Missense Polymorphisms in Matrix Metalloproteinase Genes and Skin Cancer Risk

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Abstract

Matrix metalloproteinases (MMP) degrade various components of the extracellular matrix, and their overexpression has been implicated in tumor progression. Nonsynonymous single nucleotide polymorphisms (SNPs) lead to amino acid substitutions that can alter the function of the encoded protein. We evaluated the associations of six nonsynonymous SNPs in the MMP3, MMP8, and MMP9 genes with skin cancer risk in a nested case-control study of Caucasians within the Nurses’ Health Study among 218 melanoma cases, 285 squamous cell carcinoma (SCC) cases, 300 basal cell carcinoma (BCC) cases, and 870 normal controls. We observed that the MMP9 Arg668Gln polymorphism was significantly associated with a decreased risk of SCC. Compared with the Arg/Arg group, the multivariate odds ratio was 0.67 (95% confidence interval, 0.47-0.97) for the Arg/Gln group and 0.21 (95% confidence interval, 0.05-0.97) for the Gln/Gln group (P\text{trend} = 0.004). We did not observe any association of this SNP with the risks of melanoma and basal cell carcinoma. No associations were found for other SNPs with skin cancer risk. This study provides evidence for the contribution of the MMP9 Arg668Gln to SCC development. (Cancer Epidemiol Biomarkers Prev 2008;17(12):3551–7)

Introduction

Skin cancer is the most common form of cancer in the United States, with ~1 million new cases per year (1). Among three major types of skin cancer, melanoma is the most serious form. The most common type of skin cancer is basal cell carcinoma (BCC), followed by squamous cell carcinoma (SCC). In addition to the carcinogenic effects of UV radiation, genetic factors also contribute to the development of skin cancer (2-4).

Degradation and remodeling of the extracellular matrix (ECM) and basement membrane are essential steps in tumor progression. Matrix metalloproteinases (MMP) belong to a family of over 20 zinc-dependent enzymes that degrade various protein components of the ECM. Overexpression of these enzymes leads to increased proteolytic degradation of ECM, resulting in the invasion and metastasis of many types of tumors (5-9). Based on their structure, substrate specificity, and cellular localization, MMPs can be divided into five subgroups: collagenases (including MMP8), gelatinases (including MMP9), stromelysins (including MMP3), matrilysins, and membrane-type MMPs (10). Due to their crucial roles in tumor development, MMPs have become drug targets for cancer therapy in some clinical trials (11, 12).

Single nucleotide polymorphisms (SNPs) are the most common genetic variation in the human genome, and nonsynonymous SNPs resulting in amino acid substitutions can alter the function of the encoded protein. It has been reported that MMP genetic variants including nonsynonymous polymorphisms are associated with the development of different types of tumors, such as melanoma, breast, lung, bladder, and colorectal tumors (13-17).

We evaluated the associations of six nonsynonymous SNPs in the MMP3, MMP8, and MMP9 genes with the risks of three skin cancer types (melanoma, SCC, and BCC) in a nested case-control study of Caucasians within the Nurses’ Health Study.

Materials and Methods

Study Population. The Nurses’ Health Study was established in 1976, when 121,700 female registered nurses between the ages of 30 and 55 y residing in 11 larger U.S. states completed and returned the initial self-administered questionnaire on their medical histories and baseline health-related exposures, forming the basis for the Nurses’ Health Study cohort. Updated information has been obtained by questionnaires every 2 y. From May 1989 through September 1990, we collected blood samples from 32,826 participants in the Nurses’ Health Study cohort.

The distribution of risk factors for skin cancer in the subcohort of those who donated blood samples was similar to that in the overall cohort (4). Eligible cases in this study consisted of women with incident skin cancer from the subcohort who had given a blood specimen, including SCC and BCC cases with a diagnosis any time after blood collection up to June 1, 1998, and melanoma cases up to June 1, 2000, who had no previously diagnosed skin...
cancer. A common control series was randomly selected from participants who gave a blood sample and were free of diagnosed skin cancer up to and including the questionnaire cycle during which the case was diagnosed. One or two controls were matched to each case by year of birth (±1 y). All subjects were the U.S. non-Hispanic Caucasian women in this study. The participation rates of cases and controls were 92% and 89%, respectively. The nested case-control study consisted of 218 incident melanoma cases, 285 incident SCC cases, a sample of 300 BCC cases from the large number of incident cases, and 870 age-matched controls. The study protocol was approved by the Committee on Use of Human Subjects of the Brigham and Women’s Hospital, Boston, MA.

