Polymorphisms in the DNA Repair Genes *XPC*, *XPD*, and *XPG* and Risk of Cutaneous Melanoma: a Case-Control Analysis

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Abstract

Sunlight causes DNA damage, including bulky lesions that are removed effectively by the nucleotide-excision repair (NER) pathway. There are at least eight core NER proteins participating in the pathway, and genetic variations in their genes may alter NER functions. We hypothesized that some NER variants are associated with risk of cutaneous melanoma. In a hospital-based case-control study of 602 non-Hispanic White patients with cutaneous melanoma and 603 age- and sex-matched cancer-free controls, we genotyped five common non-synonymous single-nucleotide polymorphisms identified to date and assessed their associations with risk of cutaneous melanoma. We found that a significantly increased risk of cutaneous melanoma was associated with *XPD 751Lys/Gln* [adjusted odds ratio (OR), 1.55 and 95% confidence interval (95% CI), 1.12-2.16] and *XPD 751Gln/Gln* (OR, 1.66; 95% CI, 1.03-2.68) genotypes compared with the *XPD 751Lys/Lys* genotype as well as *XPD312Asp/Asn* (OR, 1.54; 95% CI, 1.11-2.12) and *XPD312Asn/Asn* (OR, 1.75; 95% CI, 1.05-2.90) genotypes compared with the *XPD 312Asp/Asp* genotype. This increased risk was not observed in the other three *XPC* and *XPG* single-nucleotide polymorphisms. Moreover, the number of the observed *XPD* at-risk genotypes (i.e., *312Asn/Asn+Asn/Asp* and *751Gln/Gln+Lys/Gln*) was associated with cutaneous melanoma risk in a dose-response manner (OR, 1.47; 95% CI, 0.97-2.23 for one at-risk genotype; OR, 1.83; 95% CI, 1.29-2.61 for two at-risk genotypes; $P_{trend} < 0.001$). However, we found no evidence of any interaction between *XPD* genotypes with *XPC* and *XPG* genotypes or the known risk factors. We concluded that genetic variants of the *XPD* gene might serve as biomarkers for susceptibility to cutaneous melanoma. (Cancer Epidemiol Biomarkers Prev 2006;15(12):2526–32)

Introduction

Sunlight causes various kinds of DNA damage, including bulky lesions (such as photoproducts) that can lead to mutations, if not repaired efficiently. Therefore, DNA repair is central to maintaining genomic integrity in the skin (1-3). The nucleotide-excision repair (NER) pathway specifically removes bulky DNA lesions, such as pyrimidine dimers caused by UV light, bulky adducts induced by chemical carcinogens, and other helix-distorting DNA lesions (2). When the photoproducts, such as cyclobutane pyrimidine dimmers, are not repaired effectively in the skin, as seen in some NER-deficient syndromes (1-4), there will be severe consequences. For example, xeroderma pigmentosum (XP), an autosomal recessive disease characterized by a deficient NER, has a 1,000-fold increased risk of UV-induced skin cancers, including cutaneous melanoma, which is the most lethal malignant skin tumor (4). Recently, several studies have provided clues about the molecular mechanisms underlying the genetic susceptibility to cutaneous melanoma in the general population, and accumulating evidence indicates that sequence variation in NER genes may contribute to cutaneous melanoma susceptibility (for a review, see ref. 5).

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At least eight different DNA NER genes (i.e., *ERCC1, XPA, XPB/ERCC3, XPC, XPD/ERCC2, XPE, XPF/ERCC4*, and *XPG/ERCC5)*, mostly identified from XP complementation groups, play crucial roles in the NER process (6). NER starts with damage recognition done collectively by several proteins, including the XPC-HR23B and the XPA replication protein A complexes and the transcriptions factor IIH (TFIIH) complex. These complexes transiently unwind the DNA duplex, thus creating an open bubble structure around the lesion. The TFIIH complex contains two DNA helicases XPB and XPD that catalyze DNA unwinding, creating a single-strand DNA that is further cut by additional incision enzymes: the ERCC1-XPF complex that cuts at the 5′ side of the lesion and XPG that cuts at the 3′ side of the lesion (7). However, XP genes contain a number of genetic polymorphisms that can alter their functions and thus may modify risk of cutaneous melanoma induced by UV exposure (5).

Although several previous studies have investigated the association between polymorphisms in NER genes and risk of cutaneous melanoma, most of the study sizes were relatively small, and the results were not consistent (8-14). Given potential roles of XP genes in repairing UV-induced DNA damage, we hypothesized that genetic variants in XP genes may contribute individually or collectively to risk of cutaneous melanoma. We tested our hypothesis by genotyping all known common (minor allele frequency >0.05) non-synonymous single-nucleotide polymorphisms (nsSNPs) of eight core NER genes in a hospital-based study of 602 patients with cutaneous melanoma and 603 cancer-free control subjects who were frequency-matched by age, sex, and ethnicity.

Materials and Methods

Study Populations. Details of the study population and subject recruitment have been described elsewhere (15, 16).

