HHS Public Access

Author manuscript

J Invest Dermatol. Author manuscript; available in PMC 2012 November 01.

Published in final edited form as:

J Invest Dermatol. 2012 May ; 132(5): 1354–1362. doi:10.1038/jid.2012.4.

A community-based study of nucleotide excision repair polymorphisms in relation to risk of non-melanoma skin cancer

Lee Wheless[1], **Emily Kistner-Griffin**[1,2], **Timothy J. Jorgensen**[3], **Ingo Ruczinski**[4], **Yvette Berthier-Schaad**[5,8], **Bailey Kessing**[5], **Judith Hoffman-Bolton**[6], **Lesley Francis**[6], **Yin Yao Shugart**[7], **Paul T. Strickland**[8,9], **W.H. Linda Kao**[8], **Rhoda M. Alani**[10], **Michael W. Smith**[11], and **Anthony J. Alberg**[1,2,8]

[1]Division of Epidemiology and Biostatistics, Department of Medicine, Medical University of South Carolina, Charleston, SC

[2]Hollings Cancer Center, Medical University of South Carolina, Charleston, SC

[3]Department of Radiation Medicine, Georgetown University School of Medicine, Washington, DC

[4]Department of Biostatistics, The Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD

[5]Laboratory of Genomic Diversity, NCI-Frederick

[6]George W. Comstock Center for Public Health Research and Prevention, Washington County, MD

[7]Division of Intramural Research Programs, National Institute of Mental Health

[8]Department of Epidemiology, The Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD

[9]Department of Environmental Health Sciences, The Johns Hopkins University Bloomberg School of Public Health, Baltimore, MD

[10]Department of Dermatology, Boston University, Boston, MA

[11]Genetics and Genomics Group, Advanced Technology Program, SAIC-Frederick, Inc., NCI-Frederick, Frederick, MD

Abstract

Nucleotide excision repair (NER) is responsible for protecting DNA in skin cells against ultraviolet radiation-induced damage. Using a candidate pathway approach, a matched case-control study nested within a prospective, community-based cohort was carried out to test the hypothesis that single nucleotide polymorphisms (SNPs) in NER genes are associated with susceptibility to non-melanoma skin cancer (NMSC). Histologically-confirmed cases of NMSC

Corresponding Author: Anthony J. Alberg, Hollings Cancer Center, Medical University of South Carolina, 68 President Street, MSC 955, Charleston, SC 29425. Telephone: 843-876-2439; Fax: 843-876-2344; alberg@musc.edu.

CONFLICT OF INTEREST

The authors state no conflict of interest.

(n=900) were matched to controls (n=900) on age, gender, and skin type. Associations were measured between NMSC and 221 SNPs in 26 NER genes. Using the additive model, two tightly linked functional SNPs in *ERCC6* were significantly associated with increased risk of NMSC: rs2228527 (odds ratio (OR) 1.57, 95% confidence interval (CI) 1.20 – 2.05), and rs2228529 (OR 1.57, 95% CI 1.20 – 2.05). These associations were confined to basal cell carcinoma of the skin (BCC) (rs2228529, OR 1.78, 95% CI 1.30 – 2.44; rs2228527 OR 1.78, 95% CI 1.31 – 2.43). These hypothesis-generating findings suggest functional variants in *ERCC6* may be associated with an increased risk of NMSC that may be specific to BCC.

INTRODUCTION

Non-melanoma skin cancers (NMSC), comprised mainly of basal cell carcinomas (BCC) and squamous cell carcinomas (SCC), are the most common malignancy among Caucasians, with more than two million new cases diagnosed in the United States each year (Rogers *et al.* 2010). Risk of NMSC is largely determined by phenotypic characteristics, such as skin pigmentation and ability to tan, in combination with exposure to ultraviolet radiation (UVR) (Armstrong and Kricker 2001). UVR forms DNA photoproducts that can lead to signature C $\rightarrow$ T or CC $\rightarrow$ TT mutations, the major genetic lesion in NMSC (Kraemer 1997).

The mechanism responsible for removing these lesions is nucleotide excision repair (NER). NER's importance in the etiology of NMSC is clearly illustrated by Xeroderma pigmentosum (XP), a disease in which rare, highly penetrant mutations disrupt NER's ability to remove DNA photoproducts, leading to a 1,000-fold increased risk of NMSC (Kraemer *et al.*2007; Rass and Reichrath 2008). XP can be caused by mutations in eight genes (XP complementation groups A–G and variant form) with some of these mutations causing more severe skin cancer risk than others (Cleaver 1968; States *et al.* 1998). It thus stands to reason that more common, but less penetrant NER gene variants may be associated with NMSC risk in the general population.

Studies of polymorphisms in NER genes have examined their association with NMSC risk, with inconsistent findings (Applebaum et al. 2007; Vogel et al. 2001; Vogel et al. 2005; Yin et al. 2002; Han et al. 2005; Lovatt et al. 2005; Miller et al. 2006; Dybdahl et al. 1999). Many of these focused on only two or three genes, and only functional single nucleotide polymorphisms (SNPs), that is, instances where variation in a single base pair in the DNA results in a different amino acid upon translation. As the NER pathway involves products from as many as 31 different genes, a more thorough evaluation of NER gene variants in relation to NMSC may provide a clearer picture of the potential role of NER in NMSC. In addition, understanding how so-called 'silent' polymorphisms can function is advancing, such as epigenetic regulation of promoters (Esteller 2008), pre-transcriptional regulation via miRNA targeting of 3' untranslated regions (Bartel 2009), all the way through post-translational modification via synonymous polymorphisms in exons (Zhang *et al*. 2010). Including markers from each of these regions has advantages for characterizing the potential role of NER gene variants in NMSC susceptibility. The fact that different mutations in the same gene can lead to XP, Trichothiodystrohpy, and XP/Cockayne Syndrome – three very different clinical entities with marked differences in skin cancer risk – further highlights

why more thoroughly characterizing NER gene variants is advantageous for advancing understanding of the role of NER in NMSC (Kraemer *et al*. 2007). The present candidate pathway association study was carried out to more thoroughly examine the association between NER SNPs and risk of NMSC.