**Exposure Data.** We obtained information regarding skin cancer risk factors from the prospective biennial questionnaires and a retrospective supplementary questionnaire. Information on natural hair color at age 20 y and childhood and adolescent tanning tendency were collected in the 1982 prospective questionnaire. Ethnic group was ascertained in the 1992 questionnaire. In the skin cancer nested case-control study, natural skin color and other sun exposure–related information were collected by the retrospective supplementary questionnaire in 2002. In addition, the 11 states of residence of cohort members at baseline were grouped into three regions: Northeast (Connecticut, Massachusetts, Maryland, New Jersey, New York, and Pennsylvania), North-central (Michigan and Ohio), and West and South (California, Texas, and Florida). To estimate sunlight exposure for each subject, an UV database for 50 U.S. states was developed. The database used reports from the Climatic Atlas of the United States, which reported mean daily solar radiation (in Langley) at the earth’s surface for weather stations around the country (18). The records of average annual solar radiation for January and July were extracted to represent winter and summer radiation, respectively. The mean solar radiation for each individual’s past (at different age categories) and current residences was derived from the UV value measured at the nearest weather station. Both summer and winter radiation indices were developed for the residence of each age category. A cumulative lifetime sun exposure was developed by combining the residence-linked UV value and hours spent outdoors at different age categories obtained from the retrospective supplementary questionnaire. This derived variable was significantly associated with skin cancer risk (19).

**Laboratory Assays.** We genotyped 6 SNPs including MMP3 Lys45Glu (rs6796260), MMP8 Lys87Glu (rs1940475), MMP9 Ala20Val (rs1805088), MMP9 Gln279Arg (rs17576), MMP9 Pro574Arg (rs2250889), and MMP9 Arg668Gln (rs2274756) by the 5 nuclease assay (TaqMan) in 384-well format, using the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems). TaqMan primers and probes were designed using the Primer Express Oligo Design software v2.0 (ABI PRISM). Due to assay failure, we genotyped rs6032619 as a surrogate for the MMP9 rs2250889 (r² = 1.0). We calculated the r² value between the rs6032619 and the rs2250889 using the HapMap project 90 (30 trios) Caucasian samples from a U.S. Utah population with Northern and Western European ancestry collected in 1980 by the Centre d’Etude du Polymorphisme Humain (20). Laboratory personnel were blinded to case-control status, and 10% blinded quality control samples (duplicate samples) were inserted to validate genotyping procedures; concordance for the blinded quality control samples was 100%. Primers, probes, and conditions for genotyping assays are available upon request.

**Statistical Methods.** We used the χ² test to assess whether the genotypes for all six SNPs were in Hardy-Weinberg equilibrium among the controls. We evaluated the association between each genotype and skin cancer risk using unconditional logistic regression. An additive model was used to calculate the value for trend on skin cancer risk according to an ordinal coding for genotype (0, 1, or 2 copies of SNP minor allele). We compared each type of skin cancer with the common control series to increase the statistical power. For the four MMP9 SNPs, haplotype frequencies and expected haplotype counts for each individual were estimated using a simple expectation-maximization algorithm, as implemented in SAS PROC HAPLOTYPE. The analyses of possible associations between haplotypes and skin cancer risk were done using the expectation-substitution technique (21). All statistical analyses were two-sided and carried out using SAS V9.1 (SAS Institute).

**Computational Prediction for Functional Significance of Nonsynonymous SNPs.** We used five bioinformatic tools, (a) BLOSUM62 (22), (b) PMut (23), (c) PolyPhen (24), (d) SIFT (25), and (e) SNPs3D (26), to predict the functional significance of the six nonsynonymous polymorphisms. These methods are described in the Supplementary Text.

**Results**

**Descriptive Characteristics of Skin Cancer Cases and Controls.** Basic characteristics of cases and controls in this study are presented in Table 1. Detailed description was published previously (27). In brief, at the beginning of the follow-up of this nested case-control study, the nurses were between ages 43 and 68 y (mean age, 58.7 y). The mean ages at diagnosis for incident melanoma, SCC, and BCC cases were 63.4, 64.7, and 64.0 y, respectively. Skin cancer cases were more likely to possess red hair color and fair skin color. The childhood tanning ability of cases was less than that of controls. Women in the West and South regions were more likely to be diagnosed with SCC or BCC compared with those in Northeast. A family history of skin cancer was a risk factor for all three types of skin cancer. Those with skin cancers were more likely to have used sunlamps or attended tanning salons. Those with skin cancers had higher cumulative sun exposure while wearing a bathing suit and more lifetime severe sunburns that blistered. For melanoma, there were 20.4% cases on the head and neck, 29.1% on the trunk, 23.0% on the arm and hand, and 27.6% on the leg and foot. For SCC, there were 44.5% cases on the head and neck, 17.4% on the trunk, 20.0% on the arm and hand, and 18.1% on the leg and foot.