Briefly, patients with newly diagnosed and untreated cutaneous melanoma who had been referred to The University of Texas M. D. Anderson Cancer Center were recruited between May 1994 and September 2004. There were no restrictions on patients' age or tumor stage, but only those who had no metastases or other cancers and agreed to donate a blood sample were included in the study, and the response rate among the eligible patients was ~85%. All tumors of the patients were histologically classified according to the 2002 American Joint Committee on Cancer ''melanoma staging system'' (17). As previously described (15, 16), cancer-free control subjects were also recruited during the same period from self-reported cancer-free visitors to M. D. Anderson Cancer Center who were not seeking medical care but instead accompanied patients to our outpatient clinics, and the response rate among those we approached for recruitment was ~90%. The controls who were not blood related to the patients and agreed to donate a blood sample were frequency matched to the patients by age (±5 years), sex, and ethnicity. After obtaining the informed consent, we interviewed each eligible participant in person to obtain data on age, sex, ethnicity, host characteristics (e.g., color of skin, eyes, and hair), history of sun exposure (e.g., tanning ability, number of lifetime sunburns with blistering, and freckling in the sun as a child), and Fitzpatrick's sun-reactive skin type (18). At the end of the interview, a sample of blood (30 mL) was drawn into a heparinized tube from each subject. The research protocol was approved by the M. D. Anderson institutional review board.

Polymorphism Selection. We searched the National Institute of Environmental Health Science database (http://egp.gs.washington.edu) and related literature to identify all nsSNPs with a minor allele frequency >0.05 in persons of European descent in the eight core NER genes. We excluded the *XPF Ser662Pro* (rs2020955) SNP because, although its *Pro* allele was reported to have a minor allele frequency of 0.06 in a mixed population, its allele frequency has been shown to be <0.05 in White populations (http://snp500cancer.nci.nih.gov). Therefore, we included five common SNPs from three XP genes: *Ala499Val* (rs2228000) and *Lys939Gln* (rs2228001) from *XPC* on chromosome 3p25, *Asp312Asn* (rs1799793) and *Lys751Gln* (rs13181) from *XPD* on chromosome 19q13.3, and *His1104Asp* (rs17655) from *XPG* on chromosome 13q22.

Laboratory Assays. A leukocyte pellet was obtained from the buffy coat of cells by centrifugation of 1 mL of whole blood. The cell pellet was used for genomic DNA extraction with the Qiagen DNA Blood Mini kit (Qiagen, Valencia, CA). The DNA purity and concentration were determined by spectrophotometric measurement of absorbance at 260 and 280 nm. The DNA quality of three cases out of all cases we recruited was not sufficiently good for the PCR assays, whereas the DNA samples from other 602 patients and 603 controls were good enough for genotyping. A PCR was used to amplify the fragments of *XPC* containing the *Ala499Val* and *Lys939Gln* sites, the fragments of *XPD* containing the *Lys751Gln* and *Asp312Asn* sites, and the fragment of *XPG* containing the *His1104Asp* site. The previously described primers, PCR annealing time, restriction enzymes (New England Biolabs, Beverly, MA), and assay conditions for *XPC Ala499Val, XPC Lys939Gln* (19), *XPD Asp312Asn* (20), *XPD Lys751Gln* (21), and *XPG His1104Asp* (22) were followed. The sequencing-confirmed heterozygous samples were used as positive controls for the genotyping assays, ~10% of the samples were repeated, and the genotyping results were 100% concordant.

Statistical Analysis. The χ^2 test was used to evaluate differences between patients with cutaneous melanoma and control subjects in the frequency distributions of selected demographic variables, including known risk factors and each allele and genotype of the selected polymorphisms. Because skin color had been self-assessed on a screening questionnaire on a scale of 1 (light) to 10 (dark), we categorized skin color scale values of 1 to 3 as fair skin, 4 to 6 as brown skin, and 7 to 10 as dark skin, to obtain similar numbers of observations in each stratum and thus facilitate a further stratification analysis. Univariate and multivariate unconditional logistic regression analyses were used to obtain the crude and adjusted odds ratios (OR) for the risk of cutaneous melanoma and the 95% confidence intervals (95% CI). Multivariate adjustments were made, where appropriate, for age, sex, host characteristics, sun exposure history, Fitzpatrick's sun-reactive skin type, presence of moles or dysplastic nevi, and family history of cancer, for further stratification of the genotype data. To facilitate the assessment of potential interactions of the selected polymorphisms with cutaneous melanoma risk, we combined the genotypes of the *XPC* and *XPD* genes and dichotomized the variables by assuming a given genetic model (i.e., recessive, codominant, or dominant based on the observed data). We used the LDA software to calculate the linkage disequilibrium for the pairs of two *XPC* and two *XPD* polymorphisms (23) and the Phase software (version 2.0.2; University of Washington, Seattle, WA) to reconstruct the *XPC* and *XPD* haplotypes for each study participant based on their known *XPC* and *XPD* genotypes (24, 25). Finally, we assessed potential locus-locus interactions for the observed genotypes and locus-risk factor interactions for selected risk factors (e.g., color of skin, eyes, and hair; tanning ability; lifetime number of sunburns with blistering; freckling in the sun as a child; Fitzpatrick's sun-reactive skin type; the presence of moles or dysplastic nevi; and a family history of cancer). To assess any evidence of a departure from a multiplicative model, we fit the interaction terms for the pair of variables of interest by using standard unconditional logistic regression. ORs, 95% CIs, and *P*s for interactions and trend tests were obtained from multivariate logistic regression models, in which an interactive term was created as the product of each of the dichotomized genotypes and each of the dichotomized risk factors. A more-than-multiplicative interaction (i.e., $OR_{11} > OR_{01}{*}OR_{10}$) was suggested when OR_{11} was greater than $OR_{01}{*}OR_{10}$. Statistical significance was established at a P of ≤0.05, and all tests were two sided and done with the SAS software (version 8.2; SAS Institute, Cary, NC).

