Genetic variants of the ADPRT, XRCC1 and APE1 genes and risk of cutaneous melanoma

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Sunlight causes various kinds of DNA damage, including oxidative lesions that are removed effectively by the base excision repair (BER) pathway, in which ADPRT, XRCC1 and APE1 play a key role. However, genetic variation in these genes may alter their functions. We hypothesized that ADPRT, XRCC1 and APE1 polymorphisms are associated with risk of cutaneous melanoma (CM). In a hospital-based case–control study of 602 CM patients and 603 cancer-free control subjects frequency matched on age, sex and ethnicity, we genotyped for three non-synonymous single nucleotide polymorphisms (SNPs) (i.e. the ADPRT Val762Ala, XRCC1 Arg399Gln and APE1 Asp148Glu) and assessed their associations with risk of CM. We found no significant difference in the allele frequencies between cases and controls for any of these three SNPs. However, we found that, compared with the APE1 Asp/Asp genotype, a significantly decreased risk of CM was associated with the APE1 Asp/Glu (adjusted odds ratio (OR), 0.60; 95% confidence interval (CI), 0.41–0.86), Glu/Glu (OR, 0.58; 95% CI, 0.38–0.88) and combined APE1 Asp/Glu+Glu/Glu (OR, 0.59; 95% CI, 0.42–0.83) genotypes, but not for other XRCC1 variant genotypes. Moreover, there was evidence for a possible gene–gene interaction between XRCC1 and APE1 variants in the association with risk of CM (P = 0.030). We conclude that the APE1 Glu variant may have an effect or interact with XRCC1 in the etiology of CM or in linkage disequilibrium with other untyped protective alleles. Larger studies with more SNPs in the BER genes are needed to verify these findings.

Abbreviations: ADPRT, adenosine diphosphate ribosyl transferase; APE1, apurinic/apyrimidinic endonuclease/redox effector-1; CM, cutaneous melanoma; BER, base excision repair; MAF, minor allele frequency; nsSNP, non-synonymous single nucleotide polymorphisms; PCR, polymerase chain reaction; UV, ultraviolet; XRCC1, X-ray repair cross complementing group 1.

Introduction

Cutaneous melanoma (CM), the most serious form of malignant skin cancer, has been increasing in incidence in the USA for the last 30 years (1). In 2006, there were estimated 62,190 new CM cases and 7,910 deaths from CM in the USA (2). Epidemiological studies suggest that exposure to sunlight, particularly intermittent exposure (3), as well as a family history of CM and genetic variations (4) may be risk factors for CM.

Sunlight causes various kinds of DNA damage, including bulky lesions, such as pyrimidine-pyrimidine dimers, and oxidative damage, such as single DNA strand breaks, which may lead to mutations, if not repaired efficiently. Thus, DNA repair is critical for maintaining the integrity of the genome (5–7). More than 150 human DNA repair genes in several distinct pathways have been identified (8). Among these pathways, the base excision repair (BER) pathway, which possibly handles the largest number of cytotoxic and mutagenic base lesions, has only recently been associated with human cancer (9).

The BER pathway is one of the important mechanisms responsible for the repair of DNA damage resulting from exposure to various endogenous and exogenous carcinogens. The BER pathway specifically removes alterations of a single base that has been methylated, oxidized, or reduced and thus rectifies single-strand interruptions in DNA (10). Several proteins are involved in the BER process, of which poly (ADP-ribose) polymerase family member 1 (PARP1)—also known as adenosine diphosphate ribosyl transferase (ADPRT)—and X-ray repair cross-complementing group 1 (XRCC1) and apurinic/apyrimidinic endonuclease/redox effector-1 (APE1/Ref-1) play important roles (11). However, these genes are polymorphic, and genetic variation in these genes may alter BER functions (12,13).