RESULTS

Cases and controls were similar with respect to gender and skin type, but despite matching on age, cases were still an average of 3.1 years older than controls ($p < 0.01$) due to the pool of appropriate controls for the older age groups tending to be at the lower end of the ±5 year range (Table 1). Cases were less likely than controls to have been current smokers at baseline ($p < 0.01$). Individuals with any BCC were as likely to have had multiple lesions as those with any SCC (34.5% vs. 38.8%, $p = 0.21$). Compared to males, females were less likely to have had SCC (47.3% vs. 52.7%, $p = 0.01$), and more likely to have had only one lesion (73.9% vs. 65.5%, $p < 0.01$).

The SNPs with $p < 0.05$ for the additive model and their associated odds ratios for each genetic model, adjusting for principal components, age, education, BMI, and smoking status, are presented in Table 2. The top two SNPs were both functional polymorphisms in *ERCC6*: Arg1213Gly (rs2228527), and Gln1413Arg (rs2228529). The false discovery rate for these two SNPs was 12%. These two SNPs were in tight linkage disequilibrium ($r^2 = 0.97$, D' = 1). A third *ERCC6* SNP, in an intron (rs4838518), also had $p<0.05$.

The associations between the top hits from the primary NMSC analyses were assessed separately for BCC and SCC. The three *ERCC6* SNPs were the top three most significantly associated with BCC, but had null associations with SCC (Table 3). For example, the ORs for BCC versus SCC in the additive models were 1.78 (95%CI 1.30–2.44) vs. 1.09 (95%CI 0.66–1.78) for rs2228529, 1.78 (95%CI 1.31–2.43) vs. 1.11 (95%CI 0.67–1.82) for rs2228527, and 1.50 (95%CI 1.17–1.93) vs. 0.96 (95%CI 0.65–1.42) for rs4838518. Compared to 10,000 permutations for rs2228529, only two models were more significant than 0.0003, while no point estimate was greater than 1.70, versus the observed 1.78. The only SNP significantly associated with SCC was rs17495770 ($p = 0.0475$).

The analyses subsequently focused on characterizing the associations between the *ERCC6* SNPs and skin cancer risk; given the heterogeneity in the associations between these SNPs for BCC and SCC, these analyses were additionally stratified by histologic type. To further investigate the finding that multiple SNPs from the same gene met the screening criteria of $p < 0.05$, haplotypes were constructed from the unphased data for the three *ERCC6* SNPs. After adjusting for the same variables described above, the overall haplotype test for NMSC was significant ($p = 0.03$), with the GAG haplotype (rs2228529, rs4838518, rs2228527), which contained the minor allele for all three SNPs, associated with increased risk of NMSC compared to the AGA haplotype (OR 1.63, 95% CI 1.23 – 2.16) (Table 4). In analyses stratified by histologic type, the overall haplotype test was significant for BCC ($p < 0.01$) but not SCC ($p=0.99$). For BCC, compared to the haplotype of all wild type alleles (AGA), the haplotype with three variant alleles was associated with increased risk (GAG OR 1.94, 95% CI 1.40 – 2.67, $p <0.0001$) (Table 4). Of the 281 confirmed cases of SCC, there were

90 who also had a confirmed diagnosis of BCC. In the 191 with no BCC, the association between rs2228529 and SCC was OR 1.06, 95% CI 0.58 – 1.92, p = 0.85. In the 90 with confirmed BCC and SCC, the OR was 1.66 (95% CI 0.54 – 5.11, p = 0.37), more closely approximating that for BCC.

The two *ERCC6* functional polymorphisms, rs2228527 and rs2228529, were significantly associated with NMSC among both males and females (Table 5). The risks associated with these same two SNPs increased for multiple lesions. For rs2228527, the OR was 1.43 (95% CI 1.05–1.94) for a single lesion and 2.16 (95% CI1.19–3.92) for multiple lesions; the corresponding figures for rs2228529 were 1.42 (1.04–1.93) and 2.24 (1.23–4.10) (Table 5). For SCC, the ORs for both *ERCC6* SNPs, although not significant, were in the direction of increased risk among males but decreased risk among females (Table 5). This gender difference remained after excluding those with both BCC and SCC, indicating it was not driven by association with BCC.

DISCUSSION

In a community-based study, after matching on age, gender, and skin type, a thorough evaluation of NER gene variants revealed associations between two functional *ERCC6* SNPs and NMSC that to our knowledge have not been previously reported. These two SNPs, rs2228527 and rs2228529, had minor allele frequencies of 20% and the associations were compatible with increased NMSC risks of 1.6-fold for heterozygotes and 2.2-fold for those with the homozygous variant genotype. The increased risk was consistent among both males and females and increased in strength with increasing number of lesions. Individuals with NMSC will commonly develop multiple primary tumors during their lifetime. For example, the 3-year risk of developing BCC after an initial SCC is as high as 43%, while studies indicate the 3-year risk of developing SCC after BCC ranges from 1% to 19% (Marcil and Stern 2000). Both *ERCC6* SNPs were associated with increased risk of NMSC that grew stronger with an increasing number of lesions, a trend that was most pronounced among individuals with a history of BCC. Furthermore, the associations were specific to BCC.