Results

Because non-Caucasian subjects represented <1% and thus were excluded from the analyses, all subjects were non-Hispanic Whites. As a result of frequency matching, there was no difference in the frequency distribution of age and sex between the patients and control subjects. Among the patients, tumor sites included 14% on the head and neck, 26% on an upper extremity, 21% on a lower extremity, 36% on the trunk, and 3% on other sites. Based on the American Joint Committee on Cancer T-category criteria and Breslow thickness (17), 47% had tumors <1.0 mm (T_1), 24% had tumors 1.01 to 2.0 mm (T_2), 16% had tumors >2.01 (T_3-T_4), and 13% were unclassified. Determination of Clark levels (17, 26) showed that 10% were level I (*in situ*), 55% were levels II to III, and 35% were levels IV to V. As we previously described (15, 16), the classic phenotype traits (e.g., color of skin, eyes, and hair; tanning ability; lifetime number of sunburns with blistering; freckling in the sun as a child; Fitzpatrick's sun-reactive skin type; the presence of moles or dysplastic nevi; and a family history of cancer) were risk factors for cutaneous melanoma in this study population and were used for adjustment in the later multivariate logistic regression model to estimate the main effects of the SNPs.

The genotype and allele frequencies of *XPC Ala499Val* and *XPC Lys939Gln, XPD Lys751Gln* and *XPD Asp312Asn*, and

XPG His1104Asp SNPs and their association with risk of cutaneous melanoma are shown in Table 1. The ORs adjusted for age and sex were almost identical to the crude ones, suggesting an adequate frequency matching for age and sex, but ORs adjusted for other known risk factors seemed to enhance the estimates. Therefore, we presented the ORs without and with adjustment for other known risk factors. When compared with the *XPD 751Lys/Lys* genotype, a significantly increased risk was associated with *XPD Lys/Gln* (adjusted OR, 1.55; 95% CI, 1.12-2.16), *XPD Gln/Gln* (adjusted OR, 1.66; 95% CI, 1.03-2.68), and combined *Lys/Gln+Gln/Gln* genotypes (assuming a dominant model for the *XPD Gln* variant allele; adjusted OR, 1.58; 95% CI, 1.16-2.15; Table 1). Likewise, compared with the *XPD 312Asp/Asp* genotype, a significant risk was associated with *XPD Asp/Asn* (adjusted OR, 1.54; 95% CI, 1.11-2.12), *XPD Asn/Asn* (adjusted OR, 1.75; 95% CI, 1.05-2.90), and combined *XPD Asp/Asn+Asn/Asn* (adjusted OR, 1.58; 95% CI, 1.16-2.14) genotypes (assuming a dominant model for the *Asn* variant allele). This increased risk was not seen in the other three *XPC* and *XPG* SNPs (Table 1). Moreover, the distributions of these genotypes were independent of tumor histologic types, Clark levels, tumor site, and tumor thickness ($P > 0.05$ for all; data not shown).

However, the stratified analysis by age showed that the *XPD Lys751Gln* and *XPD Asp312Asn* genotypes were associated with significantly increased cutaneous melanoma risk only in young (≤45 years) subjects (adjusted OR, 2.21; 95% CI, 1.23-3.95 for *751Lys/Gln+Gln/Gln* genotypes compared with *Lys/Lys*; adjusted OR, 1.88; 95% CI, 1.06-3.35 for *XPD 312Asp/Asn+Asn/Asn* genotypes compared with *XPD 312Asn/Asn*) but not in older (>45 years) subjects (adjusted OR, 1.42; 95% CI, 0.98-2.06 for *751Lys/Gln+Gln/Gln* genotypes compared with *Lys/Lys*; adjusted OR, 1.43; 95% CI, 0.99-2.07 for *XPD 312Asp/Asn+Asn/Asn* genotypes compared with *XPD 312Asn/Asn*). Similarly, the stratified analysis by sex showed that this significantly increased cutaneous melanoma risk was only observed in the men (adjusted OR, 1.81; 95% CI, 1.18-2.77 for *751Lys/Gln+Gln/Gln* genotypes compared with *Lys/Lys*; adjusted OR, 1.65; 95% CI, 1.09-2.51 for *XPD 312Asp/Asn+Asn/Asn* genotypes compared with *XPD 312Asn/Asn*) but not in the women (adjusted OR, 1.46; 95% CI, 0.93-2.29 for *751Lys/Gln+Gln/Gln* genotypes compared with *Lys/Lys*; adjusted OR, 1.48; 95% CI, 0.95-2.30 for *XPD 312Asp/Asn+Asn/Asn* genotypes compared with *XPD 312Asn/Asn*; data not shown).

