The XPC poly-AT polymorphism in non-melanoma skin cancer

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Abstract

Signature UV-DNA lesions, cyclobutane dimers and 6–4 photoproducts, are repaired via the nucleotide excision repair pathway. NER may be subdivided into transcription-coupled repair and global genome repair, and the XPC protein is specific to this latter repair pathway recognizing helix distorting lesions and initiating their repair. Inactivating XPC mutations are associated with xeroderma pigmentosa and an extremely high risk of skin cancer. A common polymorphism in intron 9 of the XPC gene has been associated with both reduced repair of UV-DNA damage (using the host-cell reactivation assay) and increased risk of squamous cell head and neck cancer. Here, we have tested the hypothesis that the XPC PAT polymorphism is associated with non-melanoma skin cancer using a population-based case control study of skin cancer in New Hampshire (n = 1917). Overall, there was a modest decreased risk of squamous cell carcinoma (SCC) among those with the homozygous variant PAT/C genotype (OR 0.8, 95% CI 0.5–1.1) that was most evident among tanners (OR 0.4, 95% CI 0.1–1.1), however, these trends failed to reach statistical significance. There was no association of the PAT/C genotype and basal cell carcinoma (OR 1.0, 95% CI 0.7–1.3), however there was a modest, non-statistically significant, decreased risk among those with the heterozygous genotype (OR 0.8, 95% CI 0.7–1.1). We did not detect gene environment interactions for either SCC or BCC between the XPC PAT genotype and average hours of UV exposure per week, painful sunburn history, nor ionizing radiation therapy. These results suggest that the XPC PAT polymorphism does not play a major role in non-melanoma skin cancer, but that it may slightly modify the risk of SCC among individuals with a phenotype which results in low UV-DNA adduct burdens. These results require further confirmation.

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1. Introduction

Xeroderma pigmentosa is an autosomal recessive photosensitivity syndrome that confers severe skin cancer susceptibility and is attributable to genetic defects in nucleotide excision repair (NER). NER is
the primary cell mechanism for repair of UV-related DNA damage and may be conceptually segregated into transcription-coupled repair (TCR) and global genome repair (GGR). All XP cells are deficient in GGR and animal models have suggested that of the two NER pathways deficiency in global genome repair results in a higher risk of UV-induced carcinomas [1]. XPC is required for global genome repair and it is the XPC-HR23B complex that identifies target lesions in this pathway [2].

While inactivating mutations in the XPC gene confer xeroderma pigmentosa [3] more subtle changes in the XPC gene product also may impair NER efficiency and influence susceptibility to UV induced malignancy. Khan et al. [4] described two common polymorphisms in the XPC gene that are tightly linked: a Lys→Gln change at codon 939 in exon 15 and an insertion of 83 AT bases with a concurrent 5 base deletion in intron 9 (termed the PAT+ polymorphism). While this initial report did not find any association of these polymorphisms and DNA repair capacity [4], subsequent work has described an association of the XPC PAT+/+ allele and reduced repair of UV-DNA damage in the host cell reactivation assay [5,6], and elevated chromatid aberrations have been associated with the linked exon 15 polymorphism [7]. There have been two positive reports of XPC polymorphisms and solid malignancies; the PAT+/+ allele has been associated with head and neck cancer [8], and the exon 15 polymorphism with bladder cancer [9]. Given the clear role of XPC in the repair of UV damage, we have tested whether variation in the XPC gene is associated with skin cancer using a large, population-based case control study of non-melanoma skin cancer in New Hampshire.

2. Materials and methods

2.1. Study population

Cases were identified using a skin cancer registry previously described [10]. Briefly, using a collaborative network of dermatologists and pathology laboratories, all New Hampshire residents with newly diagnosed basal cell carcinoma (BCC) or squamous cell carcinoma (SCC) were identified through review of dermatology records. Population-based controls were identified by Department of Transportation (age <65) or Health Care Financing Administration (age ≥65) lists. Controls were frequency matched to cases on gender and age. Data concerning demographic traits, UV exposures, and medical history were collected using an interviewer-administered questionnaire. In addition, blood and toenail specimens were obtained for laboratory-based analyses.

