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Polymorphisms of Nucleotide Excision Repair Genes Predict Melanoma Survival

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Abstract

Melanoma is the most highly malignant skin cancer, and nucleotide excision repair (NER) is involved in melanoma susceptibility. In this analysis of 1042 melanoma patients, we evaluated whether genetic variants of NER genes may predict survival outcome of melanoma patients. We used genotyping data of 74 tagging single nucleotide polymorphisms (tagSNPs) in eight core NER genes from our genome-wide association study (including 2 in *XPA*, 14 in *XPC*, 3 in *XPE*, 4 in *ERCC1*, 10 in *ERCC2*, 8 in *ERCC3*, 14 in *ERCC4*, and 19 in *ERCC5*) and evaluated their associations with prognosis of melanoma patients. Using the Cox proportional hazards model and

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CONFLICT OF INTEREST

The authors state no conflict of interest.

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Kaplan-Meier analysis, we found a predictive role of *XPE* rs28720291, *ERCC5* rs4150314, *XPC* rs2470458 and *ERCC2* rs50871 SNPs in prognosis of melanoma patients (rs28720291: AG vs. GG, adjusted hazard ratio [adjHR] = 11.2, 95% confidence interval [CI] 3.04–40.9, $P = 0.0003$; rs4150314: AG vs. GG, adjHR = 4.76, 95% CI 1.09–20.8, $P = 0.038$; rs2470458: AA vs. AG/GG, adjHR = 2.11, 95% CI 1.03–4.33, $P = 0.040$; and rs50871: AA vs. AC/CC adjHR =2.27, 95% CI 1.18–4.35, $P = 0.015$). Patients with an increasing number of unfavorable genotypes had dramatically increased death risk. Genetic variants of NER genes, particularly *XPE* rs28720291, *ERCC5* rs4150314, *XPC* rs2470458 and *ERCC2* rs50871, may independently or jointly modulate survival outcome of melanoma patients. Because our results were based on a median follow-up of 3 years without multiple test corrections, additional large prospective studies are needed to confirm our findings.

Keywords

melanoma; nucleotide excision repair; survival; association

INTRODUCTION

Melanoma is the most lethal skin cancer, ranking the sixth most common cancer in the United States. There were estimated 76,250 new melanoma cases, in addition to 55,560 melanoma *in situ*, in 2012 (Siegel *et al.* 2012). Although surgery remains the mainstay treatment, biochemotherapy and radiotherapy are also considered in an attempt to improve local control and overall survival. Despite aggressive treatment, patient prognosis varies substantially between individuals, with a 5-year survival rate ranging from over 80% in early stages to less than 10% in patients with distant metastasis (Buettner *et al.* 2005).

Some important tumor morphological and biological characteristics are known to be associated with patients' survival, including primary tumor thickness, ulceration, mitotic activity, lymph node infiltration and distant metastasis (Spatz *et al.* 2010). However, these histopathological features of primary tumors do not provide sufficient information for assessing tumor malignancy. For example, a subset of "thin" melanoma (tumor thickness < 0.76 mm) can be lethal due to undetected metastasis (Woods *et al.* 1983). Although the underlying mechanisms are unclear, tumor genetic heterogeneity and interactions among the host and tumor factors may be responsible for rapid evolution and development of malignancies in these patients. Some somatic mutations (e.g. *BRAF* and *p16*) are commonly implicated in melanoma progression (Chin *et al.* 2006), whereas an enhanced host's immune system can efficiently suppress cancer cell spreading, contributing to prolonged survival (DiFronzo *et al.* 2002). Nevertheless, it is possible that some other unknown genetic factors, by interacting with the known clinicopathological factors, may modulate survival outcomes of melanoma patients, thus uncovering biomarker for patients' long-term survival.

Previous epidemiologic studies have supported the notion that DNA-damaging UV irradiation causes cutaneous melanoma by inducing genetic abnormality (von Thaler *et al.*). The well-studied nucleotide excision repair (NER) pathway consists of at least 23 genes/proteins that act to remove UV-induced DNA lesions. Several SNPs of the NER genes have

been shown to be associated with melanoma susceptibility (Li *et al.* 2006). However, their influence on patients' survival has not been thoroughly investigated. In a recent study of eight non-synonymous SNPs of DNA repair genes (i.e., *XPC* p.Ala499Val, *XPC*p.Lys939Gln, *ERCC2* p.Lys751Gln and *ERCC5* p.Asp1104His of NER; *APEX1* p.Asp148Glu, *XRCC1* p.Arg399Gln of base excision repair; and *XRCC3* p.Thr241Met and *NBS1* p.Glu185Gln of the homologous recombination repair), only *ERCC5* p.Asp1104His (rs17655) and *ERCC2* p.Lys751Gln (rs13181) were found to have an effect on prognosis of melanoma (Schrama *et al.* 2011), suggesting that the NER genes may be involved in melanoma outcomes, although genes involved in cell cycle checkpoint are also found to be important (Kauffmann *et al.* 2008).

Here we report our results of an analysis of prognosis of 1042 melanoma patients in association with 74 tagging SNPs of the NER genes available to us in a previously published genome-wide association study of melanoma (Amos *et al.* 2011). In the present analysis, we evaluated the association between these SNPs and survival and explored their interactions with clinicopathological risk factors in determining melanoma patient prognosis.