**Associations of the Six SNPs in the MMP3, MMP8, and MMP9 Genes with Skin Cancer Risk.** The distributions of genotypes for 6 SNPs evaluated in this study were in Hardy-Weinberg equilibrium among controls (P > 0.05).
We evaluated the main effect of each polymorphism across the three types of skin cancer (Table 2). We observed that the Gln668 allele of the MMP9 Arg668Gln polymorphism was significantly associated with a decreased risk of SCC. Compared with the Arg/Arg group, the multivariate odds ratio (OR) was 0.67 [95% confidence interval (95% CI), 0.47-0.97] for the Arg/Gln group and 0.21 [95% CI, 0.05-0.97] for the Gln/Gln group (P trend = 0.004). The multivariate estimates were essentially the same as the age-adjusted ones.

In the multivariate analyses controlling for age and skin cancer risk factors, we observed that MMP8 Pro574Arg polymorphism was associated with an increased risk of BCC (OR, 1.23; 95% CI, 1.01-1.51), and MMP9 Gln279Arg was associated with a decreased risk of SCC (OR, 0.80; 95% CI, 0.64-0.99). However, these associations were not statistically significant in the age-adjusted analyses. The ORs (95% CI) of MMP8 for BCC risk and MMP9 for SCC risk were 0.21 (95% CI, 0.05-0.97) for the Arg/Lys group and 1.23 (95% CI, 0.05-9.7) for the Lys group, respectively. We did not observe any significant associations between any of these six SNPs and melanoma risk.

Haplotypes for the MMP9 Gene and Skin Cancer Risk. We did a global test to evaluate the difference in haplotype frequencies between cases and controls (Table 3). We found a significant difference in haplotype frequency for SCC (P global test = 0.02). The haplotype that carried the Gln279Arg and Arg668Gln variant alleles was significantly associated with a decreased risk of SCC after adjusting for age (OR, 0.62; 95% CI, 0.44-0.87). This association remained significant in the multivariate model (P global test = 0.004; OR, 0.56; 95% CI, 0.40-0.80). This association was consistent with the result of single SNP analysis presented in Table 2.

Computational Prediction of the Functional Significance for the Six SNPs. The results of the computational prediction of the functional significance of the six polymorphisms are summarized in Table 4. Results based on BLOSUM62 show that only the MMP9 Pro574Arg polymorphism has a negative score, which is indicative of a nonconservative change (28). This suggests that MMP9 Pro574Arg is likely to be deleterious. Results based on PMut show that MMP9 Pro574Arg and MMP9 Arg668Gln had pathogenicity prediction scores of >0.5, suggesting that these 2 nonsynonymous SNPs may be pathologic, whereas the other SNPs were predicted to be neutral. Only the MMP9 Pro574Arg polymorphism displayed a reliability index of >5. Results based on PolyPhen show that, except for the MMP9 Gln279Arg polymorphism, which had a PSIC score Δ of 1.537 (“possibly damaging”), all other SNPs had PSIC score Δ values <1.0, (“benign”). SIFT showed that the 6 SNPs had TI scores ranging from 0.35 to 1.00, with MSCS values below 3.25 across the board; therefore, all the six SNPs are considered “tolerated.” SNPs3D prediction scores, such as svm profile score and svm structure score, were calculated for each SNP. All six SNPs had positive svm profile scores, showing that they are not highly conserved in the respective protein families. Only the MMP9 Arg668Gln polymorphism received a negative svm structure score and was predicted to cause losses of both hydrogen bond and salt bridge; therefore, this SNP may have a deleterious effect on protein structural stability.