The ADPRT gene is located at chromosome 1q41–q42 that encodes a chromatin-associated enzyme—poly(ADP-ribose) transferase—which modifies various nuclear proteins by poly(ADP-ribosylation) (14). There are a number of reported polymorphisms in ADPRT (http://egp.gs.washington.edu/data/adprt/adprt.csnps.txt), but only five are non-synonymous single nucleotide polymorphisms (nsSNPs) that cause an amino acid change, only one of which is a common SNP [i.e. minor allele frequency (MAF) > 0.05] (http://egp.gs.washington.edu/data/adprt/adprt.pph-sift.txt). This common ADPRT nsSNP is a base T→C transition at site 40,676 that causes Val to Ala amino acid substitution at codon 762 of exon 17 (i.e. Val762Ala; rs1136410), located in the sixth helix of the catalytic domain. Recent studies suggest that the Val762Ala
polymorphism is associated with altered ADPRT function, with the Ala allele contributing to significantly low poly (ADP-ribose)ation activities in an allele dosage-dependent manner (14). The low ADPRT activity due to this polymorphism is thought to reduce its ability to recruit XRCC1 and other related proteins.

XRCC1 plays a direct role in BER, because it interacts with a complex of DNA repair proteins, including poly (ADP-ribose) polymerase, DNA ligase 3, and DNA polymerase β (15,16). Located at chromosome 19q13.2, XRCC1 has numerous SNPs (http://egp.gs.washington.edu/data/xrcc1/xrcc1.cnps.txt), of which eight are nsSNPs but the only three common SNPs are Arg194Trp, Arg280His, and Arg399Gln (http://egp.gs.washington.edu/data/xrcc1/xrcc1.pph-sift.txt). The Arg399Gln polymorphism has the highest MAF (0.23), a base G→A transition at site 25897 that causes Arg to Gln amino acid substitution at codon 399 of exon 10 (rs25487).

The Arg399Gln polymorphism, which occurs at a conserved residue in the poly(ADP-ribose) polymerase-binding domain of XRCC1, may alter the efficiency of the repair process (17). In a recent report, it was found that the 399Gln allele was in complete linkage disequilibrium (LD) with the 280His allele (D’ = 1.0) and the 280His allele was in complete LD with the 194Arg allele (D’ = 1.0) in both whites and African Americans (18).

APE1, also known as APEX1, is located at chromosome 14q11.2-q12 that encodes a protein involved in both BER and regulation of gene expression as a redox co-activator of different transcription factors, such as p53 and AP-1 (19). APE1 only has three nsSNPs (http://egp.gs.washington.edu/data/apex/apexx.pph-sift.txt), of which only one has an MAF >5%, a base T→G transversion polymorphism at site 2573 that changes Asp to Glu at codon 148 of exon 5 of the APE1 gene (i.e. Asp148Glu; rs3136820). Individuals carrying the Glu allele are thought to have a higher sensitivity to ionizing radiation than those who carry the Asp allele (20).

Most of the published studies of the association between the ADPRT, XRCC1 and APE1 polymorphisms and cancer risk relate to cancers of the breast (18), lung (21,22), esophagus (23) and skin (24,25). Only two studies have investigated the association with CM, but the sample sizes in those studies were relatively small and the studies included only XRCC1 polymorphisms (26,27). We hypothesized that selected common nsSNPs of the BER genes—specifically ADPRT, XRCC1 and APE1—may interact to contribute collectively to risk of CM. We tested this hypothesis in a study of 602 patients with CM and 603 cancer-free control subjects frequency-matched by age, sex and ethnicity.

Materials and methods

Subjects

The subject recruitment was described elsewhere (28). Briefly, this study included patients newly diagnosed with CM who were referred to The University of Texas M. D. Anderson Cancer Center (MDACC) between May 1994 and September 2004, who agreed to donate one 30-ml blood sample for our ongoing melanoma study. These patient subjects accounted for ~20% of all patients seen at MDACC, because those referred cases who were treated elsewhere were not eligible for the phenotype assays using peripheral blood lymphocytes. All patients with CM recruited for this study were histologically classified according to the 2002 American Joint Committee on Cancer ‘melanoma staging system’ (29). Control subjects were recruited during a similar period from visitors to MDACC who were not seeking medical care but were accompanying patients to our outpatient clinics. We first approached potential control subjects by using a screening questionnaire to determine their willingness to participate in research studies and to obtain information about their demographic characteristics for frequency-matching purposes and their personal history of cancer for eligibility. We then recruited eligible control subjects from those willing respondents, who constituted approximately 90% of control screened. Control subjects were self-reported cancer-free, agreed to provide a one-time blood sample for this study, were not related by blood to one another or any patient entered in the study, and were frequency-matched to patients with CM in this study by age (±5 years) and sex. The research protocol was approved by the MDACC institutional review board.