To our knowledge, no previous evidence has been reported on the association between either rs2228527 or rs2228529 and NMSC. Several genome-wide association studies (GWAS) of NMSC have been conducted, so the fact the results for these two SNPs have yet to be reported indicates they were not among the most significant SNPs (Gudbjartsson et al. 2008; Nan et al. 2011; Rafnar et al. 2008; Stacey et al. 2008; Stacey et al. 2009, Zhang et al. 2011a, Zhang et al. 2011b). In studies based in Iceland, the association between risk of NMSC and SNPs observed to be associated with pigmentation within this study population (Gudbjartsson et al. 2008), and the most significant 35 SNPs from a genome-wide scan have been reported (Stacey et al. 2008, Rafnar et al. 2009, Stacey et al. 2009). Similarly, a GWAS using the Nurses' Health Study (NHS) and the Health Professionals Follow-up Study (HPFS) has reported the most significant SNPs overall (Nan et al. 2011), as well as select SNPs from within two Kyoto Encyclopedia of Genes and Genomes pathways shown to be enriched for significant associations (Zhang et al. 2011a, Zhang et al. 2011b); for the latter, the NER pathway was not significant for enrichment (p = 0.33), but the associations for individual genes and SNPs were not presented (Zhang et al. 2011b). There were also

important between-study differences in case definition. The present study was comprised of histologically confirmed NMSC cases whose NMSC was not preceded by any other cancer, the Icelandic cohort included histologically confirmed BCC cases regardless of other cancer diagnoses, and the NHS/ HPFS included self-reported BCC that was the patient's only known cancer, excluding those with both BCC and SCC. The SNPs identified in the present study could possibly be associated with NMSC in these GWASs but did not reach the stringent GWAS significance threshold.

The rs2228529 SNP falls within a highly-conserved ubiquitin-binding domain in *ERCC6* that is necessary for the transcription-coupled repair branch of NER (Anindya 2010). The function of rs2228527 is currently unknown, though its observed association with NMSC in the present study could be due solely to its tight linkage with rs2228529. *ERCC6*, also called *CSB*, is mutated in Cockayne Syndrome (CS), which presents as progressive sensorineural degeneration and sun sensitivity. CS patients are not reported to have an increased risk of NMSC (Kraemer *et al*. 2007), however, the average lifespan of individuals with CS is only 12 years, thus they simply may not survive long enough for an increased NMSC risk to manifest (Hoeijmakers 2009). Alternatively, the global genome repair branch of NER may compensate for a defective *ERCC6* in CS, as suggested by mouse models, and attenuate NMSC risk (Tornaletti 2009; Nouspikel 2009; Pines *et al.* 2010; van der Horst *et al.* 1997). Knockdown of *ERCC6* in human keratinocytes decreased repair of UV-induced cyclobutane dimers as well as 8-oxo-deoxyguanine, providing mechanistic evidence of a role for *ERCC6* in skin carcinogenesis (Javeri et al. 2011). In the absence of a larger-scale, hypothesis-driven study such as the present study, an increased risk of NMSC associated with *ERCC6*, if true, may not have been detected.

In the present study, nonsignificant increased risks of NMSC were observed for rs13181 (OR 1.10, 95% CI 0.88 – 1.37, p = 0.3975) and rs1800975 (OR 1.15, 95% CI 0.92 – 1.44, p = 0.2328). Due to a low genotyping platform design score, rs1799793 was not genotyped. Many studies have measured the association between these three *XPD* and *XPA* polymorphisms and NMSC (Applebaum *et al.* 2007; Vogel *et al*. 2001; Vogel *et al*. 2005; Yin *et al*. 2002; Han *et al*. 2005; Lovatt *et al*. 2005; Miller *et al*. 2006; Dybdahl *et al*. 1999). The largest of these, drawing from a population-based study in New Hampshire with nearly 900 cases of BCC and 700 of SCC, observed a 15–25% decreased risk of either BCC or SCC among those with the *XPA* A23G (rs1800975) polymorphism (Miller *et al.* 2006), as well as a 20% decreased risk of SCC and 10% decreased risk of BCC among those carrying variant forms of both *XPD* Lys751Gln and Asp312Asn (Applebaum *et al.* 2007), after adjusting for age, sex, skin pigmentation, and number of severe sunburns. There was a significant 30–40% reduced risk of SCC among carriers of the *XPD* Lys751Gln (rs13181) variant in the Nurses Health Study (n = 286 SCC cases, n = 874 controls), and a roughly 20% decreased risk for *XPD* Asp312Asn (rs1799793), while neither was associated with BCC (n = 300 cases), adjusting for age, race, skin type, sunburns, family history, geography, tanning bed use, and sun exposures (Han *et al.* 2005). Some studies support a non-significantly decreased risk of NMSC associated with these variants (Vogel *et al.* 2005; Lovatt *et al.* 2005), while others have found non-significant increases in risk (Vogel *et al.* 2001; Vogel *et al.* 2005; Yin *et al.* 2002).