Discussion
The MMPs play an important role in proteolytic degradation and remodeling of various ECM components in both physiologic and pathologic situations, including tissue repair, angiogenesis, and tumor cell invasion and metastasis. Under normal physiologic conditions, MMPs are expressed at very low levels,
### Table 2. Six SNPs in the MMP3, MMP8, and MMP9 genes and skin cancer risk

<table>
<thead>
<tr>
<th></th>
<th>Melanoma</th>
<th>SCC</th>
<th>BCC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MMP3, Lys45Glu</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>211 (25.2)</td>
<td>45 (21.3)</td>
<td>1.00</td>
</tr>
<tr>
<td>AG</td>
<td>428 (51.1)</td>
<td>120 (56.9)</td>
<td>1.31 (0.89-1.92)</td>
</tr>
<tr>
<td>GG</td>
<td>198 (23.7)</td>
<td>46 (21.8)</td>
<td>1.08 (0.68-1.70)</td>
</tr>
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</table>

#### Additive OR

<table>
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<th>BCC</th>
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<td>211 (25.2)</td>
<td>45 (21.3)</td>
<td>1.00</td>
</tr>
<tr>
<td>AG</td>
<td>428 (51.1)</td>
<td>120 (56.9)</td>
<td>1.04 (0.83-1.29)</td>
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<tr>
<td>GG</td>
<td>198 (23.7)</td>
<td>46 (21.8)</td>
<td>0.74</td>
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#### Trend OR

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<td>AA</td>
<td>211 (25.2)</td>
<td>45 (21.3)</td>
<td>1.00</td>
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<td>AG</td>
<td>428 (51.1)</td>
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<tr>
<td>GG</td>
<td>198 (23.7)</td>
<td>46 (21.8)</td>
<td>0.74</td>
</tr>
</tbody>
</table>

### Additional Information

- **MMP3, MMP8, and MMP9** genes and skin cancer risk
- **Melanoma, SCC, BCC**
- **Unconditional logistic regression adjusted for the age.**
- **Unconditional logistic regression adjusted for age, constitutional susceptibility score (tertiles), family history of skin cancer (yes/no), the number of lifetime severe sunburns that blistered (none, 1-5, 6-11, >11), sunlamp use or tanning salon attendance (yes/no), cumulative sun exposure while wearing a bathing suit (tertiles), and geographic region.**

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*Unconditional logistic regression adjusted for age, constitutional susceptibility score (tertiles), family history of skin cancer (yes/no), the number of lifetime severe sunburns that blistered (none, 1-5, 6-11, >11), sunlamp use or tanning salon attendance (yes/no), cumulative sun exposure while wearing a bathing suit (tertiles), and geographic region.*
whereas overexpression and activation have been found in many types of tumors (8, 29-31). Several previous studies examined the associations between the MMP polymorphisms and the development of tumors, such as lung cancer, breast cancer, and melanoma metastasis (11, 13-17).

Here, we assessed the associations between six nonsynonymous SNPs in the MMP3, MMP8, and MMP9 genes and the risk of three types of skin cancer. We observed a significant inverse association between the MMP9 Gln668 allele and SCC risk, mirrored in the haplotype analysis as well. MMP9 (gelatinase B) is produced by multiple cells including keratinocytes (32). Expression of the MMP9 gene was detected in malignantly transformed keratinocytes in SCC but not in normal keratinocytes (32). Furthermore, overexpression of MMP9 has been linked to the dyskeratotic foci of Bowen’s disease, which is cutaneous SCC in situ (32-34). In addition, MMP9 protein expression was up-regulated in SCC of the skin when compared with BCC (35). In a transgenic mouse model, the absence of MMP9 was shown to delay angiogenesis and to reduce the frequency of SCC (33).

The results of the computational prediction of the functional significance for the SNPs in this study indicated the possible functional effects of the MMP9 nonsynonymous SNPs, such as Pro574Arg and Arg668Gln, on the MMP9 protein. We showed that the MMP9 Pro574Arg and Arg668Gln had two predictions of being less evolutionarily conservative or deleterious. The MMP9 Pro574Arg is predicted by BLOSUM62 to be evolutionarily less acceptable and by PMut to be pathologic to protein function; the MMP9 Arg668Gln is predicted by PMut to be pathologic and by SNPs3D to be deleterious to the protein structural stability. Similarly, a synthetic three-dimensional structure of MMP9 derived by Cotignola et al. (13) reflects the potential alteration of MMP9 protein function due to an amino acid change of nonsynonymous SNPs in the MMP9 gene including Arg668Gln, Pro574Arg, and Gln279Arg evaluated in this study. Presumably, the Gln allele of the MMP9 Arg668Gln is associated with reduced activity of the MMP9 protein, reducing the degradation of ECM. This could help explain our finding that women carrying the variant allele of MMP9 Arg668Gln have lower risk of SCC than those carrying the wild-type allele. On the other hand, compared with melanocytes and basal cells, keratinocytes are less tolerant of DNA damage and have a lower apoptotic threshold, which makes easier to enter the apoptosis pathway as a protective mechanism for SCC (36-38). This further supports the effect of MMP9 protein on SCC risk rather than melanoma or BCC risks because it has been proposed that MMP9 contributes to apoptosis (39-41). However, further biochemical studies are needed to confirm this because the role of MMPs in tumorigenesis is much more complex. Individual MMPs may play different roles in different cell types or in different states of transformation. For example, it has been shown that MMP9 can induce tumorigenesis and also inhibit it by enhancing angiostatin production (42-44).