After we obtained their informed consent, we interviewed each eligible patient and each control and each control subject in person to obtain data on their age, sex, ethnicity, host characteristics (e.g. color of skin, eyes and hair), history of sun exposure (e.g. tanning ability, number of sunburns with blistering in their lifetime, and freckling in the sun as a child), and Fitzpatrick’s sun-reactive skin type (created based on the questionnaire data) (30). At the end of the interview, blood (30 ml) drawn from each patient and control subject was collected in heparinized tubes.

Genotyping

A leukocyte cell pellet was obtained from the buffy coat by centrifugation of 1 ml of whole blood. The cell pellet was used for genomic DNA extraction by using the Qiagen DNA Blood Mini Kit (Valencia, CA). The DNA purity and concentration were determined by spectrophotometric measurement of absorbance at 260 and 280 nm. We had 3 cases whose DNA samples were run out. Due to our frequency matching design, we could not identify enough matched controls among the hospital visitors during the study period for another 147 cases who were either <30 or >70 years old. Therefore, the DNA samples from a total of 602 CM patients and 603 controls were available for genotyping. Polymerase chain reaction (PCR) was used to amplify the fragments of ADPRT that contained the sites of Val762Ala (rs1136410), the fragments of XRCC1 that contained the sites of Arg399Gln (rs25487), and the fragments of APEI that contained the sites of Asp148Glu (rs3136820). The ADPRT Val762Ala wild type (Val) and variant (Ala) alleles were identified by using the forward primer 5’-TTGGCTCCTCCAGGCAACGAGC-3’ and the reverse primer 5’-CATGATGAGTGATCTTGTGTGT-3’ to amplify a 110-bp PCR product. A 5-μl aliquot was digested with 1.5 U of BstUI restriction enzyme (New England Biolabs, Beverly, MA) at 60°C for 2 h and then separated on a 3% Metaphor agarose gel. The three possible genotypes were defined by three distinct patterns of bands seen on the gel: Val/Val (110 bp); Val/Ala (110 and 90 bp); and Ala/Ala (90 bp). The XRCC1 Arg399Gln wild type and variant alleles were identified by using the forward primer 5’-ATTGCCACGACAGGATAAG-3’ and the reverse primer 5’-GCCCTTCAGATCACACCTAA-3’ to amplify a 213-bp PCR product. A 5-μl aliquot was digested with 1.5 U of NciI restriction enzyme (New England Biolabs) at 37°C for 16 h and then separated on a 2% agarose gel. The three possible genotypes were defined by three distinct patterns of bands seen on the gel: Gln/Gln (213 bp); Gln/Arg (213, 161 and 52 bp); and Arg/Arg (161 and 52 bp). The APE1 Asp148Glu allele wild-type (Glu) and variant (Asp) allele were identified by using the forward primer 5’-ACGGCATAGGGTACACCTAA-3’ and the reverse primer 5’-GCTGTTACGACGACAAAGC-3’ to amplify a 206-bp PCR product. A 5-μl aliquot was digested with 1.5 U of BstZ1 restriction enzyme (New England Biolabs), at 60°C for 16 h and then was separated on a 2% agarose gel. The three possible genotypes were defined by three distinct patterns of bands visualized on the gel: Glu/Glu (206 bp); Glu/Asp (206, 153, and 53 bp); and Asp/Asp (153 and 53 bp). The genotyping assays for ~10% of the samples were repeated, and the results were 100% concordant.

Statistical analysis

The χ²-test was used to evaluate differences in the frequency distributions of selected demographic variables, known risk factors, and each allele and genotype of the ADPRT, XRCC1 and APE1 polymorphisms between patients with CM and control subjects. On the screening questionnaire, skin color was self-assessed on a scale of 1 (light)–10 (dark); skin color values of 1–3 were categorized as fair skin, values of 4–6 as brown skin, and values of 7–10 as dark skin. Univariate and multivariate logistic regression analyses were used to obtain the crude and adjusted odds ratios (ORs) for the risk of CM and 95% confidence intervals (CIs). The multivariate adjustment included age, sex and host characteristics (e.g. color of skin, eyes and hair), sun exposure history (e.g. tanning ability, lifetime number of sunburns with blistering in their lifetime, and freckling in the sun as a child), and Fitzpatrick’s sun-reactive skin type (created based on the questionnaire data) (30). At the end of the interview, blood (30 ml) drawn from each patient and control subject was collected in heparinized tubes.

