XPD Polymorphism and Risk of Subsequent Cancer in Individuals with Nonmelanoma Skin Cancer

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Abstract

Background: Individuals with nonmelanoma skin cancer (NMSC) are at increased risk of developing subsequent cancers. Genetic predisposition to reduced DNA repair capacity may be an underlying susceptibility factor explaining the excess risk of malignancies. To test this hypothesis, a cohort study was conducted to examine the association between XPD Lys751Gln polymorphism and risk of a second primary cancer in individuals with NMSC. Methods: A subgroup of 481 individuals with a history of NMSC who participated in the CLUE II community-based cohort was followed for the development of a second primary cancer. Blood specimens donated in 1989 were genotyped for the XPD Lys751Gln polymorphism using the 5’ nuclease assay. Cox proportional regression with delayed entry was used to calculate the incidence rate ratio (IRR) and 95% confidence interval (95% CI) for risk of developing a second primary cancer according to XPD genotype. All statistical tests were two sided. Results: Eighty individuals developed a second primary cancer. The most frequent occurring cancers were of the prostate (18%), lung (15%), and breast (15%). Persons with at least one Gln allele had an increased risk of a second primary cancer compared with the reference Lys/Lys genotype (adjusted IRR 2.22, 95% CI 1.30-3.76). When the reference category was limited to never smokers with the Lys/Lys genotype, the risk of developing a second primary cancer associated with having at least one Gln allele was increased >3-fold in both never smokers (IRR 3.93, 95% CI 1.36-11.36) and ever smokers (IRR 6.14, 95% CI 2.17-17.37). Conclusion: These findings suggest that individuals with NMSC who have the variant XPD Gln allele are at increased risk of developing a second primary cancer. (Cancer Epidemiol Biomarkers Prev 2004;13(8):1271–5)

Received 12/29/03; revised 3/3/04; accepted 3/11/04.

Grant support: National Institute of Aging grant 5UL1AG018033, National Cancer Institute grant 5U01CA096038, and National Institute of Environmental Health Sciences grant P30 ES05819. Minority Supplement of Clinical Oncology Research Career Development Program (K12-CA01709) from the National Cancer Institute (A.M. Brewster) and K07 award (CA2390) from the National Cancer Institute (A.J. Alberg).

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The overall prognosis of NMSC is excellent, but several studies have shown that affected individuals are at increased risk of developing subsequent cutaneous and noncutaneous malignancies (6-19) and have a higher overall cancer mortality rate (20, 21) compared with the general population. The excess incidence of noncutaneous malignancies in individuals with a prior NMSC is unexplained. One hypothesis is that genetic predisposition to reduced DNA repair capacity may be an underlying susceptibility factor, increasing the risk of a subsequent malignancy (19, 22).

Polymorphisms of genes involved in the major NER pathway are of particular interest as candidate genes that may contribute to interindividual variations in DNA repair capacity and cancer susceptibility. In addition to the removal of mutagenic UV-induced photoproducts, the NER pathway is responsible for the removal of bulky and helical distorting DNA adducts induced by chemical carcinogens and cellular metabolites (3, 23).

Given the involvement of the NER pathway in the removal and repair of a wide variety of carcinogenic DNA lesions, defects in this pathway may predispose an individual to both cutaneous and noncutaneous malignancies. Within this pathway, a plausible candidate susceptibility gene is the common XPD polymorphism in exon 23 that causes an amino acid substitution of lysine for glutamine (24). The normal functioning XPD protein...
plays an essential role in NER and participates in the unwinding of DNA at the site of a deleterious DNA lesion (25, 26). Rare recessively inherited mutations in XPD as seen in individuals with xeroderma pigmentosum syndrome are associated with defects in NER and an elevated risk of skin cancers and internal cancers (27). Functional studies, although not entirely consistent (28), tend to show that the XPD Lys751Gln polymorphism is associated with reduced DNA repair capacity (29-31).

To test the hypothesis that reduced DNA repair capacity may explain the association between NMSC and subsequent cancers, we followed a cohort of individuals with a history of NMSC to examine the association between XPD Lys751Gln genotype status and risk of developing a second primary cancer.