Further analysis suggested that there was linkage disequilibrium between *XPC Ala499Val* and *XPC Lys939Gln* SNPs ($D' = 0.830$, $R^2 = 0.165$, $P < 0.001$ for *Ala* and *Lys* alleles) and between *XPD Lys751Gln* and *XPD Asp312Asn* SNPs ($D' = 0.608$, $R^2 = 0.329$, $P < 0.001$ for *Lys* and *Asp* alleles), suggesting that a joint effect between the two *XPC* and two *XPD* SNPs may exist. However, differences in the overall distributions of the estimated *XPC* or *XPD* haplotypes between the patients and control subjects were not statistically significant ($P > 0.05$). We further did the tests for each haplotype separately for case-control frequency differences and the trend tests for the number of at-risk haplotypes and

Table 1. Genotype and allele frequencies of the *XPC*, *XPD*, and *XPG* polymorphisms among non-Hispanic White patients with cutaneous melanoma and control subjects and their association with the risk of cutaneous melanoma

Variables	Patients (n = 602), n (%)	Control subjects (n = 603), n (%)	P*	Crude OR (95% CI)	Adjusted OR (95% CI)†
XPC Ala499Val					
Ala/Ala	338 (56.1)	318 (52.7)	0.080‡	1.00	1.00
Ala/Val	214 (35.6)	248 (41.1)		0.81 (0.64-1.03)	0.75 (0.55-1.03)
Val/Val	50 (8.3)	37 (6.1)		1.27 (0.81-2.00)	1.61 (0.82-3.16)
Ala/Val+Ala/Ala	552 (91.7)	566 (93.9)	0.146§	1.00	1.00
Val/Val	50 (8.3)	37 (6.1)		1.39 (0.89-2.15)	1.82 (0.94-3.52)
Val allele	0.261	0.267	0.773‖		
XPC Lys939Gln					
Lys/Lys	223 (37.0)	195 (32.3)	0.183‡	1.00	1.00
Lys/Gln	281 (46.7)	311 (51.6)		0.79 (0.62-1.02)	0.78 (0.55-1.09)
Gln/Gln	98 (16.3)	97 (16.1)		0.88 (0.63-1.24)	1.08 (0.67-1.73)
Lys/Lys+Lys/Gln	504 (83.7)	506 (83.9)	0.928§	1.00	1.00
Gln/Gln	98 (16.3)	97 (16.1)		1.01 (0.75-1.38)	1.26 (0.82-1.92)
Gln allele	0.396	0.419	0.268§		
XPD Lys751Gln					
Lys/Lys	219 (36.4)	255 (42.3)	0.110‡	1.00	1.00
Lys/Gln	297 (49.3)	270 (44.8)		1.28 (1.00-1.64)	1.55 (1.12-2.16)
Gln/Gln	86 (14.3)	78 (12.9)		1.28 (0.90-1.83)	1.66 (1.03-2.68)
Lys/Gln+Gln/Gln	383 (63.6)	348 (57.7)	0.036§	1.28 (1.02-1.62)	1.58 (1.16-2.15)
Gln allele	0.390	0.353	0.066‖		
XPD Asp312Asn					
Asp/Asp	242 (40.2)	273 (45.3)	0.163‡	1.00	1.00
Asp/Asn	290 (48.2)	259 (43.0)		1.26 (0.99-1.61)	1.54 (1.11-2.12)
Asn/Asn	70 (11.6)	71 (11.8)		1.11 (0.77-1.61)	1.75 (1.05-2.90)
Asp/Asn+Asn/Asn	360 (59.8)	33 (54.7)	0.075§	1.23 (0.98-1.55)	1.58 (1.16-2.14)
Asn allele	0.357	0.333	0.232‖		
XPG His1104Asp					
His/His	373 (62.0)	370 (61.4)	0.847‡	1.00	1.00
His/Asp	206 (34.2)	206 (34.2)		0.99 (0.78-1.26)	0.88 (0.64-1.21)
Asp/Asp	23 (3.8)	27 (4.5)		0.85 (0.48-1.50)	0.77 (0.36-1.67)
His/Asp+Asp/Asp	229 (38.0)	233 (38.6)	0.830§	0.97 (0.78-1.23)	0.87 (0.64-1.18)
Asp allele	0.209	0.216	0.711‖		

NOTE: The observed genotype frequency among the control subjects was in agreement with the Hardy-Weinberg equilibrium ($\chi^2 = 1.55$, $P = 0.213$ for *XPC Ala499Val*; $\chi^2 = 2.13$, $P = 0.144$ for *XPC Lys939Gln*; $\chi^2 = 0.242$, $P = 0.623$ for *XPD Lys751Gln*; $\chi^2 = 0.632$, $P = 0.427$ for *XPD Asp312 Asn*; and $\chi^2 = 0.061$, $P = 0.805$ for *XPG His1104/Asp*).