In general, more of the NER SNPs associated with NMSC were associated with BCC than SCC, adding supportive evidence for the hypothesis that risk of BCC and SCC may be associated with different NER genes and pathophysiology. For example, XP patients with mutations in *XPC* are known to have a preponderance of SCCs (Kraemer *et al.* 2007). In the present study, no SNPs in *XPC* were associated with risk of either BCC or SCC. However, in the majority of UVR-induced SCCs *XPC* expression is lost (de Feraudy *et al.* 2010a, de Feraudy *et al.* 2010b), and this inactivation has been shown to be sufficient for neoplastic transformation (Rezvani *et al.* 2011). Therefore, individuals with SCC could possibly have an XP phenotype acquired through somatic mutation that was not measured in the present study of germline SNPs, potentially contributing to the weaker associations for SCC than BCC.

The strengths of the present study include its community-based study design, matching on important NMSC risk factors, its broad coverage of the NER pathway, and its sample size of histologically-confirmed cases of NMSC comparable to the largest studies addressing this question. Skin type is the strongest phenotypic predictor of skin cancer risk, and is also correlated with sun exposures in this population, suggesting there was also some control for environmental sun exposures (Wheless *et al.* 2009). The associations with NMSC for those missing skin type (rs2228529 OR 1.80, 95% CI 1.18 – 2.74, rs2228527 OR 1.78, 95% CI 1.17 – 2.72) were stronger than those who had skin type data (rs2228529 OR 1.49, 95% CI 1.03 – 2.15, rs2228527 OR 1.51, 95% CI 1.05 – 2.18), indicating there may be residual confounding among those who were matched on the “missing” category for skin type. Although >50% of the study population was missing skin type data, both *ERCC6* SNPs still had ORs>1.0 and $p < 0.05$ upon stratification by those with and without skin type data, suggesting the missing data did not impact the overall conclusion of increased risk associated with these two SNPs.

A number of limitations should be kept in mind when drawing inferences from this study. Despite a rigorous matching protocol, residual case-control age differences remained. In analyses stratified according to age <65 versus ≥65 years at baseline, the *ERCC6* functional variants were significant ($p < 0.05$) within both age groups (data not shown). Moreover, the median age of diagnosis for cases was 64 years (mean 63.4 ±; 13.0, range 22 – 93), an age the controls surpassed on average midway through the follow-up period without developing NMSC. As such, the age difference between cases and matched controls most likely did not substantially bias the results. The age difference also led to the exclusion of more than 100 cases for which there were no appropriate controls. The allele frequencies for the two *ERCC6* functional polymorphisms among these excluded cases were no different than among the included cases of the same age group (rs2228527 $p = 0.95$, rs2228529 $p = 0.91$), suggesting these exclusions likely did not impact the overall associations. In regards to number of NMSCs, those categorized as having multiple lesions are confirmed to have had more than one NMSC. The follow-up was incomplete, so some individuals classified with one lesion may in fact have had more than one, as individuals with a previous NMSC are at a greatly increased risk of developing additional lesions (Marcil and Stern 2000). To the extent this was true, it would have biased against detecting a trend in risk with number of lesions, so the estimates of the trend in the present study are likely conservative.

Sunburns are markers of acute, intermittent sun exposure, the pattern most strongly associated with BCC (Armstrong and Kricker 2001). Data from the 2007 follow-up questionnaire concerning blistering sunburns (ever vs. never) were available for 142 matched case-control sets. In this subset, the associations between rs2228529 and NMSC and BCC actually increased slightly after adjusting for history of blistering sunburns, although the confidence intervals were wide (data not shown). These results, though limited by being from a small, potentially highly selected sample, suggest that further adjusting for sunburn history is unlikely to attenuate the observed association between the *ERCC6* SNPs and BCC.

The strength and internal consistency of the associations between these functional SNPs and NMSC in the present study suggest a closer examination of these SNPs is warranted. Only two of 10,000 permutations for rs2228529 were more significant than the observed p-value, and no permutations had ORs as strong as the observed ORs, making it highly unlikely these findings were due to chance. The findings that two nonsynonymous coding SNPs in *ERCC6* are associated with NMSC, and perhaps specifically to BCC have not to our knowledge been previously reported. Before these findings can be assessed for public health or clinical relevance, they will first need to be replicated and functional significance established to clarify the role of *ERCC6* in NMSC. If these associations prove to be genuine in the long run, they will advance understanding of skin carcinogenesis and could be of potential relevance to risk stratification and targeting preventive measures.

The results of the present study suggest that two functional polymorphisms in *ERCC6* may play a role in risk of NMSC, especially BCC. While known for its causative role in Cockayne Syndrome, previously *ERCC6* has been associated with risk of skin cancer in mice, but not humans. Using a candidate pathway-based approach, combined with a methodologically sound population-based, matched case-control study, the results identify *ERCC6* as a new target for study as a potential skin cancer risk factor.

MATERIALS AND METHODS

Study population

This study was approved by the Institutional Review Boards of the Johns Hopkins University Bloomberg School of Public Health and the Medical University of South Carolina and was conducted according to Declaration of Helsinki Principles, including written informed consent. This case-control study was nested within the larger parent study, the “Give Us a Clue to Cancer and Heart Disease” (CLUE II) study, established in 1989 in Washington County, MD. At baseline, participants provided demographic information and gave a 20 mL blood sample that was collected in a heparinized 20 mL vacutainer tube. All blood samples were immediately refrigerated until centrifugation, which usually took place within 6 hours and never exceeded 24 hours. Aliquots of plasma, red blood cells, and buffy coats were then separated and stored at −70° C.