We also assessed interactions between the combined XRCC1 and APE1 genotypes and selected sunlight exposure-related variables. ORs, 95% CIs, and
Results

As a result of frequency matching, there was no difference in the frequency distribution of age ($P = 0.132$) and sex ($P = 0.207$) between patients and control subjects (Table I). Among the patients, tumor sites included 14% on the head and neck, 26% on an upper extremity, 21% on a lower extremity, 36% on the trunk and 3% on other sites. Based on the American Joint Committee on Cancer T-category criteria (29) and Breslow thickness (29,33), 47% had tumors <1.0 mm (T1), 24% had tumors 1.01–2.0 mm (T2), 16% had tumors >2.01 (T3–4) and 13% were unclassified. Determination of Clark levels (29,33) showed that 10% were level I (in situ), 55% level II–III and 35% level IV–V (data not shown). In this study population, the frequencies of classic phenotype traits (except for skin color, tanning ability and family history of any cancer) that are known risk factors for CM were significantly higher in patients with CM than in control subjects after adjustment for age and sex. However, for skin color, the association was borderline significant ($P = 0.061$) (Table I). Therefore, these risk factors appeared to be involved in the etiology of CM in this population and were used in the later stratification and gene–environment interaction analysis.

Genotype distribution among patients with CM and control subjects

The genotype and allele frequencies of the ADPRTVal762Ala, XRCC1Arg399Gln and APE1Asp148Glu polymorphisms and their associations with risk of CM are shown in Table II. The genotype distribution of control subjects was in agreement with the Hardy–Weinberg equilibrium. We found no significant differences in the allele frequency between cases and controls for any of the three polymorphisms. However, the ADPRT variant Ala, XRCC1 variant Gln and APE1 variant Glu allele frequencies were lower among patients (0.152, 0.351 and 0.461, respectively) than among controls (0.172, 0.355 and 0.495, respectively) ($P = 0.202$ for the ADPRT Ala allele; 0.871 for the XRCC1 Gln allele, and 0.103 for the APE1 Glu allele). These results suggest that the Ala, Gln, and Glu alleles may be protective alleles in this study population.

For the three polymorphisms, the difference in genotype frequency distributions was not statistically significant between the cases and controls. However, the frequency of combined ADPRT Val/Ala+Ala/Ala genotype was lower in the cases (27.4%) than in the controls (31.5%; $P = 0.119$), the frequency of XRCC1 combined Arg/Gln+Glu/Gln genotype was lower among the cases (57.5%) than among the controls (58.7%; $P = 0.665$), and the frequency of combined APE1 Asp/Glu+Glu/Glu genotype was significantly lower among the cases (68.8%) than among the controls (74.1%; $P = 0.040$). Taken together, these data suggest that the ADPRT Ala/Ala, Val/Ala+Ala/Ala, XRCC1 Gln/Gln, Arg/Gln+Gln/Gln, and APE1Asp148Glu Glu/Glu, Asp/Glu +Glu/Glu genotypes are probably protective against CM. However, the distributions of these genotypes were independent of tumor histological types, Clark levels, tumor site, and tumor thickness ($P > 0.05$ for all; data not shown).

Association between the ADPRTVal762ala, XRCC1arg399gln and APE1asp148glu polymorphisms and risk of CM

When the ADPRT Val/Val genotype was used as the reference group, no significant risk was associated with Val/Ala, Ala/Ala, or combined Val/Ala+Ala/Ala genotypes, assuming a dominant model for the Ala variant allele. This assumption was made because only a few subjects carried the Ala/Ala genotype and to be consistent with the other two variants. Similarly, when the XRCC1 Arg/Arg genotype was used as the reference group, no significant risk was associated with Arg/Gln, or the combined Arg/Gln+Gln/Gln genotypes, assuming a dominant

