Identification of a melanoma susceptibility locus and somatic mutation in TET2

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rs4698934 was nominally significantly associated with melanoma risk. The combined odds ratio per T allele = 1.18; 95% confidence interval (1.10–1.25); combined P = 7.70 × 10⁻². This SNP is located in the intron of the TET2 gene on chromosome 4q24. In addition, a novel somatic mutation of TET2 was identified by next-generation sequencing in 1 of 22 sporadic melanoma cases. TET2 encodes a member of TET family enzymes that oxidizes 5-methylcytosine to 5-hydroxymethylcytosine (5mC). It is a putative epigenetic biomarker of melanoma as we previously reported, with observation of reduced TET2 transcriptional expression. This study is the first to implicate TET2 genetic variation and mutation in melanoma.

Introduction

Melanoma is the leading cause of skin cancer-related mortality and, in advanced stages, is characterized by poor prognosis. The incidence of melanoma has steadily increased worldwide (1,2). Much of this increase has been seen in relatively young adults, and consequently, the number of life years lost per melanoma death is higher than that of most other solid tumors (3). Susceptibility to melanoma is determined by both environmental risk factors, including excessive exposure to ultraviolet radiation and genetically controlled phenotypic traits such as nevus propensity, red or blonde hair, light-colored eyes, fair skin, and limited tanning ability (4–6). Common low-risk predisposing alleles probably account for most of the melanoma burden (7,8). Many putative risk alleles involved in cellular pathways such as pigmentation, DNA repair, telomere maintenance, oxidation stress, apoptosis, cell growth, and melanocyte differentiation and migration have been implicated in melanoma susceptibility (9–11). Genome-wide association studies (GWASs) have propelled this field forward by identifying many novel low- to moderate-risk loci that may cause sporadic melanoma. Within the last several years, several GWASs have reported their findings on melanoma risk (11–18), and most of the loci identified in earlier GWASs were within pigmentation genes (19,20), such as MCIR and TYR. Recent melanoma GWASs identified new loci not related to pigmentation, including ATMi, MX2, CASP8, PARP1, CCND1, and a locus (probably SETDB1 and ARNT) at 1q21.3 (15–17). However, all currently identified loci are believed to account for only a small proportion of the genetic susceptibility to melanoma (21,22). To identify additional melanoma risk alleles, we conducted a multistage melanoma GWAS in Caucasians. In addition, somatic gene mutational status was detected by next-generation sequencing in sporadic melanoma samples.

Materials and methods

Harvard cohorts

Description of study populations. Nurses’ Health Study. The Nurses’ Health Study (NHS) was established in 1976, when 121 700 female registered nurses between the ages of 30 and 55 years residing in 11 larger US states completed and returned an initial self-administered questionnaire on their medical histories and baseline health-related exposures. Biennial questionnaires with collection of exposure information on risk factors have been collected prospectively. Every 2 years, along with exposures, outcome data with appropriate follow-up of reported disease events are collected. Overall, follow-up has been high; after more than 20 years, >90% of participants continue to complete questionnaires. From May 1989 through September 1990, we collected blood samples from 32 826 participants in the NHS. Information on melanoma development was first collected in the 1984 questionnaire.

Health Professionals Follow-up Study. In 1986, 51 529 men from all 50 US states in health professions (dentists, pharmacists, optometrists, osteopathic physicians, podiatrists, and veterinarians) aged 40–75 years answered a detailed mailed questionnaire, forming the basis of the study. The average follow-up rate for this cohort over 10 years is >90%. On each biennial questionnaire, we obtained disease- and health-related information. Between 1993 and

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Melanoma cases and controls in the discovery set. Eligible cases in the NHS and the Health Professionals Follow-up Study (HPFS) consisted of participants with pathologically confirmed invasive melanoma, diagnosed any time after baseline up to the 2008 follow-up cycle for both cohorts. All subjects were United States non-Hispanic Caucasians. We have previously conducted several GWASs on different disease outcomes (NHS: breast cancer, coronary heart disease, type 2 diabetes, kidney stone, pancreatic cancer, and glaucoma; HPFS: coronary heart disease, type 2 diabetes, kidney stone, advanced prostate cancer, and glaucoma). The study description for eight GWAS sets of the discovery set is presented in Supplementary Methods, available at Cancerogenesis Online. We included only controls in each GWAS, except for the kidney stone GWAS, in which we used both cases and controls. Participants without melanoma diagnosis were the controls in the current study, and those with melanoma diagnosis were the cases. In addition, we genotyped the rest of the melanoma cases in both cohorts who were not included in these previous GWASs. A detailed description of the numbers of cases and controls included and the platform for each study are shown in Table I. Finally, we included 494 melanoma cases and 5628 controls.