Ascertainment of NMSC cases was determined through linkage with the Washington County Cancer Registry, which registers NMSC diagnoses. The follow-up period concluded on December 31st, 2008.

Selection of Cases and Controls

From the 28,594 individuals who at baseline had no history of cancer other than NMSC, a subset of 6,589 individuals was selected for genotyping, including 1,391 histologically-confirmed incident cases of NMSC and 2,586 cancer-free controls. The remaining individuals in the genotyping cohort had cancers other than NMSC and thus were not included in the present study. Of the NMSC cases and cancer-free controls, genotyping was never attempted due to DNA quality or availability in n = 212 individuals, n = 132 were excluded due to low genotyping rates, and an additional n = 64 were excluded on the basis of principle components analysis clustering by genetic ancestry. This latter exclusion was to minimize the potential for population stratification by genetic admixture. Finally, 242 cases were excluded based on diagnosis of another cancer before diagnosis of NMSC, as the NMSC potentially could have been caused by treatment-related factors (Perkins *et al.* 2005). After these exclusions, this left a total of 1,027 cases and 2,300 cancer-free controls available for matching. Cases and controls were matched on age +/− 5 years (+/− 10 years for those 75 and older), gender, and skin type. Race and ethnicity were accounted for in the initial principle components analysis of genetic ancestry by restricting to a single, genetically homogeneous cluster. Moreover, the full cohort was almost exclusively Caucasian of northern European descent.

Based on this protocol, 887 cases were matched to 887 controls. An additional 13 cases were matched to controls whose skin type was off by one category, bringing the total to 900 in both groups. The majority of cases who were excluded due to the lack of a suitable matching control were males ages 65 and older (n = 92).

NER Genotyping

From a larger study examining SNPs from all DNA repair mechanisms as a potential explanation for the increased risk of second primary cancers after NMSC (Jorgensen *et al.* 2009), the present study focuses on the association between NER pathway SNPs and NMSC. A complete description of the selection of genes and SNPs analyzed in the parent study was previously reported (Jorgensen *et al.* 2009). Briefly, the SNP selection strategy was to: 1) identify all known NER genes, 2) select all validated functional SNPs, i.e. those that change the amino acid sequence of the protein product, and 3) select tagging SNPs to obtain as complete coverage as possible. Using this method, 347 SNPs in 28 genes of the NER pathway were selected.

Genotyping was conducted at the Laboratory of Genomic Diversity at the National Cancer Institute using the Illumina Golden Gate© assay with a custom designed microarray chip.

After excluding SNPs due to genotyping failure (<95% success, n = 28), deviation from Hardy-Weinberg equilibrium (HWE) ($p < 0.001$, n = 33), or low minor allele frequency (<0.01, n = 65), 221 SNPs in 26 genes remained for analyses. A full listing of the RefSNP (rs) number, gene, and p-value for the association with NMSC is provided in Supplemental Table 1.

Covariate Information

Demographic information collected at baseline included age, self-reported race, gender, years of education, BMI and smoking history. Follow-up questionnaires were periodically mailed to participants, with those in 2003 and 2007 containing items relevant to skin cancer, including skin type and sun exposure. The item concerning the skin type variable that was used in the matching protocol was included in both 2003 and 2007 and asked, “If you spent an hour in the midday sun for the first time without sunscreen, which of these reactions best describes what would happen to your skin?” The five possible responses were: “blistering sunburn,” “sunburn without blisters,” “mild sunburn that becomes a tan,” “tan or darken with no sunburn,” or “no change in skin color.” As tanning ability tends to decrease with age (Gilchrest *et al.* 1979), the response from the 2003 survey was used to determine skin type, except when there was no response in 2003, in which case the 2007 data were used.

Statistical Analysis

Genetic heterogeneity was assessed using principal components in the statistical environment R (R Project, www.r-project.org). Comparison with the three HapMap2 populations identified subjects of non-Caucasian background (largely identical to self-reported race, data not shown). To avoid potential population stratification among the Caucasians, the first three principal components were recorded and used in the data analyses.

HWE was assessed among the controls using PLINK v1.07 (Shaun Purcell, http://pngu.mgh.harvard.edu/purcell/plink/) (Purcell *et al.* 2007). A two-step strategy was used to assess the association between NER SNPs and NMSC. First a macro in SAS version 9.1 (SAS Institute, Cary, NC) was used to screen each SNP for its association with NMSC using the additive genetic model in a conditional logistic regression, which is on a multiplicative scale. In the additive model, each individual is coded as having 0, 1, or 2 copies of the minor allele for each SNP (Sasieni 1997). The covariates adjusted for in these models were the first three principle components from the population stratification analysis, and baseline age (continuous), education (<12 years, 12 years, >12 years), body mass index (BMI, continuous), and smoking (never, former, current). Second, for SNPs with additive genetic model $p < 0.05$, the mode of inheritance (dominant, recessive, or genotype) was assessed. Conditional logistic regression was used to calculate odds ratios (OR) and 95% confidence intervals (CI).