<table>
<thead>
<tr>
<th>Variables</th>
<th>Cases (N = 602)*</th>
<th>Controls (N = 603)*</th>
<th>P-valueb</th>
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</tr>
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<td>&gt;45</td>
<td>427</td>
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</tr>
<tr>
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<td>94</td>
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<tr>
<td>Fair</td>
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<td>57.0</td>
<td>259</td>
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<tr>
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<td>Poor (low)</td>
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<tr>
<td>≥1</td>
<td>434</td>
<td>73.8</td>
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<td>Freckling in the sun as a child</td>
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<td>263</td>
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<tr>
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<td>373</td>
<td>63.9</td>
<td>279</td>
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*The sum of the numbers of subjects in some of the strata was fewer than the total number of subjects because some subjects did not provide the information.

bTwo-sided χ²-test.
model for the Gln variant allele. When the APE1Asp/Asp genotype was used as the reference group, however, a significantly decreased risk for CM was associated with the Asp/Glu (adjusted OR, 0.60; 95% CI, 0.41–0.86), Glu/Glu (adjusted OR, 0.58; 95% CI, 0.38–0.88), and the combined Asp/Glu+Glu/Glu (OR, 0.59; 95% CI, 0.42–0.83) genotypes, assuming a dominant model for the Glu variant allele (Table II).

Gene–gene interaction between ADPRTVal762Ala, XRCC1Arg399Gln, and APE1Asp148Glu genotypes

Considering potential interactions among variants in genes involved in the same BER pathway, we further evaluated the association between the combined genotypes of ADPRT, XRCC1 and APE1 and risk of CM. When the combined genotype of ADPRT (Val/Val)/XRCC1 (Arg/Arg) was used as the reference, a non-significantly reduced risk for CM was observed in those who carried the other genotypes. However, when the combined genotype of ADPRT (Val/Val)/APE1 (Asp/Asp) was used as the reference, a significantly decreased risk for CM was associated with either the ADPRT (Val/Val)/APE1(Glu/Glu+Asp/Glu) genotype (adjusted OR, 0.55; 95% CI, 0.37–0.83) or ADPRT (Val/Ala+Ala/Ala)/APE1 (Glu/Glu+Asp/Glu) genotype (adjusted OR, 0.57; 95% CI, 0.36–0.90), and the trend test was significant (P = 0.005). When the combined genotype of APE (Asp/Asp)/XRCC1 (Arg/Arg) was used as the reference, we only found a borderline-significantly reduced risk for CM in those who carried the other genotypes, although the trend test was significant (P = 0.006) (Table III).

In the analysis of the gene–gene (i.e. among ADPRT, XRCC1 and APE1) interaction in the multivariate logistic regression models, we used the dichotomized genotypes (i.e. Val/Val versus Val/Ala+Ala/Ala for ADPRT; Arg/Arg vs. Gln/Gln+Arg/Gln for XRCC1; and Asp/Asp versus Glu/Glu+Asp/Glu for APE1). The only evident interaction was between the XRCC1 and APE1 variant genotypes (P = 0.030) (Table III), because when the combined genotype of APE (Asp/Asp)/XRCC1 (Arg/Arg) was used as the reference, the combined genotype of APE (Asp/Asp)/XRCC1 (Glu/Gln+Arg/Gln) was associated with a non-significantly increased risk for CM but other genotypes were associated with a non-significantly reduced risk for CM (Table III).

Finally, we evaluated possible three-way interaction among these SNPs. As shown in Table IV, the most frequent combined XRCC1Arg/Arg+ADPRTVal/Val+APE1Asp/Glu genotype was 14.5% in the cases and 12.6% in the controls and many of the combined genotypes were <5%, which was combined into one group named as ‘Others’ (26.4% for the cases and 27% for the controls). Apparently reduced risk was associated with those combined genotypes with at least one APE1 Glu allele, although only one was statistically significant (adjusted OR, 0.41; 95% CI, 0.19–0.89 for the XRCC1Arg/Arg+ADPRTVal/Val+APE1Glu/Glu genotype). However, no statistical evidence for a three-way interaction was identified (P = 0.553) as assessed in the multivariate logistic model. It is possible that this study had a limited study power to detect such an interaction.

