOA6, and MX2 genotypes, but

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not the PARP1 haplotype (P = 0.14), remained significantly associated with MPM after adjusting for baseline features. After additionally adjusting for phenotypic index and back nevi, the ORs did not appreciably change, although the association with NCOA6 rs4191442 became insignificant (P = 0.07). In a multivariable model further adjusting for genotypes and the PARP1 haplotype, the TERT/CLPTM1L, TYR, MTAP, TYR, and MX2 genotypes remained significant, but the NCOA6 genotype (P = 0.06) and PARP1 haplotype (P = 0.22) did not; none of the ORs appreciably changed.

In an exploratory stratified analysis adjusted for baseline features, no evidence was found of effect modification by phenotypic index or back nevus counts on the association of genotypes with MPM (Supplementary Table S5). However, there was evidence of effect modification by back nevus, but not phenotypic index, on the association between the PARP1 haplotype and MPM (P for interaction = 0.01). The PARP1 haplotype AG was negatively associated (P = 0.02) with MPM when 0 to 10 back nevi were present, whereas both the AG (P = 0.03) and AC (P = 0.01) haplotypes were negatively associated with MPM when >10 back nevi were present.
Germline Variants Associated with Multiple Primary Melanoma

Table 2. Association of a PARP1 haplotype with MPM in the GEM Study

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>SNP</th>
<th>Haplotype frequency</th>
<th>OR (95% CI)&lt;sup&gt;a,b&lt;/sup&gt;</th>
<th>Global P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PARP1: rs3219090 (SNP1), rs2695238 (SNP2)</td>
<td>1</td>
<td>G</td>
<td>0.672</td>
<td>1.00 (reference)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>A</td>
<td>0.300</td>
<td>0.93 (0.83–1.05)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>A</td>
<td>0.017</td>
<td>0.53 (0.54–0.81)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>G</td>
<td>0.011</td>
<td>0.93 (0.56–1.56)</td>
</tr>
</tbody>
</table>

aThe reference category is the haplotype with the highest frequency.
bAdjusted for age, sex, age by sex, and study center.

Discussion

In the international GEM Study, we found that SNPs in TERT/CLPTM1L, TYR, MITF, TYR, NCOA6, and MX2 and a PARP1 haplotype were associated with the occurrence of MPM. TERT/CLPTM1L rs401681, TYR rs1408799, MTPAP rs1335510, TYR rs10830253, and MX2 rs45430 were associated with MPM independently of each other and of phenotypic index and back nevi. NCOA6 rs4911442 and the PARP1 haplotype were not significant in the multivariable model, possibly as a result of diminished statistical power as there was little change in the ORs. There was no evidence for effect modification of SNP associations with MPM by patient phenotype; however, back nevi did modify the association of the PARP1 haplotype with MPM.

The single SNP associations reported in GEM are in the same direction as those reported in the literature (Supplementary Table S2). In a recent large meta-analysis, Chatzinasiou and colleagues (22) found a similar trend toward protection from melanoma with a colorectal cancer genotype (rs1885120 (MYH7B) and rs3219090 (TERT/CLPTM1L)). Variants in each of these loci reached genome-wide significance in the GEM Study, although the association of the PARP1 haplotype with MPM was not significant. There was no evidence for effect modification of SNP associations with MPM by patient phenotype; however, back nevi did modify the association of the PARP1 haplotype with MPM.

We know of only one other study addressing associations with MPM of common genetic variants in the loci discussed here. Helsing and colleagues (23) found no association of API2 rs1015362 and rs4911414, TYR rs1126809, and TYR rs1408799 with MPM in patients identified through the Norwegian Melanoma Registry compared with melanoma-free blood donors. Although this design differs from GEM, we also found no association of API2 rs4911414 and TYR rs1408799 with MPM. We did not genotype API2 rs1015362 and TYR rs1126809.

Strengths of our study include its large size, population-based case ascertainment, homogeneous questionnaire administration, and high ascertainment of cases and controls. The multivariable model, examination of risk stratified by phenotype, and evidence for the GEM Study’s magnitudes of associations with lower minor allele frequencies (e.g., SLC45A2 rs1408799 with MPM) in patients identified through the Norwegian Cancer Registry compared with melanoma-free blood donors. Although this design differs from GEM, we also found no association of API2 rs4911414 and TYR rs1408799 with MPM. We did not genotype API2 rs1015362 and TYR rs1126809.