Ancillary analyses examined differences between histology (BCC vs. SCC), number of NMSCs (0, 1, >1), and gender. Construction of haplotypes from unphased markers was conducted using PLINK, with subsequent conditional regression analyses conducted in SAS. Unless otherwise specified, SAS was used for all analyses. The Bonferroni correction does not account for the reality that in the present study some of the genotyped SNPs were in high linkage disequilibrium (LD), which would make this test overly conservative. Therefore, to account for the actual LD present among the SNPs genotyped empirical significance levels for the top SNPs were generated using 10,000 permutations. Permutations were conducted using PLINK to assign random case-control status within each matched stratum, followed by

modeling in SAS as before. The false discovery rate was calculated to estimate the proportion of significant findings expected to be false positives.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENTS

This research was made possible by funding from the National Cancer Institute (NCI) (R01 CA105069), NIH/NCCR (TL1 RR029881), and the Abney Family Foundation. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. government. The project described in this manuscript has been funded in part with federal funds from the National Cancer Institute, National Institutes of Health, under contract N01-CO-12400.

Abbreviations used

BMI	body mass index
BCC	basal cell carcinoma
CI	confidence interval
CS	Cockayne Syndrome
NER	nucleotide excision repair
NMSC	non-melanoma skin cancer
SCC	squamous cell carcinoma
SNP	single nucleotide polymorphism
UVR	ultraviolet radiation
XP	Xeroderma pigmentosum

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Table 1

Characteristics of NMSC cases and matched[1] controls, Washington County, MD.

Characteristic	NMSC cases % (n = 900)	Controls % (n = 900)	p[2]
Age at baseline[3]	59.7 +/− 13.0	56.6 +/− 13.1	<0.01
Mean +/− SD	4.4	5.9	
≤34	9.4	13.8	
35–44	18.4	19.7	
45–54	27.7	31.0	
55–64	27.6	24.0	
65–74	12.4	5.7	
≥75			
Gender	46.3	46.3	1.0
Male	53.7	53.7	
Female			
Education[3]	12.1 +/− 3.0	12.4 +/− 2.9	0.48
Mean +/− SD	27.9	26.0	
<12 years	41.7	41.1	
12 years	30.4	32.9	
>12 years			
Body Mass Index[3]	25.9 +/− 4.3	26.2 +/− 4.6	0.36
Mean +/− SD	1.3	0.8	
<18.5	42.9	41.8	
18.5 – 24.9	41.0	40.2	
25.0 – 29.9	14.8	17.2	
≥30.0			
Cigarette Smoking†	51.1	43.0	<0.01
Never	35.0	30.7	
Former	13.9	26.3	
Current			
Skin Type[4]	14.5	14.5	0.89
Blistering burn	34.0	31.6	
Burn without blisters	38.0	40.4	
Burn that tans	10.2	10.9	
Tan without burn	3.3	2.6	
No change	(n = 479)	(n = 479)	
Missing			
Basal Cell Carcinoma	65.5	--	
1	34.5	--	
>1	61.2	--	
Squamous Cell Carcinoma	38.8	--	
1			
>1			

[1] Controls were matched on age (+/− 5 years, +/− 10 years for those ≥75), gender, and skin type. 13 cases were matched to controls with skin type off by one category.

[2] Chi Squared test

[3] Measured at baseline in 1989

[4] Measured in 2003 and 2007

Table 2

SNPs associated with NMSC at p < 0.05 using the additive model.

SNP	Gene	MAF	Genotype (Case/control)	HWE p	P[2]	Additive OR (95% CI)	Genotype OR (95% CI)	Dominant OR (95% CI)	Recessive OR (95% CI)
rs2228527	*ERCC6* Arg1213Gly	0.1983	AA (568/600) AG (283/264) GG (48/35)	0.3781	0.0007	1.57 (1.20 – 2.05)	AA: 1.0 (referent) AG: 1.65 (1.18 – 2.31) GG: 2.17 (1.06 – 4.46)	1.71 (1.23 – 2.37)	1.80 (0.89 – 3.62)
rs2228529	*ERCC6* Gln1413Arg	0.2013	AA (563/592) AG (290/272) GG (47/34)	0.6646	0.0008	1.57 (1.20 – 2.05)	AA: 1.0 (referent) AG: 1.62 (1.16 – 2.27) GG: 2.26 (1.08 – 4.72)	1.69 (1.22 – 2.33)	1.88 (0.92 – 3.85)
rs17495770	*RPA3* intron (LOC729852)	0.1403	GG (645/682) CG (233/208) CC (22/10)	0.2272	0.0017	1.68 (1.21 – 2.34)	GG: 1.0 (referent) CG: 1.65 (1.14 – 2.39) CC: 3.13 (0.86 – 11.40)	1.71 (1.19 – 2.45)	2.74 (0.76 – 9.87)
rs4647709	*DDB2* Intron	0.08528	GG (768/736) AG (127/158) AA (5/6)	0.5589	0.0066	0.59 (0.40 – 0.87)	GG: 1.0 (referent) AG: 0.54 (0.36 – 0.81) AA: 1.61 (0.19 – 14.01)	0.55 (0.37 – 0.83)	1.57 (0.19 – 13.40)
rs4134822	*XAB2/XPA* Val126Ile	0.01426	GG (858/879) AG (34/17) AA (0/0)	*1*	0.0186	2.69 (1.13 – 6.40)	GG: 1.0 (referent) AG: 2.69 (1.13 – 6.40) AA: --	2.69 (1.13 – 6.40)	--
rs4150530	*GTF2H1* 5'UTR	0.1042	CC (737/706) AC (155/182) AA (7/12)	0.8702	0.0334	0.67 (0.47 – 0.97)	CC: 1.0 (referent) AC: 0.66 (0.44 – 0.99) AA: 0.52 (0.11 – 2.33)	0.65 (0.44 – 0.96)	0.56 (0.13 – 2.52)
rs4838518	*ERCC6* intron	0.485	GG (237/245) AG (438/451) AA (224/204)	0.9467	0.0329	1.26 (1.02 – 1.57)	GG: 1.0 (referent) AG: 1.10 (0.77 – 1.57) AA: 1.62 (1.05 – 2.48)	1.24 (0.89 – 1.73)	1.52 (1.06 – 2.17)
rs876430	*ERCC5* flanking	0.2626	GG (467/505) AG (372/337) AA (61/57)	0.9294	0.0313	1.31 (1.02 – 1.67)	GG: 1.0 (referent) AG: 1.24 (0.89 – 1.73) AA: 1.85 (1.02 – 3.38)	1.33 (0.97 – 1.82)	1.70 (0.95 – 3.06)
rs1264302	*VARS2* synonymous (GTF2H4)	0.3598	GG (354/376) AG (420/421) AA (124/102)	0.3774	0.0347	1.27 (1.02 – 1.59)	GG: 1.0 (referent) AG: 1.22 (0.88 – 1.69) AA: 1.66 (1.03 – 2.67)	1.31 (0.96 – 1.78)	1.48 (0.96 – 2.30)