For melanoma and BCC risks, we did not observe any suggestive associations with the six nonsynonymous SNPs we evaluated. Previous studies focused on the association between the SNPs in the MMP2, MMP3, and MMP9 genes and melanoma progression, showing significant inverse associations of the three MMP9 SNPs (Gln279Arg, Pro574Arg, and Arg668Gln) with metastasis of melanoma (13, 45). No studies have assessed the effects of SNPs in the MMP9 genes on the risks of SCC and BCC. To our knowledge, this is the first simultaneous evaluation of the associations between nonsynonymous SNPs in the MMP9 genes and the risks of three most common types of skin cancers.

In summary, we found an inverse association between the MMP9 Arg668Gln polymorphism and SCC risk. The nested case-control design, high follow-up rate, and high response rate for the retrospective supplementary questionnaire are among strengths of this study. The limitations of the study include the misclassification of the self-reported assessment of pigmented phenotypes and sun exposure–related information. Our previous report showed that the retrospective assessment of risk factors was not likely to substantially bias the estimate of risk in this study (19). Because we selected incident cases after blood collection at the mean age of 58.7 years, the cases had an older age at diagnosis than that in the general population. The sample size in each cancer type in this study was modest, and additional studies are warranted to confirm these associations. Further

### Table 3. Haplotypes for four SNPs in the MMP9 gene and skin cancer risk

<table>
<thead>
<tr>
<th>SNP</th>
<th>Controls</th>
<th>Melanoma</th>
<th>SCC</th>
<th>BCC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>A</td>
<td>B</td>
<td>C</td>
<td>D</td>
<td>Cases</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>925 (60.9)</td>
</tr>
<tr>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>318 (20.9)</td>
</tr>
<tr>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>222 (14.6)</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>49 (3.2)</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>6 (0.4)</td>
</tr>
</tbody>
</table>

**A:** Ala20Val; **B:** Gln279Arg; **C:** Pro574Arg; **D:** Arg668Gln

NOTE: 0, common allele; 1, rare allele. Logistic regression adjusted for age. P value for global test for melanoma is 0.12; for SCC is 0.02; and for BCC is 0.53.
Table 4. Computational prediction of the functional significance of six SNPs in the MMP9, MMP3, and MMP9 genes

<table>
<thead>
<tr>
<th>SNP</th>
<th>BLOSUM62 score</th>
<th>PMut pathogenicity</th>
<th>PSIC score</th>
<th>Tolerance Index</th>
<th>SVM profile</th>
<th>SVM structure</th>
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<tbody>
<tr>
<td>Lys87Glu</td>
<td>1.00 (neutral)</td>
<td>0.143 (benign)</td>
<td>1.00 (tolerated)</td>
<td>1.99 (molecular effects)</td>
<td></td>
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<tr>
<td>Ala20Val</td>
<td>0.00</td>
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<td></td>
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<tr>
<td>Gln279Arg</td>
<td>0.2577 (neutral)</td>
<td>1.537</td>
<td>0.38 (tolerated)</td>
<td>2.61 (molecular effects)</td>
<td></td>
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<tr>
<td>/C0</td>
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<td></td>
<td></td>
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<tr>
<td>Cys211His</td>
<td>0.68</td>
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*A Score < 0 signals an evolutionarily less acceptable substitution, and a Score >0 signals an evolutionarily more acceptable substitution.

TI, tolerance index; the median sequence conservation score is shown in the square brackets for each SNP. An median sequence conservation score of >3.25 is indicative of a low confidence because of an insufficient number of homologous sequences.

References


21. Kraft P, Cox DG, Paynter RA, Hunter D, De Vivo I. Accounting for both profile of the functional prediction PSIC score "0.00-0.99" and "1.50-1.99" are indicative of "Benign" and "Possibly damaging," respectively. The Reliability Index is shown in the square brackets for each SNP. The prediction becomes more reliable as the Reliability Index becomes higher.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

We thank Dr. Hardeep Ranu and Pati Soule of the Dana-Farber/Harvard Cancer Center High-Throughput Polymorphism Detection Core for their laboratory assistance, and Carolyn Guo for her programming support, and the participants in the Nurses’ Health Study for their dedication and commitment.