Laboratory assays. Genotyping in eight GWAS of the discovery set. We performed genotyping in the breast cancer GWAS in NHS using the Illumina HumanHap550 array, as part of the National Cancer Institute’s Cancer Genetic Markers Of Susceptibility Project. For the coronary heart disease and type 2 diabetes GWASs of the discovery set, we performed genotyping using the Affymetrix 6.0 array. For the glaucoma GWAS, we performed genotyping using the Illumina HumanHap660 array. For the kidney stone, advanced prostate cancer, and melanoma GWASs, we performed genotyping using the Illumina HumanHap610 array. The quality control procedures for eight GWAS sets of the discovery set are presented in Supplementary Methods, available at Cancerogenesis Online.

Imputation and meta-analysis. On the basis of the genotyped single-nucleotide polymorphisms (SNPs) and haplotype information in the National Center for Biotechnology Information build 35 of phase II Hapmap CEU data, we imputed genotypes for >2.5 million SNPs using the program MACH (25). Only SNPs with imputation quality R² > 0.95 in each study were included in the final analysis. A total of 1,579,307 SNPs were included in the final meta-analysis of the NHS and HPFS (Table I). Betas from each study of the discovery set were combined in a meta-analysis with weights proportional to the inverse variance of the beta in each study.

MD Anderson Cancer Center

The study participants for the discovery analysis were from a hospital-based case–control study of melanoma, for which cases were recruited from among non-Hispanic white patients at MD Anderson between March 1998 and August 2008. Samples and data were available from 931 melanoma patients and 1026 cancer-free controls (friends of other patients reporting to clinics), who were frequency matched on age and sex, completed a comprehensive skin lifestyle questionnaire, and passed quality control filters for genotyping. This questionnaire was administered by an interviewer to 70% of patients and controls and was self-administered for the remaining 30%. An additional case series comprising 873 individuals presenting for treatment for melanoma at MD Anderson was also included, bringing the total number of melanoma patients to 1804. The study protocols were approved by the Institutional Review Board at MD Anderson, and informed consent was obtained from all participants. Tissue samples were collected as whole blood, with various DNA extraction methods (including Gentra, Qiagen, and phenol–chloroform). DNA samples for the first-stage GWAS were genotyped using the Illumina OmniQuad array and were called using the Bead Studio algorithm at the John Hopkins University Center for Inherited Disease Research. Detailed information about the MD Anderson melanoma GWAS can be found elsewhere (17).

Table I. Melanoma cases and controls (SNPs genotyped by studies in the NHS and HPFS of the discovery phase)

<table>
<thead>
<tr>
<th>Disease</th>
<th>Control</th>
<th>Case</th>
<th>Genotyped SNPs</th>
<th>Platform</th>
<th>HPFS</th>
<th>Control</th>
<th>Case</th>
<th>Genotyped SNPs</th>
<th>Platform</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melanoma</td>
<td>0</td>
<td>264</td>
<td>6209091</td>
<td>Illumina 610</td>
<td></td>
<td>0</td>
<td>136</td>
<td>6209091</td>
<td>Illumina 610</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>840</td>
<td>5</td>
<td>546666</td>
<td>Illumina 550</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coronary heart disease</td>
<td>557</td>
<td>13</td>
<td>721316</td>
<td>Affymetrix 6.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glaucoma</td>
<td>337</td>
<td>5</td>
<td>495161</td>
<td>Illumina 660</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kidney stone</td>
<td>359</td>
<td>5</td>
<td>6209091</td>
<td>Illumina 610</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type 2 diabetes</td>
<td>1220</td>
<td>23</td>
<td>704409</td>
<td>Affymetrix 6.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pancreatic cancer</td>
<td>64</td>
<td>2</td>
<td>559865</td>
<td>Illumina 550</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>3377</td>
<td>317</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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</tr>
</tbody>
</table>

We regressed a binary coding for melanoma case or control (0 or 1) on each SNP (dosage file) that passed quality control filters. The five largest principal components of genetic variation were nominally significantly associated with melanoma (P < 0.05), and we adjusted for them along with age. These principal components were calculated for all individuals on the basis of ~10,000 unlinked markers using the EIGENSTRAT software. We performed a meta-analysis to combine the replication sets and the discovery set. From the discovery stage, SNPs with P values <1 × 10⁻⁴ were selected for replication, and in the replication stages, P values < .05 were considered nominally statistically significant.