Abbreviations used: CI, confidence interval; HWE, Hardy-Weinberg equilibrium; MAF, Minor allele fraction; OR, odds ratio; UTR, untranslated region

All models are adjusted for the first three principle components, and baseline age, education, body mass index, and smoking status

[1] Numbers do not add up to 1800 due to genotyping failures

[2] Permutation p-value from the additive model

Table 3

For the top SNPs associated with NMSC, the odds ratios (and 95% CI and p-values) of each SNP with BCC and with SCC.

		Basal Cell Carcinoma (n=701 cases)				Squamous Cell Carcinoma (n=281 cases)			
SNP	**Gene**	**p[1]**	**Frequency Case/Control**	**Additive OR (95% CI)**	**Genotype[2] OR (95% CI)**	**p[1]**	**Frequency Case/Control**	**Additive OR (95% CI)**	**Genotype[2] OR (95% CI)**
rs2228529	*ERCC6* Gln1413Arg	0.0003	AA (436/463) AG (230/210) GG (35/27)	1.78 (1.30 – 2.44)	AA: 1.0 (referent) AG: 1.90 (1.28 – 2.80) GG: 2.78 (1.22 – 6.33)	0.7481	AA (175/182) AG (87/87) GG (19/11)	1.09 (0.66 – 1.78)	AA: 1.0 (referent) AG: 1.02 (0.56 – 1.87) GG: 1.42 (0.33 – 6.14)
rs2228527	*ERCC6* Arg1213Gly	0.0003	AA (440/469) AG (224/204) GG (36/28)	1.78 (1.31 – 2.43)	AA: 1.0 (referent) AG: 1.94 (1.31 – 2.88) GG: 2.67 (1.20 – 5.93)	0.6908	AA (178/186) AG (84/83) GG (19/11)	1.11 (0.67 – 1.82)	AA: 1.0 (referent) AG: 1.05 (0.57 – 1.94) GG: 1.43 (0.33 – 6.15)
rs4838518	*ERCC6* Intron	0.0016	GG (177/195) AG (344/352) AA (179/154)	1.50 (1.17 – 1.93)	GG: 1.0 (referent) AG: 1.42 (0.93 – 2.15) AA: 2.26 (1.36 – 3.75)	0.8333	GG (79/74) AG (130/141) AA (72/66)	0.96 (0.65 – 1.42)	GG: 1.0 (referent) AG: 0.62 (0.32 – 1.22) AA: 0.97 (0.44 – 2.16)
rs17495770	*LOC729852* (*RPA3*)	0.0166	GG (502/523) CG (177/168) CC (22/10)	1.56 (1.08 – 2.23)	GG: 1.0 (referent) CG: 1.50 (0.99 – 2.26) CC: 2.94 (0.82 –10.57)	0.0475	GG (201/215) CG (77/66) CC (3/0)	2.10 (1.01 – 4.37)	GG: 1.0 (referent) CG: 2.03 (0.97 – 4.26) CC: --
rs4647709	*DDB2* Intron	0.0203	GG (591/566) AG (105/131) AA (5/4)	0.61 (0.40 – 0.93)	GG: 1.0 (referent) AG: 0.54 (0.34 – 0.84) AA: 1.99 (0.20 –19.41)	0.0820	GG (241/238) AG (39/41) AA (1/2)	0.49 (0.22 – 1.10)	GG: 1.0 (referent) AG: 0.47 (0.20 – 1.08) AA: 0.93 (0.00 – 847.3)
rs1264302	*VARS2* Synonymous	0.0256	GG (274/303) AG (324/317) AA (102/81)	1.33 (1.04 – 1.71)	GG: 1.0 (referent) AG: 1.37 (0.93 – 2.00) AA: 1.74 (1.03 – 2.94)	0.1710	GG (116/121) AG (131/132) AA (33/27)	1.38 (0.87 – 2.19)	GG: 1.0 (referent) AG: 1.24 (0.67 – 2.28) AA: 2.15 (0.77 – 5.98)
rs4134822	*XAB2/XPA* Val126Ile	0.0366	GG (667/684) AG (28/13) AA (0/0)	2.83 (1.07–7.49)	GG: 1.0 (referent) AG: 2.83 (1.07–7.49) AA: --	0.2510	GG (267/273) AG (11/7) AA (0/0)	2.32 (0.55 – 9.80)	GG: 1.0 (referent) AG: 2.32 (0.55 – 9.80) AA: --
rs4150530	*GTF2H1* 5’ UTR	0.0730	CC (574/554) AC (121/140) AA (6/7)	0.68 (0.45 – 1.04)	CC: 1.0 (referent) AC: 0.66 (0.42 – 1.04) AA: 0.62 (0.11 – 3.56)	0.3240	CC (227/212) AC (51/63) AA (2/6)	0.73 (0.39 – 1.37)	CC: 1.0 (referent) AC: 0.79 (0.40 – 1.57) AA: 0.18 (0.00 – 7.42)
rs876430	*ERCC5* Flanking	0.0824	GG (362/390) AG (293/264) AA (46/46)	1.28 (0.97 – 1.69)	GG: 1.0 (referent) AG: 1.30 (0.89 – 1.89) AA: 1.59 (0.81 – 3.13)	0.1849	GG (150/157) AG (107/109) AA (24/15)	1.38 (0.86 – 2.23)	GG: 1.0 (referent) AG: 0.97 (0.51 – 1.85) AA: 3.62 (1.05 – 12.51)