UV-related variables were created from interview-derived information. Skin type was defined as the acute response to the first sun exposure of the season: always tans, tans than burns, or always burns. Using a pre-interview completed residential history, participants were asked about their lifetime sun exposures (data available for the subset of individuals enrolled during the first phase of the study, \( n = 1156 \)). Individuals reported on painful sunburns that lasted 2 or more days. Cumulative UV exposure was calculated, as was the average number of UV hours/week. In addition, self-report of therapeutic ionizing radiation (verified through medical records) [11] was included in the XPC data analyses.

2.2. Genotyping

DNA was isolated from 200 µl of buffy coat using Qiagen QiaAMP extraction kits. The XPC PAT polymorphism was genotyped as described by Shen et al. [8]. Primers were (1) 5’-TAGCACCCAGCAGTCAAAG, and (2) 5’-TGTGAATGTGCTTAATGCTG. Following a 94 °C incubation for 3 min, 40 cycles of PCR were performed [94 °C 30 s → 66 °C 3 min] followed by a 3 min 66 °C incubation. PCR products were electrophoresed through 2% agarose and visualized with ethidium bromide staining. The XPC poly-AT insertion positive allele (PAT+) produced a PCR product of 344 bp, and the XPC PAT− allele was 266 bp in length.

2.3. Statistical analysis

Crude and adjusted odds ratios and 95% confidence intervals for the association of XPC genotype and case status were calculated using unconditional logistic regression. All adjusted models included age, sex, and tendency to sunburn (always burn, burn then
The ‘joint’ effects of gene and environment were examined using those with both low exposure and the PAT $K/K$ genotype as the referent group. Multiplicative interaction was tested using the log-likelihood score of a ‘full’ model that contained genotype and environment plus a (gene*environment) interaction term and a ‘reduced’ model that did not contain the cross-product variable.

3. Results

1917 individuals were studied (613 controls, 572 SCC, 732 BCC). NMSC is primarily a disease of Caucasians, and there were very few non-Caucasian participants, thus, the analysis was restricted to Caucasians. The demographic description of participants is presented in Table 1.

4. Discussion

Overall, we did not observe a risk effect for the XPC PAT+ allele in non-melanoma skin cancer, whereas polymorphisms in this gene have been associated with risk for head and neck as well as bladder cancer [8,9]. We did observe a modest, non-statistically significant, reduced risk associated with...
the PAT+/+ polymorphism in SCC, and the trend for risk reduction was limited to those with a tendency to tan, rather than burn. No significant interactions with known risk traits or exposures were identified, although we had limited power to detect such interactions.

Our findings of no increased risk associated with the PAT+/+ genotype are contrary to our a priori hypothesis based on the results reported by Shen et al. [8] and Qiao et al. [5]. There is no clear indication as to why our findings in skin cancer contradict these earlier reports. One possibility is that the mechanism of repair, and the role of XPC, may differ based on both the type and amount of exposure.

The XPC-HR23B protein complex recognizes helix-distorting lesions as part of the global genome NER repair pathway [2]. This complex has a strong binding affinity for 6–4PPs, and a rather weak affinity for CPD’s [12,13] and this is reflected in the much slower repair kinetics for CPDs compared to 6–4PPs (T1/2 15 vs. 5 h), and the persistence of CPDs as much as 3 weeks post-exposure [14,15]. It has been postulated that it is the persistent CPD lesions that are more likely to promote cancer induction [15], and XPC is not primarily involved in their repair. Our findings of no elevated risk for non-melanoma skin cancer with the XPC PAT+ polymorphism are consistent with this model and suggest that the formation and repair of CPDs may be more relevant to cancer risk than 6–4PPs. It would be of interest to discern whether individuals with a tanning phenotype have an altered ratio of CPD:6–4PPs, supporting our observation of effect modification for the XPC polymorphism by tanning phenotype. Further, these subtleties regarding the spectra of adducts and in vivo repair kinetics may help explain the apparent discrepancies between our study and the results from the host-cell reactivation assay (which would measure both 6–4PP and CPD repair) and head and neck cancer (where cigarette smoking exposure might result in a stronger emphasis on the global genome repair mechanism).

Finally, while we had significant power to detect a modest risk effect for the XPC polymorphism, our findings are based only on New Hampshire residents, and additional studies are necessary to confirm the role of the XPC PAT+ polymorphism in non-melanoma skin cancer.

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