Somatic mutation detection by targeted next-generation sequencing in sporadic melanoma cases

DNA was isolated from formalin-fixed melanoma samples using standard methods. In brief, samples were incubated in proteinase K overnight, followed by subsequent purification of the DNA according to manufacturer’s instructions (QIAamp DNA Mini Kit, QIAGEN, Gaithersburg, MD). Then, DNA concentration was assessed using PicoGreen dsDNA detection (Life Technologies, Carlsbad, CA). Then, DNA was sequenced using the Illumina HiSeq 2500 sequencer (Illumina, San Diego, CA). Targeted next-generation sequencing was performed using cancer genomic assay to detect mutations in 275 cancer target genes. The complete coding sequence of the target genes, plus selected introns for 30 of the genes, was captured using a custom-designed Agilent SureSelect hybrid capture kit (Agilent Technologies, Santa Clara, CA) and massively parallel sequencing was performed on the Illumina HiSeq 2500 sequencer (Illumina, San Diego, CA). Data analysis was performed with a bioinformatics pipeline that included open-sourced software (Mutect and GATK, Broad Institute, Cambridge, MA) as well as internally developed software (VisCap Cancer, Breakmer).

Results

In the discovery stage of the GWAS, we further selected the 69 SNPs most significantly associated with melanoma risk (P < 5.0 × 10⁻⁵) and not in high linkage disequilibrium (R² > 0.8) with each other or with published melanoma risk SNPs for in silico replication in two independent GWAS datasets (Australian GWAS on melanoma risk and GenoMEL GWAS on melanoma risk; 5149 cases and 12 795 controls in total). Seven SNPs were nominally significantly associated with melanoma risk in the combined replication set (Supplementary Table I, available at Cancerogenesis Online). The most significant SNP identified was on chromosome 3, rs13097028, P = 9.99 × 10⁻⁷ in the combined set. Another SNP identified on chromosome 3 was
rs1031925, \( P = 4.27 \times 10^{-6} \). Two SNPs on chromosome 2 were identified, rs11901831 (\( P = 1.48 \times 10^{-5} \)) and rs13404035 (\( P = 4.15 \times 10^{-6} \)). One SNP on chromosome 4 was identified, rs4698934 (\( P = 5.21 \times 10^{-6} \)). One SNP on chromosome 6 was identified, rs1889497 (\( P = 2.45 \times 10^{-6} \)). One SNP on chromosome 13 was also identified, rs4773180 (\( P = 5.69 \times 10^{-5} \)). None of the other 62 SNPs reached nominal significance in the combined replication set (Supplementary Table 1, available at Carcinogenesis Online).

We further genotyped these seven SNPs in a nested case–control study within the NHS II of 234 melanoma cases and 238 controls. The SNP rs4698934 was replicated with a \( P \) value of 0.009. The OR per T allele was 1.18 (95% CI: 1.10–1.25, combined \( P = 7.70 \times 10^{-5} \)) (Table II). Regional association plot of SNPs at the TET2 locus is displayed in Figure 1.

We examined the results of the previously identified melanoma susceptibility loci in the discovery set, and they were comparable with previous findings (Table III). All but two (CASP8 and CCND1) of the known loci were nominally replicated. For the CASP8 locus, the direction of association was consistent with prior publications. Among the novel SNPs we identified in this study, SNPs at TET2 locus is the most significantly associated with melanoma susceptibility. As we reported previously, TET2-mediated global loss of 5-hmC in melanoma genome is an epigenetic hallmark event (26). Then, we sequenced 22 melanoma samples to a depth of 110 reads on average for each melanoma sample. A c.650C>T nucleotide mutation was identified in one melanoma sample at an allelic fraction of 15.5%. The variant affects Serine nucleotide 217 to Phenylalanine, resulting from the substitution to missense of amino acid p.S217F (Figure 2).