*Two-sided χ^2 test for either genotype distribution or allele frequency.

†Adjusted for age, sex, skin color, eye color, hair color, tanning ability, lifetime number of sunburns with blistering, freckling in the sun as a child, presence of moles or dysplastic nevi, and family history of cancer.

‡Distribution of three genotypes.

§Distribution of combined genotypes.

‖Allele distribution.

diplotypes and found that none of the *XPC* or *XPD* haplotypes or diplotypes was significantly associated with risk of cutaneous melanoma (data not shown). When the two *XPC* and two *XPD* SNPs were further combined separately based on the number of the observed at-risk genotypes (i.e., *XPC 499Val/Val, XPC 939Gln/Gln*, and *XPD 312Asn/Asn+Asn/Asp* and *XPD 751Gln/Gln+Lys/Gln*), we found that cutaneous melanoma risk increased as the number of *XPD* at-risk genotypes increased (adjusted OR, 1.47; 95% CI, 0.97-2.23 for one at-risk genotype and adjusted OR, 1.83; 95% CI, 1.29-2.61 for two at-risk genotypes, $P_{trend} < 0.001$). When the number of the observed at-risk genotypes was dichotomized, the subjects who carried one to two at-risk genotypes had a 1.7-fold increased risk of cutaneous melanoma (adjusted OR, 1.70; 95% CI, 1.23-2.36), compared with those who carried zero at-risk genotypes (Table 2).

XPC, XPD, and *XPG* all play roles in NER. However, only two *XPD* SNPs seemed to have a main effect on cutaneous melanoma risk in this study population. Although the *XPC* and *XPG* SNPs had no effects on cutaneous melanoma risk, we were interested in whether the main effects of the observed *XPD* genotypes on cutaneous melanoma risk were modified by *XPC* and *XPG* genotypes and the known risk factors. As shown in Table 3, we dichotomized the combined *XPC* genotypes and *XPG* genotypes to stratify the dichotomized *XPD* combined genotypes. We found that although a significantly increased risk associated with the combined *XPD* at-risk genotypes was only evident among those who did not carry any *XPC* at-risk genotype (adjusted OR, 1.62; 95% CI, 1.07-2.44), there was no evidence of a locus-locus interaction ($P_{interaction} = 0.771$). Similarly, a significantly increased risk associated with the combined *XPD* at-risk genotypes was only evident among those who carried the *XPG* 1104 *His/His* genotype (adjusted OR, 1.85; 95% CI, 1.21-2.83), but no significant locus-locus interaction was evident ($P_{interaction} = 0.636$; Table 3).

Finally, we did stratification analysis for the association between dichotomized *XPD* genotypes (zero versus one to two at-risk genotypes) by selected known risk factors for cutaneous melanoma. The ORs in different strata were shown in Table 4. The risk of cutaneous melanoma associated with one to two at-risk *XPD* genotypes was more pronounced among some strata, suggesting possible gene-environment or gene-gene interactions in the etiology of cutaneous melanoma. However, further analysis showed no statistical evidence of such interactions on a multiplicative scale, as assessed in the multivariate logistic regression models (Table 4).

Discussion

In this hospital-based case-control study of cutaneous melanoma, we showed an increased risk of cutaneous melanoma associated with *XPD 751Gln* and *XPD 312Asn* variant alleles. Moreover, when *XPD Lys751Gln* and *XPD Asp312Asn* SNPs were combined, risk of cutaneous melanoma increased as the number of *XPD* at-risk genotypes increased, suggesting that these two *XPD* SNPs may interact to contribute collectively to risk of cutaneous melanoma. However, it seemed that they did not interact with the selected *XPC* and *XPG* SNPs or the known risk factors for cutaneous melanoma in the etiology of cutaneous melanoma. Although exactly how these *XPD* SNPs work to influence risk of cutaneous melanoma remains unknown, the risk variant alleles could be either functional themselves or in linkage disequilibrium with other functional or disease-causing alleles involved in the etiology of cutaneous melanoma.

Human skin is constantly exposed to carcinogenic agents, especially UV radiation, that cause various kinds of DNA damage, such as cyclobutane pyrimidine dimers, that may lead to the development of skin cancers, including cutaneous melanoma (27, 28). In previous studies, we have shown that cutaneous melanoma was likely to occur in individuals whose UV light-induced DNA damage was not repaired effectively (29). It is possible that variations in DNA repair phenotype in the general population are probably caused by polymorphisms of DNA repair genes (30). Most of UV light–induced DNA damage is repaired by the NER pathway in a multi-step process involving at least 25 proteins, of which at least eight core proteins (i.e., ERCC1, XPA, XPB, XPC, XPD, XPF, XPF, and XPG) play crucial roles (1-4). However, we only identified and tested five common nsSNPs in *XPC, XPD*, and *XPG* out of these eight NER genes. Further studies using the hypothesis-free approach to select tagging SNPs, which should capture most of the identified SNPs to date, may advance our understanding of the roles of SNPs in these NER genes in the etiology of cutaneous melanoma. In addition, further correlation studies of NER genotypes and phenotype are