Abbreviations used: CI, confidence interval; OR, odds ratio; UTR, untranslated region

All models are adjusted for the first three principle components, and baseline age, education, body mass index, and smoking status

[1] P-value from the additive model

[2] Numbers of cases and controls with each genotype. Numbers do not add up to 1402 for BCC or 562 for SCC due to genotyping failures

Table 4

Odds ratios (and 95% CIs) of NMSC, BCC, and SCC according to *ERCC6* haplotypes (rs2228527, rs4838518, rs2228529).

Haplotype	Frequency case/control	NMSC OR (95% CI)	Frequency BCC cases	BCC OR (95% CI)	Frequency SCC cases	SCC OR (95% CI)
AGA	50.3/52.0	1.0 (referent)	49.3	1.0 (referent)	50.7	1.00 (referent)
AAA	28.2/29.0	1.11 (0.87 – 1.41)	29.0	1.24 (0.94 – 1.63)	27.1	0.94 (0.59 – 1.49)
AAG	0.1/0.1	1.28 (0.09 – 17.73)	0.1	1.60 (0.12 – 21.80)	0	--
GGA	0.4/0.3	1.88 (0.35 – 10.14)	0.4	2.08 (0.35 – 12.28)	0.5	0.71 (0.05 – 10.74)
GAG	20.9/18.5	1.63 (1.23 – 2.16)	21.0	1.94 (1.40 – 2.67)	21.7	1.07 (0.64 – 1.80)
Overall p-value		0.03		<0.01		0.99

Abbreviations: CI, confidence interval; OR, Odds ratio

All models were adjusted for the first three principle components, and baseline age, education, body mass index, and smoking
Six of the possible eight haplotypes were observed, with one occurring only among controls (GAA, n = 2)
Numbers do not add to 100 due to rounding

J Invest Dermatol. Author manuscript; available in PMC 2012 November 01.

Table 5

For the top two (*ERCC6*) SNPs associated with NMSC, odds ratios (and 95% CI) of NMSC, BCC, and SCC, and stratified according to sex and number of lesions.

	Non-melanoma skin cancer		Basal Cell Carcinoma		Squamous Cell Carcinoma	
***ERCC6* SNP**	**n cases/controls**	**OR (95% CI)**	**n cases/controls**	**OR (95% CI)**	**n cases/controls**	**OR (95% CI)**
rs2228527	899/899	1.57 (1.20 – 2.05)	700/700	1.78 (1.31 – 2.43)	280/280	1.11 (0.67 – 1.82)
Sex	417/417	1.78 (1.07–2.97)	317/317	1.78 (0.88–3.57)	148/148	1.74 (0.72–4.20)
Males	482/482	1.41 (1.03–1.95)	383/383	1.68 (1.13–2.48)	132/132	0.87 (0.45–1.65)
Females	899	1.0 (referent)	700	1.0 (referent)	280	1.0 (referent)
# of Lesions	629	1.43 (1.05 – 1.94)	458	1.72 (1.19 – 2.48)	171	0.92 (0.50 – 1.70)
None	270	2.16 (1.19 – 3.92)	242	2.07 (1.12 – 3.80)	109	1.70 (0.59 – 4.86)
1						
>1						
rs2228529	898/898	1.57 (1.20 – 2.05)	700/700	1.78 (1.30 – 2.44)	280/280	1.09 (0.66 – 1.78)
Sex	416/416	1.75 (1.04–2.94)	316/316	1.88 (0.92–3.86)	148/148	1.67 (0.69–4.01)
Males	482/482	1.42 (1.03–1.96)	384/384	1.70 (1.15–2.52)	132/132	0.86 (0.45–1.64)
Females	898	1.0 (referent)	700	1.0 (referent)	280	1.0 (referent)
# of Lesions	628	1.42 (1.04 – 1.93)	458	1.70 (1.17 – 2.47)	171	0.93 (0.50 – 1.72)
None	270	2.24 (1.23 – 4.10)	242	2.15 (1.16 – 3.99)	109	1.73 (0.60 – 4.99)
1						
>1						

Abbreviations: BCC, basal cell carcinoma; CI, confidence interval; NMSC, non-melanoma skin cancer; OR, Odds ratio; SCC, squamous cell carcinoma

Numbers do not sum to 1800 due to genotyping failures;
ORs and 95% CIs are from the additive model, adjusted for the first three principle components, age at baseline, education, body mass index, and smoking status
Both BCC and SCC may be present among those with >1 lesion.