**Discussion**

We replicated most of the known loci, and we identified several novel loci predisposing to melanoma. The SNP rs13097028 on chromosome 3q26 locus was the most significant, but was not replicated in the NHS II. The SNP rs4698934 locus on chromosome 4q24 was replicated. The NHS II has a modest sample size, and the statistical power for this replication is limited. The SNP rs13097028 is not located in gene region, and genes near this locus are ACTRT3, MYNN, LRRC34, LRRIQ4, LRRC31, and MECOM.

The SNP rs4698934 is within the intron of TET2 gene at position of 106139387. Interestingly, the somatic mutation of exon 3 at position of 106155749 is identified in our study. TET2 (tet oncogene family member 2, or Ten-Eleven Translocation 2) is a family member of genes with enzymatic activity. As we reported previously, TET2-mediated 5-hmC loss in melanoma progression with diminished gene expression as a possible molecular mechanisms (26).

TET2 is a key intermediate of DNA demethylation. Mutations to the TET gene have been reported to be the most frequently mutated gene in myelodysplastic syndrome and tightly associated with reduced overall survival in hematologic malignancies (28,29). TET2-mediated 5-hmC loss recently has also been reported in various solid malignancies, including breast cancer (30,31), oral squamous cell carcinoma (32), gastrointestinal stromal tumor (33,34), hepatocellular carcinoma (35), and brain tumor (36). In melanoma, several groups and we reported that the TET2-mediated 5-hmC loss is associated with prognosis and as a putative biomarker (26,37–39).

In addition, we reported that increasing 5-hmC levels via overexpressing TET2 reversed the genome-wide 5-hmC loss toward a benign nevus-like pattern (26). We functionally characterized the significant impact of TET2-mediated 5-hmC loss in melanoma progression with diminished TET2 gene expression as a possible molecular mechanisms underlying global loss of 5-hmC previously (26). The findings of germline and somatic mutations in this study provide further evidence for a possible novel mechanism of 5-hmC loss is due to inactivation of TET2 enzymatic function due to TET2 gene mutation.

The major limitation of this study is its modest statistical power, which prevents detection of the modest effects of some genetic variants. With the current sample size, we have 80% power to detect an effect of 1.44. Furthermore, constitutional and environmental risk factors...
Table III. Effect sizes for previously published melanoma risk loci in our melanoma GWAS