Stratified analyses of the combined genotypes of XRCC1Arg399Gln and APE1Asp148Glu and known risk factors for CM

Because our study sample size was not large enough to allow for the test of a three-way gene–gene–environment interaction, we performed stratified analyses for the gene–gene interaction between the APE1 and XRCC1 variant genotypes by the known risk factors for CM (Table V). It was apparent that the APE1–XRCC1 gene–gene interaction was more pronounced in the subgroups having dark skin color (P = 0.008), black or brown hair color, good tanning ability (P = 0.027), freckles in the sun as a child (P = 0.005), no dysplastic nevi (P = 0.031) or first-degree relatives of any cancer (P = 0.010). For example, compared with the combined genotype of APE

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Table II. Genotype and allele frequencies of the ADPRT, XRCC1 and APE1 polymorphisms among non-Hispanic white patients with CM and control subjects and their associations with risk of CM

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Cases (N = 602)</th>
<th>Controls (N = 603)*</th>
<th>P-valueb</th>
<th>Crude OR (95% CI)</th>
<th>Adjusted OR (95% CI)c</th>
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<tr>
<td></td>
<td>No.</td>
<td>%</td>
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<td>ADPRT Val762Ala</td>
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<td>Val/Val</td>
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<td>Ala/Ala</td>
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<td>1.00 (0.51–1.97)</td>
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<td>Val/Ala+Ala/Ala</td>
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<td>Ala allele</td>
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<td>0.2%</td>
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<td>XRCC1 Arg399Gln</td>
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<td>Arg/Gln</td>
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</tr>
<tr>
<td>APE1 Asp148Glu</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asp/Asp</td>
<td>188</td>
<td>31.2</td>
<td></td>
<td></td>
<td>1.00</td>
</tr>
<tr>
<td>Asp/Glu</td>
<td>273</td>
<td>45.4</td>
<td></td>
<td></td>
<td>0.76 (0.58–1.00)</td>
</tr>
<tr>
<td>Glu/Glu</td>
<td>141</td>
<td>23.4</td>
<td></td>
<td></td>
<td>0.78 (0.57–1.07)</td>
</tr>
<tr>
<td>Asp/Glu+Glu/Glu</td>
<td>414</td>
<td>68.8</td>
<td></td>
<td></td>
<td>0.77 (0.60–0.99)</td>
</tr>
<tr>
<td>Glu allele</td>
<td>0.461</td>
<td>0.4%</td>
<td></td>
<td></td>
<td>0.103</td>
</tr>
</tbody>
</table>

*The observed genotype frequency among the control subjects was in agreement with the Hardy–Weinberg equilibrium (χ² = 0.048, P = 0.827 for ADPRT; χ² = 0.120, P = 0.729 for XRCC1; and χ² = 0.133, P = 0.716 for APE1).

bTwo-sided χ²-test for either genotype distribution or allele frequency.

cORs were adjusted for all selected variables included in Table I.
In this hospital-based case–control study of CM, we demonstrated a reduced risk of CM associated with the variant APE1-148Glu genotypes. It is also possible that the APE1-148Glu variant may interact with the ADPRT-Val and XRCC1-399Gln variants in the etiology of CM. However, these variants appeared not to be associated with tumor histological types, tumor sites, Clark levels, or tumor thickness, nor did they appear to be involved in interactions with the known risk factors for CM. Although exactly how the XRCC1-399Gln and APE1-148Gln variants work to influence the risk explored the gene–environment interactions. However, there was no statistical evidence for gene–environment interactions either in multivariate logistic regression models or in additive interaction models (data not shown).

**Discussion**


of CM is not known, these variants either could be functional themselves or could be in LD with other functional variants involved in the etiology of CM. Several lines of evidence support the role of the BER pathway genes in the etiology of CM. The human skin is constantly exposed to environmental pro-oxidative agents such as UV radiation, and sunlight has been implicated as a major environmental contributor to the development of most cases of CM (34). Although most of the UV-induced DNA damage is repaired by the nucleotide excision repair pathway (5–7), BER also plays an important role in protecting against the occurrence of tumors, including CM (41). Considering the involvement of BER in repairing oxidative damage to DNA, particularly damage induced by UVA, it is likely that the BER plays a role in UV-induced skin mutagenesis (42).