**Subjects and Methods**

Study participants were residents of Washington County, Maryland, who took part in a cohort study called CLUE II based on the slogan “Give us a clue to cancer and heart disease” (32). The CLUE II cohort was established in 1989 when 25,080 Washington County adult residents donated a blood specimen and completed a brief questionnaire, which included a history of previous cancer, smoking habits, and demographic information. In the questionnaire, participants were asked if they ever had cancer, the organ of involvement, where it was diagnosed, and the date of diagnosis. Compared with the Washington County’s population, participation in the CLUE II cohort was greater among women, the better educated, and individuals in the 45 to 74 age range. Blood specimens were centrifuged, separated as buffy coat, RBC, and plasma, and stored at –70°C. Cancers diagnosed among cohort participants are identified by computerized record linkage to the Washington County cancer registry. This registry was established in 1958, and cases of cancer that occur in county residents are identified from discharge records and pathologic specimens from the Washington County Hospital as well as from death records.

The present study was limited to 509 CLUE II participants who self-reported a cancer history that consisted only of basal and squamous carcinoma of the skin prior to entry into the study. The XPD Lys751Gln genotype status was not available for 17 participants. Reasons for missing data included no available blood specimen (1 individual) and inadequate DNA extracts (16 individuals). An additional 11 individuals did not provide date at NMSC diagnosis. This left 481 individuals in the analytic cohort for the present study.

A second primary cancer was defined as the first cancer diagnosed among cohort participants who did not develop a second primary cancer for participants with XPD genotypes Gln/Gln and Lys/Gln using as the reference group the Lys/Lys genotype (34). Person-year ratio (IRR) and 95% confidence interval (95% CI) of developing a second primary cancer for participants with the XPD genotypes Gln/Gln and Lys/Gln using as the reference group the Lys/Lys genotype (34). Proportionality of hazards over time for comparison models and fully adjusted models that included age at baseline. For stratified analyses, individuals heterozygous for the variant Lys/Gln were compared using the Student’s t test.

The association between genotype and subsequent cancer risk was examined stratifying by smoking status at baseline. For stratified analyses, individuals heterozygous and homzygous for the variant Gln allele were combined, because functional studies have shown a decreased repair capacity associated with at least one Gln allele (31). Proportionality of hazards over time for
the Lys/Lys group compared with the Lys/Gln and Gln/Gln combined group was assessed quantitatively by a test of Schoenfeld residuals and was not shown to be violated.

Analyses were done using Stata Statistical Software version 7.0 (Stata Corporation, College Station, TX).

Results

The baseline characteristics of study participants are shown in Table 1. There were 233 (48%) women and 248 (52%) men. The majority of participants were White, reflecting the racial composition of the county. At entry into the CLUE II study, 52% of participants were current or former smokers. During the follow-up period, 80 participants developed a second primary cancer. The cancer types were prostate (17.5%), lung and pleura (15.0%), breast (15.0%), bladder (8.7%), colorectal and stomach (8.7%), melanoma (7.5%), lymphoma (7.5%), liver (7.5%), larynx and tonsil (2.5%), leukemia (2.5%), and other epithelial (7.5%). The mean time from diagnosis of NMSC skin cancer to CLUE II study entry was 8 years. There were 113 deaths among the 481 participants. In this cohort of persons with a history of NMSC, the age-adjusted risk of developing a second primary cancer was statistically significantly higher in former smokers (IRR 1.84, 95% CI 1.13-2.99) and current smokers (IRR 2.44, 95% CI 1.22-4.88) compared with never smokers. Men were at higher risk of a second primary cancer compared with women (IRR 1.35, 95% CI 0.86-2.11), and participants with <12 years of education compared with ≥12 years (IRR 1.36, 95% CI 0.85-2.16) had slightly elevated risks that were not statistically significant (data not shown).

The frequency of the variant Gln allele was 38%, which was consistent with the frequency observed in previous studies of Caucasian populations (35-38), and the distribution of genotypes in the study population was in Hardy-Weinberg equilibrium (Table 2). In the fully adjusted model, individuals who had a variant Gln allele were 2.22 times more likely (95% CI 1.30-3.76) to develop a second primary cancer compared with individuals homozygous for the Lys/Lys genotype (Table 2). Risk was similar for individuals heterozygous or homozygous for the Gln allele but was statistically significant only in the heterozygotes.