Table 2. Main effects of combined *XPC Ala499Val, Lys939Gln* and *XPD Asp312Asn, Lys751Gln* genotypes on cutaneous melanoma risk

Genotypes	Cases (n = 602), n (%)	Controls (n = 603), n (%)	Crude OR (95% CI)	Adjusted OR (95% CI)*
XPC Ala499Val+Lys939Gln				
No. at-risk genotype†				
0	456 (75.8)	471 (78.1)	1.00	1.00
1	144 (23.9)	130 (21.6)	1.14 (0.87-1.50)	1.34 (0.95-1.91)
2	2 (0.3)	2 (0.3)	1.03 (0.15-7.36)	1.45 (0.15-7.82)
P_{trend}			0.342	0.062
Dichotomized groups				
0	456 (75.8)	471 (78.1)	1.00	1.00
1-2	146 (24.3)	132 (21.9)	1.14 (0.87-1.49)	1.43 (0.91-2.09)
XPD Lys751Gln+Asp312Asn				
No. at-risk genotype‡				
0	157 (26.1)	197 (32.7)	1.00	1.00
1	147 (24.4)	134 (22.2)	1.38 (1.01-1.89)	1.47 (0.97-2.23)
2	298 (49.5)	272 (45.1)	1.38 (1.05-1.79)	1.83 (1.29-2.61)
P_{trend}			0.026	<0.001
Dichotomized groups				
0	157 (26.1)	197 (32.7)	1.00	1.00
1-2	455 (73.9)	406 (67.3)	1.38 (1.07-1.76)	1.70 (1.23-2.36)

*Adjusted for age, sex, skin color, eye color, hair color, tanning ability, lifetime number of sunburns with blistering, freckling in the sun as a child, presence of moles or dysplastic nevi, and family history of cancer.

†The at-risk genotypes used for the calculation were *XPC 499Val/Val* and *XPC 939Gln/Gln*.

‡The at-risk genotypes used for the calculation were *XPD 312Asn/Asn+Asn/Asp* and *XPD 751Gln/Gln+Lys/Gln*.

Table 3. Stratification analysis of the combined *XPD* genotypes associated with cutaneous melanoma risk by *XPC* and *XPG* genotypes

Genotypes	Combined *XPD* genotypes* (case/control), *n* (%)		Crude OR (95% CI)	Adjusted OR (95% CI)†
	0 at-risk genotypes	1-2 at-risk genotypes		
Combined *XPC* genotypes‡				
0 at-risk genotype	100/134 (63.7/68.0)	274/265 (61.6/65.3)	1.55 (1.16-2.07)	1.62 (1.07-2.44)
1-2 at-risk genotypes	57/63 (36.3/32.0)	171/141 (38.4/34.7)	0.94 (0.56-1.57)	1.74 (0.99-3.05)
$P_{interaction}$			0.096	0.771
XPG His1104Asp				
His/Asp+Asp/Asp	54/77 (34.4/39.1)	175/156 (39.9/38.4)	1.60 (1.06-2.41)	1.54 (0.90-2.63)
His/His	103/120 (65.6/60.9)	270/250 (60.7/61.6)	1.26 (0.92-1.72)	1.85 (1.21-2.83)
$P_{interaction}$			0.362	0.636

*The at-risk genotypes used for the calculation were *XPD 312Asn/Asn+Asn/Asp* and *XPD 751Gln/Gln+Lys/Gln*.
†Adjusted for age, sex, skin color, eye color, hair color, tanning ability, lifetime number of sunburns with blistering, freckling in the sun as a child, presence of moles or dysplastic nevi, and family history of cancer.
‡The at-risk genotypes used for the calculation were *XPC 499Val/Val* and *XPC 939Gln/Gln*.

warranted to clarify their independent and interactive roles in the etiology of cutaneous melanoma.

Because XPC protein is involved in the earliest damage recognition and initiation of NER (31), a number of studies have investigated the association of *XPC* polymorphisms with risk of several cancers (32-38). However, only three studies investigated the association between *XPC Ala499Val* and *XPC Lys939Gln* SNPs and risk of cutaneous melanoma. In a German hospital-based case-control study of 294 White patients with cutaneous melanoma and 375 healthy control subjects, no association was found between *XPC 499Val* variant genotypes and risk of cutaneous melanoma (9), which is consistent with the findings in our present study. In another study from the same group, the *XPC 939Gln* variant genotype was shown to be associated with an increased risk of cutaneous melanoma (10). In contrast, we did not find any association between *XPC 939Gln* variant genotypes and risk of cutaneous melanoma in our present study with a much larger sample

Table 4. Stratification analysis of the combined *XPD* genotypes associated with cutaneous melanoma risk by selected variables