Although there have been no previous reports of the association between the ADPRT polymorphisms and risk of CM, a recent study found that overexpression of ADPRT is potentially a novel molecular marker of aggressive CM and that there is a direct correlation between ADPRT-mediated inhibition of apoptosis associated with CM and the biologic behavior of CM (43). In the present study, we found that the 762Ala variant genotypes (i.e. Val762Ala + Ala762Ala) were associated with a non-significantly increased risk of CM. Although larger studies are needed to substantiate this finding, XRCC1 participates directly in both BER and single-strand break repair (44,45). The association of XRCC1 polymorphisms with cancer risk at many sites has been studied extensively (11), but there are only four studies of skin cancers, two of which included CM. In a study of 125 CM cases and 211 controls (26), the 399Gln/Gln genotype was found to be associated with a non-significantly increased risk of CM. In contrast, in a nested case–control study within the Nurses’ Health Study of 219 melanoma and 873 controls, the 399Gln/Gln genotype was associated with a non-significantly reduced risk of CM (27). Consistent with that study, our study of 602 CM cases and 603 controls also showed that both variant genotypes (399Arg/Gln+399Gln/Gln) were associated with a non-significantly reduced risk of CM.
Reported studies of non-melanoma skin cancer afford interesting findings regarding the association of polymorphisms with the occurrence of skin cancers. Although a study of 97 patients with basal-cell carcinoma and 97 controls did not find any evidence for an association of the 399Gln/Glu genotype (24), another population-based case–control study of non-melanoma skin cancer (499 basal-cell carcinoma, 246 squamous-cell carcinoma, and 431 controls) (25) showed that the 399Gln/Glu genotype was associated with a lower risk in general, but a high risk in those patients who had a history of painful sunburn. That finding provides an excellent example of gene–environment interaction, but like all previously published CM studies, our study showed no evidence for a gene–environment interaction in CM, although much larger studies are needed to confirm this finding.

No previously reported study has investigated the association between APE1 polymorphisms and risk of CM. It has been shown that, when exposed to stresses, such as UV radiation, oxidative agents (such as H2O2 and reactive oxygen species) can generate injuries, promoting a transient induction that correlates with an increase of endonuclease APE1 variant genotypes leading to altered BER might be associated with hypersensitivity to ionizing radiation (20). However, our data suggest that the 148Glu variant genotypes (i.e. 148Glu/Asp, 148Glu/Glu and 148Glu/Asp+Glu/Glu) were associated with a reduced risk of CM.

It is conceivable that genes involved in the same pathway may have a collective effect on DNA repair outcomes. Indeed, when we analyzed the combined genotypes of the three selected nsSNPs, it was apparent that ADPRT and APE1 collectively contribute to a reduced risk for CM. Moreover, XRCC1 and APE1 polymorphisms may also interact to have a collective effect on risk of CM. We had speculated that the variant genotypes leading to altered BER might be associated with an increased risk of CM, because reduced BER may result in cumulative damage to DNA and thereby an increased frequency of mutations. However, our data does not appear to support this hypothesis. Alternatively, cells without efficient BER may be more prone to apoptosis, a mechanism that may possibly reduce the risk of carcinogenesis (25). Indeed, this paradox phenomenon was reflected in the observed interaction between the known risk factors and the observed genotypes. For example, the APE1 genotypes containing the Glu allele appeared to be associated with a lower risk of developing CM than other APE1 genotypes. However, the combined variant genotypes of XRCC1 and APE1 had a statistically significant interactive effect on risk of CM in subgroups of those who had dark or brown skin or black or brown hair, good tanning ability, freckling, family history of cancer or no dysplastic nevi, although most of these significant results disappeared after adjustment for multiple tests. Although the study sample size did not provide enough statistical power, these findings suggest that XRCC1 and APE1 polymorphisms may interact to have a collective effect on risk of CM. Further studies are necessary to identify the actual mechanisms underlying the association between the ADPRT, XRCC1, and APE1 variant alleles and risk of CM. Because of uncontrolled biases in the selection of subjects and limited sample size, larger and population-based studies with inclusion of more SNPs in genes involved in the BER pathway are warranted to confirm these findings.

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Conflict of Interest Statement: None declared.

References


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