The fully adjusted risk of developing a smoking-related second primary cancer such as lung and pleura cancer (n = 12), bladder (n = 7), larynx and tonsil (n = 2), and colorectal (n = 6) was also evaluated by XPD genotype. Individuals with the variant Gln allele were at higher risk of developing a smoking-related cancer (Lys/Gln versus Lys/Lys IRR 1.62, 95% CI 0.68-3.84; Gln/Gln versus Lys/Lys IRR 1.72, 95% CI 0.50-5.87), but the finding was not statistically significant. For the non-smoking-related cancers, the association observed was Lys/Gln versus Lys/Lys IRR 3.06, 95% CI 1.51-6.18 and Gln/Gln versus Lys/Lys IRR 2.29, 95% CI 0.87-6.05 (data not shown).

The joint effect of ever smoking and carrying the Gln allele on risk of developing a second primary cancer was assessed (Table 3). The results showed that, compared with never smokers with the Lys allele, the variant Gln allele was associated with a statistically significant increased risk of a second primary cancer in never smokers (IRR 3.93, 95% CI 1.36-11.36) and was even more pronounced in ever smokers (IRR 6.14, 95% CI 2.17-17.37). The test for interaction was not statistically significant (P = 0.1).

Conclusion

In a cohort with a history of NMSC, the risk of developing a second primary cancer was approximately doubled in those with the variant Gln allele compared with those homozygous for the Lys allele. These data suggest that reduced DNA repair capacity at least partly explains the higher risk of cancer among individuals with a diagnosis of NMSC.

Ten population-based registry studies have observed that, after adjusting for age, overall cancer incidence is greater in persons with a history of NMSC compared with the general population (6-8; 10-15; 18). The preponderance of evidence from these studies indicates that the increased cancer risk in those with a history of NMSC not only is due to the incidence of malignancies with shared risk factors such as recurrent NMSC, incident melanoma, or lip cancer (6, 7, 12-15) but also includes an increased risk of numerous other primary cancer sites. Furthermore, not only cancer incidence but also cancer mortality is higher in those with a personal history of NMSC (21). The increased cancer mortality is due to poorer prognosis in addition to the increased risk of cancer. Among patients diagnosed with cancers of the
colon, breast, lung, prostate, and non-Hodgkin’s lymphoma, survival is significantly worse in those with prior history of NMSC than those with no previous NMSC (20).

Immune suppression induced by UV light (10, 13), infection with the human papilloma virus (15), Epstein-Barr virus (8), cigarette smoking (6, 7), and poor DNA repair capacity (19, 21) have all been postulated as possible etiologic links to multiple cancers in individuals with a history of NMSC. There have been no studies to date, however, that provide empirical evidence that any of these factors contribute to the increased risk of a second primary cancer. The results of this study suggest that the elevated risk of second cancer in persons with a history of NMSC is at least partly due to genetic susceptibility mediated by reduced DNA repair capacity.

There is evidence to support the hypothesis that reduced repair of DNA damage from exogenous and endogenous insults is an important determinant of susceptibility to cancers of several sites (23). Polymorphisms of important genes in DNA repair pathways that decrease DNA repair capacity may contribute to the interindividual variation in DNA repair capacity that has been observed in skin cancer development and other malignancies. The XPD Gln allele has been associated with increased DNA adduct levels (35-37) and reduced DNA repair capacity either on its own (31) or in combination with other DNA repair gene variant alleles including the variant Asn allele of XPD Asp312Asn (29, 30). Alternatively, suboptimal repair of X-ray DNA damage has been shown with Lys allele (28). The Lys751Gln variant has been associated with increased risk of lung cancer (29, 36, 38) and the head and neck cancers (39) with some reported inconsistencies (40).

Although the test of interaction was not statistically significant, the risk of developing a second primary cancer was observed to be highest in ever smokers with the Gln allele compared with never smokers with the Lys/Lys genotype. The risk was similar among never smokers with the Gln allele and ever smokers homozygous for the Lys/Lys allele. This finding suggests that the variant Gln allele may be as significant as a smoking history in increasing the risk of a second primary cancer among individuals with a history of NMSC skin cancer. The subset analysis did not suggest a stronger association between XPD Gln allele and smoking-related cancers compared with the non-smoking-related cancers. In fact, the three most frequent cancers that developed among the study participants were prostate, lung, and breast. This was not unexpected as they are also the most frequent occurring malignancies in the U.S. population (1). It is of interest, however, that reduced DNA repair capacity and presence of DNA adducts have been associated with both lung and breast cancer (41, 42).