Subsequent melanomas are a major problem for melanoma patients, but few studies have explored their genetic predisposition. Our results provide evidence that several putative low-penetrance susceptibility loci for melanoma are generalizable to other populations.

Table 3. Genotype and haplotype associations with MPM (N = 1,074) compared with SPM (N = 2,137) for participants with no missing data in the GEM Study

<table>
<thead>
<tr>
<th>Gene neighborhood</th>
<th>SNP(s)</th>
<th>Model</th>
<th>OR (95% CI)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>P</th>
<th>OR (95% CI)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>P</th>
<th>OR (95% CI)&lt;sup&gt;c&lt;/sup&gt;</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotypes</td>
<td>TERT/CLPTM1L</td>
<td>rs401681</td>
<td>ADD</td>
<td>1.21 (1.08–1.35)</td>
<td>0.001</td>
<td>1.20 (1.07–1.35)</td>
<td>0.0006</td>
<td>1.19 (1.06–1.33)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs2735832</td>
<td>ADD</td>
<td>0.83 (0.74–0.94)</td>
<td>0.0020</td>
<td>0.85 (0.75–0.95)</td>
<td>0.0009</td>
<td>0.85 (0.76–0.96)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs1335510</td>
<td>ADD</td>
<td>0.80 (0.71–0.89)</td>
<td>0.00010</td>
<td>0.80 (0.71–0.90)</td>
<td>0.0004</td>
<td>0.81 (0.72–0.91)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs10830253</td>
<td>ADD</td>
<td>1.33 (1.09–1.62)</td>
<td>0.0005</td>
<td>1.19 (1.06–1.33)</td>
<td>0.003</td>
<td>1.20 (1.06–1.34)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs4911442</td>
<td>ADD</td>
<td>1.38 (1.03–1.81)</td>
<td>0.03</td>
<td>1.18 (0.99–1.35)</td>
<td>0.07</td>
<td>1.16 (0.99–1.35)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs45430</td>
<td>ADD</td>
<td>0.87 (0.77–0.97)</td>
<td>0.02</td>
<td>0.87 (0.77–0.98)</td>
<td>0.02</td>
<td>0.85 (0.75–0.96)</td>
</tr>
<tr>
<td>Haplotype</td>
<td>PARP1</td>
<td>rs3219090; rs2695238</td>
<td>AC&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.97 (0.85–1.09)</td>
<td>0.001</td>
<td>0.99 (0.87–1.12)</td>
<td>0.009</td>
<td>0.99 (0.88–1.13)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs3219090; rs2695238</td>
<td>AG&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.52 (0.29–0.92)</td>
<td>0.34</td>
<td>0.55 (0.31–0.97)</td>
<td>0.22</td>
<td>0.55 (0.31–0.97)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>rs3219090; rs2695238</td>
<td>GC&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.13 (0.64–1.97)</td>
<td>1.09 (0.62–1.91)</td>
<td>1.10 (0.62–1.95)</td>
<td>1.09 (0.62–1.95)</td>
<td>1.10 (0.62–1.95)</td>
</tr>
</tbody>
</table>

NOTE: Participants were included who had no missing data for phenotypic index, back nevus counts, or genotypes or haplotypes included in the table.

Abbreviation: ADD, additive.

aAdjusted for age at diagnosis, sex, age by sex, and study center.
bAdjusted for age at diagnosis, sex, age by sex, study center, phenotypic index, and back nevi (0–10, >10).
cAdjusted for age at diagnosis, sex, age by sex, study center, phenotypic index, back nevi (0–10, >10), and all genotypes/haplotypes in the table.
dReference category determined by the haplotype with the highest frequency.
risk of subsequent melanoma. Also, validation of genetic associations in the large international population-based GEM study adds further credibility that these loci are melanoma risk–associated. Knowledge of genetic risk factors for subsequent melanoma could inform screening algorithms, future risk estimation modeling, and future prevention studies for melanoma survivors.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors’ Contributions


Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): I. Orlow, A. Kricker, B.K. Armstrong, H. Anton-Culver, S.B. Gruber, L.D. Marrett, R.P. Gallagher, S. Rosso, T. Dwyer, A. Sharma, E. La Pilla, L. From, K.J. Buxam, D.W. Ollila, M. Berwick, N.E. Thomas


Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): D.C. Gibbs, R.P. Gallagher, M. Berwick, N.E. Thomas


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