Variable	Combined *XPD* genotypes (case/control), *n* (%)		Crude OR (95% CI)	Adjusted OR (95% CI)†	*P*‡
	0 at-risk genotypes*	1-2 at-risk genotypes*			
Age					
≤45	51/55 (32.5/27.9)	127/97 (28.5/23.9)	1.41 (0.89-2.25)	2.06 (1.12-3.78)	0.144
>45	106/142 (67.5/72.1)	318/309 (71.5/76.1)	1.38 (1.03-1.85)	1.55 (1.04-2.30)	
Sex					
Men	97/122 (61.8/61.9)	254/255 (57.1/62.8)	1.25 (0.91-1.72)	1.85 (1.19-2.60)	0.119
Women	60/75 (38.2/38.1)	191/151 (42.9/37.2)	1.58 (1.06-2.36)	1.58 (0.96-2.60)	
Hair color					
Black or brown	98/135 (63.6/81.3)	280/271 (64.7/81.1)	1.42 (1.05-1.94)	1.73 (1.18-2.54)	0.829
Blond or red	56/31 (36.4/18.7)	153/63 (35.3/18.9)	1.34 (0.79-2.28)	1.57 (0.80-3.10)	
Eye color					
Other	92/123 (59.7/73.2)	248/266 (57.4/79.6)	1.25 (0.90-1.72)	1.49 (0.99-2.23)	0.120
Blue	62/45 (40.3/26.8)	184/68 (42.6/20.4)	1.96 (1.22-3.16)	2.22 (1.26-3.94)	
Skin color					
Dark or brown	63/86 (40.9/51.2)	189/157 (43.7/46.6)	1.64 (1.12-2.42)	1.84 (1.17-2.92)	0.266
Fair	91/82 (59.1/48.8)	244/180 (56.3/53.4)	1.22 (0.86-1.74)	1.58 (0.98-2.57)	
Tanning ability					
Good (high)	94/107 (61.0/64.1)	257/189 (59.4/56.3)	1.55 (1.11-2.16)	1.82 (1.22-2.72)	0.333
Poor (low)	60/60 (39.0/35.9)	176/147 (40.6/43.7)	1.20 (0.79-1.82)	1.45 (0.80-2.63)	
No. lifetime sunburns with blistering					
None	35/80 (22.7/47.6)	118/163 (27.3/48.8)	1.66 (1.04-2.63)	2.00 (1.12-3.54)	0.500
≥1	119/88 (77.3/52.4)	314/171 (72.7/51.2)	1.36 (0.97-1.89)	1.52 (1.01-2.29)	
Freckling in the sun as a child					
No	64/98 (41.6/58.3)	198/194 (45.9/58.1)	1.56 (1.08-2.27)	1.85 (1.17-2.93)	0.485
Yes	90/70 (58.4/41.7)	233/140 (54.1/41.9)	1.29 (0.89-1.89)	1.41 (0.87-2.29)	
Moles					
No	35/61 (23.3/48.8)	101/104 (23.6/43.5)	1.69 (1.03-2.78)	1.71 (0.98-2.97)	0.659
Yes	115/64 (76.7/51.2)	327/135 (76.4/56.5)	1.35 (0.94-1.94)	1.70 (1.12-2.57)	
Dysplastic nevi					
No	129/117 (87.8/96.7)	377/214 (90.0/97.3)	1.60 (1.18-2.16)	1.71 (1.22-2.39)	
Yes	18/4 (12.2/3.3)	42/6 (10.0/2.7)	1.56 (0.39-6.19)	0.001 (0.001-999)	
First-degree relatives with any cancer					
No	45/63 (29.6/40.9)	165/131 (38.4/41.1)	1.76 (1.13-2.76)	2.15 (1.25-3.68)	0.169
Yes	107/91 (70.4/59.1)	265/188 (61.6/58.9)	1.20 (0.86-1.68)	1.47 (0.97-2.24)	

NOTE: The number of subjects in some of the strata was less than the total number of subjects because some subjects did not provide the information.
*The at-risk genotypes used for the calculation were *XPD 312Asn/Asn+Asn/Asp* and *XPD 751Gln/Gln+Lys/Gln*.
†Adjusted for age, sex, skin color, eye color, hair color, tanning ability, lifetime number of sunburns with blistering, freckling in the sun as a child, presence of moles or dysplastic nevi, and family history of cancer in a logistic regression model.
‡Test for multiplicative interaction obtained from logistic regression models with adjustment for age, sex, and the main effects of the interactive variables.

size. Interestingly, in another large study that analyzed 1,238 secondary or higher-order primary (cases) versus 2,485 single primary (control) cases of cutaneous melanoma, the *XPC 939Gln* variant genotype was not associated with risk of secondary or higher-order primary cutaneous melanoma (8).