<table>
<thead>
<tr>
<th>Ref</th>
<th>Gene</th>
<th>CHR</th>
<th>SNP</th>
<th>MA</th>
<th>Other studies</th>
<th>Our study</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Barrett et al. (15)</td>
<td>—</td>
<td>1</td>
<td>rs7412746</td>
<td>C</td>
<td>9.0 \times 10^{-11}</td>
<td>0.89 (0.85–0.95)</td>
</tr>
<tr>
<td>Barrett et al. (15)</td>
<td>PARP1</td>
<td>1</td>
<td>rs3219090</td>
<td>T</td>
<td>9.3 \times 10^{-8}</td>
<td>0.82 (NA)</td>
</tr>
<tr>
<td>Falchi. et al. (14)</td>
<td>CASP8</td>
<td>2</td>
<td>rs13016963</td>
<td>A</td>
<td>8.6 \times 10^{-10}</td>
<td>1.14 (1.09–1.19)</td>
</tr>
<tr>
<td>Amos et al. (17)</td>
<td>CLPTM1L/TERT</td>
<td>5</td>
<td>rs401681</td>
<td>C</td>
<td>0.004</td>
<td>0.73 (0.59–0.91)</td>
</tr>
<tr>
<td>Brown et al. (12)</td>
<td>MTAP/CDKN2A</td>
<td>9</td>
<td>rs7023329</td>
<td>G</td>
<td>4.0 \times 10^{-7}</td>
<td>0.85 (0.80–0.91)</td>
</tr>
<tr>
<td>Bishop. et al. (13)</td>
<td>MTAP</td>
<td>9</td>
<td>rs10757257</td>
<td>G</td>
<td>3.4 \times 10^{-8}</td>
<td>1.23 (1.15–1.30)</td>
</tr>
<tr>
<td>Nan et al. (10)</td>
<td>TYR</td>
<td>11</td>
<td>rs1126809*</td>
<td>A</td>
<td>2.8 \times 10^{-7}</td>
<td>1.21 (1.13–1.30)</td>
</tr>
<tr>
<td>Brown et al. (12)</td>
<td>TYR</td>
<td>11</td>
<td>rs1393350</td>
<td>A</td>
<td>2.4 \times 10^{-13}</td>
<td>1.29 (1.21–1.38)</td>
</tr>
<tr>
<td>Falchi. et al. (14)</td>
<td>CCND1</td>
<td>11</td>
<td>rs1485933</td>
<td>A</td>
<td>0.0012</td>
<td>1.11 (1.04–1.18)</td>
</tr>
<tr>
<td>Falchi. et al. (14)</td>
<td>ATM</td>
<td>11</td>
<td>rs1801516</td>
<td>A</td>
<td>3.4 \times 10^{-9}</td>
<td>0.84 (0.78–0.90)</td>
</tr>
<tr>
<td>Brown et al. (12)</td>
<td>MCI1R</td>
<td>16</td>
<td>rs258322</td>
<td>A</td>
<td>2.5 \times 10^{-27}</td>
<td>1.67 (1.52–1.83)</td>
</tr>
<tr>
<td>Gudbjartsson et al. (11)</td>
<td>PIGU</td>
<td>20</td>
<td>rs910873</td>
<td>C</td>
<td>9.9 \times 10^{-16}</td>
<td>1.75 (1.53–2.01)</td>
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<tr>
<td>Gudbjartsson et al. (11)</td>
<td>MTHF7B</td>
<td>20</td>
<td>rs1885120</td>
<td>G</td>
<td>9.8 \times 10^{-16}</td>
<td>1.78 (1.54–2.04)</td>
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<tr>
<td>Falchi. et al. (14)</td>
<td>MX2</td>
<td>21</td>
<td>rs45430</td>
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<td>2.9 \times 10^{-8}</td>
<td>0.88 (0.85–0.92)</td>
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<tr>
<td>Bishop. et al. (13)</td>
<td>PLA2G6</td>
<td>22</td>
<td>rs29385</td>
<td>C</td>
<td>2.6 \times 10^{-7}</td>
<td>1.23 (1.15–1.30)</td>
</tr>
</tbody>
</table>

CHR, chromosome; MA, minor allele and also test allele; NA, not available; OR, odds ratio; our study, Harvard and MD Anderson melanoma GWAS; Ref, reference.

*SNP rs1126809 was not included in our GWAS, and we used SNP rs1847134 as substitution, the LD between the two SNPs is 0.96. SNP rs1485933 was not included in our GWAS, nor any SNPs in high linkage disequilibrium with it.

Fig. 1. Regional association plot of SNPs with melanoma risk. The vertical axis represents the $-\log_{10} P$ values. Recombination rates in this region are plotted in the background. LD is represented by R2 as five levels: 0–0.2, 0.2–0.4, 0.4–0.6, 0.6–0.8, and 0.8–1.0. The significance level of the SNP rs4698934 was shown for both discovery set and discovery and replication combined. The plot was generated based on Hapmap Build 37.3

Fig. 2. Somatic mutation of TET2 detection by next-generation sequencing in sporadic melanoma. Plot of copy number variation by chromosomes, sex chromosomes are excluded from the analysis. The vertical axis is the ratio of number of reads for this specimen and a panel of normal in log base 2 scales. A value of 0 denotes no difference from normal (diploid). When the sample contains 100% tumor cells, a value of −1 equals to 1 copy loss and 0.58 is 1 copy gain.
for melanoma, such as pigmentation and sun exposure, vary among European populations. These may underlie previously noted differences between the US and Australian or GenoMEL study findings (17). Further meta-analysis of the existing GWASs is warranted to identify additional melanoma susceptibility loci. Although the identification of low-risk alleles has stimulated much enthusiasm in the field, gaining a deeper understanding of the biological consequences and clinical utility of these findings remains challenging. Future prospective studies to elucidate whether germline TET2 SNP correlates with somatic TET2 mutation in melanoma patients will provide critical insight, which will require larger clinical study with multiple institutions.

**Supplementary material**

Supplementary Methods and Supplementary Table 1 can be found at http://carcin.oxfordjournals.org/.

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**Conflict of Interest Statement:** None declared.

**References**


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