The strengths of our study include its community-based setting and our ability to ascertain cancer outcomes through record linkage of study participants to the Washington County cancer registry. We were able to adjust for potential confounders of subsequent cancer risk such as age, gender, cigarette smoking, and education level as a surrogate of socioeconomic status. Data on sunlight exposure were lacking; however, it may be assumed that the whole cohort was exposed to substantial doses of UV radiation given that UV radiation is the major risk factor for NMSC (2). Nevertheless, our inferences would be more certain if the study had incorporated individual data on sunlight exposure. Because similar excess risks of developing a second primary cancer have been found in individuals with a history of squamous and basal cell skin cancer (10, 11), these histologic subtypes have a shared susceptibility factor responsible for the increased risk of subsequent cancer.

### Table 2. IRRs of second primary cancer by XPD genotype in a cohort of individuals with a history of NMSC, Washington County, Maryland, 1989 to 2002

<table>
<thead>
<tr>
<th>XPD genotypes</th>
<th>Developed second primary cancer</th>
<th>Incident cases per 100 person-years</th>
<th>Age-adjusted IRR (95% CI)</th>
<th>Multivariate adjusted IRR* (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No, n (%)</td>
<td>Yes, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lys/Lys</td>
<td>160 (39.9)</td>
<td>18 (22.5)</td>
<td>0.92</td>
<td>1.00 (reference)</td>
</tr>
<tr>
<td>Lys/Gln</td>
<td>184 (45.9)</td>
<td>51 (63.8)</td>
<td>2.10</td>
<td>2.27 (1.32-3.89)</td>
</tr>
<tr>
<td>Gln/Gln</td>
<td>57 (14.2)</td>
<td>11 (13.7)</td>
<td>1.50</td>
<td>1.87 (0.88-3.98)</td>
</tr>
<tr>
<td>Lys/Gln and Gln/Gln</td>
<td>1.96</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Adjusted for age at NMSC diagnosis, sex, years of education in 1989, and cigarette smoking status in 1989 (never, former, or current).

### Table 3. IRRs of second primary cancer according to XPD genotype and cigarette smoking status in a cohort of individuals with a history of NMSC, Washington County, Maryland, 1989 to 2002

<table>
<thead>
<tr>
<th>Smoking status*</th>
<th>XPD genotypes</th>
<th>Developed second primary cancer</th>
<th>Incident cases per 100 person-years</th>
<th>Multivariate adjusted IRR (95% CI)†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No, n (%)</td>
<td>Yes, n (%)</td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>Lys/Lys</td>
<td>4 (5.0)</td>
<td>83 (20.7)</td>
<td>0.40</td>
</tr>
<tr>
<td></td>
<td>Lys/Gln and Gln/Gln</td>
<td>24 (30.0)</td>
<td>119 (29.7)</td>
<td>1.52</td>
</tr>
<tr>
<td>Ever</td>
<td>Lys/Lys</td>
<td>14 (17.5)</td>
<td>77 (19.3)</td>
<td>1.42</td>
</tr>
<tr>
<td></td>
<td>Lys/Gln and Gln/Gln</td>
<td>36 (47.5)</td>
<td>121 (30.3)</td>
<td>2.30</td>
</tr>
</tbody>
</table>

*Data missing for one participant for cigarette smoking status.
†Adjusted for age at diagnosis of NMSC, sex, and years of education in 1989.
cancers. To test this hypothesis, we would have analyzed the data stratified by histologic subtype of NMSC. Unfortunately, information on NMSC histologic subtype was not known; therefore, future studies would be needed to address this issue.

In summary, the results of this study suggest that reduced DNA repair capacity may be an underlying risk factor responsible for the observed increased incidence of secondary cancers in individual with a history of NMSC. Additional relevant polymorphisms of the XPD gene such as XPD Ast312A3sp and genes in other DNA repair pathways should also be examined as a logical next step as well as replication of the findings in other larger populations.

References

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