XPD is an ATP-dependent DNA helicase involved in NER and in basal transcription as part of the transcription factor TFIIH (39). Seven polymorphisms in exons 6, 8, 10, 17, 22, and 23 of the *XPD* gene have been identified by direct sequencing (40-42). However, among these seven SNPs, three of these polymorphisms (*Ala575Ala, Asp711Asp*, and *Arg156Arg*) are silent, and the remaining four result in amino acid changes, of which the *IIe199Met* and *His201Tyr* polymorphisms are rare (allele frequency <1%), and *Lys751Gln* and *Asp312Asn* are the only common functional SNPs. Studies have shown that the *XPD 751Gln* variant modified the amino acid electronic configuration in a domain important for the interaction with the helicase activator p44 (43, 44). In addition, *XPD 751Gln* and *XPD 312Asn* variants were reportedly associated with suboptimal removal of DNA adducts (45). Therefore, these two SNPs are likely to be functional. To date, five reported studies have investigated the association between these two SNPs and risk of cutaneous melanoma. In the earliest study of 56 patients and 66 control subjects conducted in 2001, it was found that *XPD 751Gln* variant genotypes were associated with a significantly increased risk of cutaneous melanoma (OR, 2.8; 95% CI, 1.2-7.0; ref. 14). In a later study with 176 patients and 177 control subjects, no significant association was found between *XPD Lys751Gln* and *XPD Asp312Asn* SNPs and cutaneous melanoma risk, except for an increased risk in older (>50 years) subjects (13). In contrast, a nested case-control study within the Nurses' Health Study found a nonsignificant negative association between both *XPD 751Gln* and *XPD 312Asn* genotypes and risk of cutaneous melanoma (11). More recently, in a large study, *XPD* variant genotypes were found to be associated with an increased risk of secondary or higher-order primary cutaneous melanoma when compared with the single primary cutaneous melanoma (8). In the present study, by far the largest case-control study of primary cutaneous melanoma cases, we found a significantly increased risk of cutaneous melanoma associated with both *XPD 312Asn* and *XPD 751Gln* variant genotypes, but no obvious age difference in risk estimates was observed. However, we did observe this significantly increased cutaneous melanoma risk in men but not in women, but this sex difference in risk estimates was not statistically significant. Moreover, when we combined these two *XPD* polymorphisms, cutaneous melanoma risk increased as the number of the risk genotypes increased in a dose-response manner, suggesting *312Asn* and *751Gln* variants may interact to play a joint role in the etiology of cutaneous melanoma.

XPG cleaves various artificial DNA substrates, including bubbles, splayed arms, and stem-loops (46, 47). In addition, XPG has a structural function independent of its cleavage activity in the assembly of the NER DNA-protein complex (48). Only two published studies, one with 294 patients and 375 control subjects (10) and the other with 1,238 patients with second- or higher-order primary melanomas and 2,485 patients with a single primary melanoma (8), have investigated the association between *XPG* polymorphisms and risk of cutaneous melanoma. In both studies, the *XPG 1104Asp* allele was associated with a nonsignificant decrease in the risk of cutaneous melanoma, a finding that is consistent with our study.

It is conceivable that functional polymorphisms of the same gene may have a collective effect on DNA repair outcomes. Indeed, when we analyzed the combined genotypes of the two selected nsSNPs of *XPD*, it was apparent that the two *XPD* SNPs interacted to contribute collectively to risk of cutaneous melanoma. We speculate that the increased risk associated with the combined *XPD* genotypes may be due to the at-risk allele-mediated altered NER capacity or that these genotypes are likely in linkage disequilibrium with untyped risk or disease-causing alleles. Because reduced NER may cause cumulative damage to DNA and a resultant increased frequency of mutations, these genetic alterations may lead to melanogenesis. However, we did not find any interactions between *XPD* SNPs with *XPC* and *XPG* SNPs or the selected known risk factors, possibly due to limited study power. Larger population-based studies and mechanistic studies are necessary to confirm the roles of XP SNPs in the etiology of cutaneous melanoma and to identify the actual mechanisms underlying the association between *XPD* genotypes and risk of cutaneous melanoma. Once validated, these functional SNPs, together with SNPs in other genes in different biological pathways (15, 16), may be considered in future risk assessment and modeling of cutaneous melanoma (49).

Like all other case-control studies, inherent biases in the present study may have led to some spurious findings. First, of the eight core NER genes (i.e., *ERCC1, XPA, XPB, XPC, XPD, XPE, XPF*, and *XPG*), we used common functional SNPs instead of tagging SNPs. Therefore, there is genetic variation within other NER genes we did not evaluate and the selected genes we studied in the study. Second, because it is a hospital-based case-control study, the control subjects in this study may not be representative of the general population. Although our study is the largest study with 602 primary cutaneous melanoma cases and 603 cancer-free controls, our sample size was still not large enough to identify significant associations in different strata in some subgroups, let alone the power to evaluate gene-environment interactions adequately. Finally, the control subjects in the present study lacked examinations by dermatologists, and the risk factors reported were self-administered. These limitations can only be overcome in large prospective studies.

In summary, the results of this hospital-based case-control study showed that *XPD 751Gln* and *XPD 312Asn* variant genotypes were associated with a significantly increased risk of cutaneous melanoma, compared with those with *751Lys/Lys* and *312Asp/Asp* genotypes, respectively. Furthermore, the combined *XPD* genotypes containing one to two at-risk genotypes were associated with a significantly greater risk of cutaneous melanoma than were those containing zero at-risk genotypes. These findings suggest the two *XPD* polymorphisms may interact to contribute collectively to risk of cutaneous melanoma. Because of uncontrolled biases in the selection of subjects in a retrospective study, such as ours, these results could be biased. Therefore, larger, population-based studies are warranted to confirm our findings.

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