RESULTS

Patient Characteristics

The analysis consisted of 1042 patients with primary cutaneous melanoma (Table 1), who had available data from questionnaire, genotyping, and survival. The patients were aged between 18 and 84 years at diagnosis with a mean of 50.8 years and standard deviation of 13.1 years. There were slightly more women than men (58.8% vs. 41.2%); 83.1% of the patients had early-stage melanoma (*in situ* and stages I and II), and 16.9% had later-stage melanoma (stages III and IV). We also collected complete information about tumor morphology, including primary tumor thickness, ulceration, metastasis to local lymph nodes, mitotic rate (mitoses/mm^2) of tumor cells [because there was no association with mitotic rate by the American Joint Committee on Cancer (AJCC) staging system of mitoses $\geq 1/mm^2$ versus $<1/mm^2$, we used $3/mm^2$ as the cutoff as shown in Table 1], anatomic site of the tumor and patient biological characteristics, including colors of the skin, hair and eyes, tanning ability after sun exposure, lifetime sunburns with blistering, moles and family history of skin cancer. The median follow-up time was 35.7 months, during which 52 (5.0%) of the 1042 patients had died at the last follow-up.

To determine if there was any confounding factor influencing patients' death or survival time, we performed Cox proportional hazards regression analysis to assess the association between overall survival (OS) and clinicpahtological characteristics. In the univariate analysis, older age, dark color of hair, freckling in the sun as a child, advanced tumor stages, thick tumor, presence of tumor ulceration, and increased primary tumor mitotic rate were significant predictors for poor survival. When all of these variables were included in a Cox proportional hazards regression model for adjustment to calculate hazards ratio (HR), only dark color of the skin (HR = 4.55), freckling in the sun as a child (HR = 2.90), advanced AJCC stage (HR = 5.60), and presence of tumor ulceration (HR = 2.72) remained statistically significant predictors for poor survival (Table 1).

Determination of melanoma survival prediction model

We performed the stepwise multivariate Cox proportional hazards regression analysis to further screen for optimal predictors of survival in melanoma patients, using covariates listed in Table 1 and the 74 selected SNPs of the 8 NER core genes (i.e., two SNP for *XPA*, 14 SNPs for *XPC*, three SNPs for *XPE/DDB1*, four SNPs for *ERCC1*, 10 SNPs for *ERCC2/XD*, eight SNPs for *ERCC3/XPB*, 14 SNPs for *ERCC4/XPF*, and 19 SNP for *ERCC5/XPG*). As shown in Table 2, clinicopathological factors of age (≤50 vs. >50), stage (*in situ*/I/II vs. III/IV), ulceration (no vs. yes) and mitotic rate (≤3 vs. >3/mm^2), and SNPs of rs28720291 (GG vs. AG), rs4150314 (GG vs. AG), rs2470458 (AG+GG vs. AA) and rs50871 (AC+CC vs. AA) were selected as the most significant predictors of survival, among which covariates of later stages (III/IV) (HR = 6.34; 95% CI 3.11–11.9), rs28720291 GG genotype (HR = 6.69; 95% CI 1.83–23.7), and rs4150314 GG genotype (HR= 6.15; 95% CI 1.46–28.5) were of the strongest predictors. Older age, ulceration, increased mitotic rate, rs2470458 AG/GG genotypes, and rs50871 AC/CC genotypes were of low or moderate risk factors (1< HR <3).

NER genetic polymorphisms as independent survival risk factors

The initial stepwise Cox proportional hazards regression analysis suggested four SNPs (*XPE* rs28720291, *ERCC5* rs4150314, *XPC* rs2470458 and *ERCC2* rs50871) as important and independent predictors for survival of melanoma patients. We further performed univariate and multivariate Cox proportional hazards regression analyses to evaluate their effects on risk of death or in the presence of other clinicopathological covariates. In the univariate analysis, *XPE* rs28720291AG and *ERCC2* rs50871AA genotypes were associated with increased hazards of early death (AG vs. GG: HR = 4.92, 95% CI 1.77–13.70, $P = 0.002$; and AA vs. AC+CC: HR = 2.18, 95% CI 1.26–3.77, $P = 0.005$, respectively). In the multivariate analyses performed with adjustment for age, sex, tumor Breslow thickness, tumor stage, ulceration, tumor cell mitotic rate, involvement of lymph nodes, and **tumor** anatomic site, the four SNPs remained significantly associated with survival outcome of melanoma patients [i.e., rs28720291: AG (no AA was observed) vs. GG 11.2, 95% CI 3.04–40.9, $P = 0.0003$; rs4150314: AG (no AA was observed) vs. GG 4.76, 95% CI 1.09–20.8, $P = 0.038$; rs2470458: AA vs. AG+GG 2.11, 95% CI 1.03–4.33, $P = 0.040$; and rs50871: AA vs. AC+CC 2.27, 95% CI 1.18–4.35, $P = 0.015$] (Table 3).

Survival of melanoma patients and combined genetic risk factors

To assess the joint effect of the four SNPs on patient prognosis, we combined their unfavorable genotypes (i.e., *XPE* rs28720291AG, *ERCC5* rs4150314AG, *XPC* rs2470458AA, and *ERCC2* rs50871AA genotypes). The frequencies of patients with 0, 1, 2 and 3 unfavorable genotypes were 276, 566, 188 and 10, respectively; no patient had all four unfavorable genotypes. Patients with an increasing number of unfavorable genotypes had dramatically increased risk of death by over 30-fold (HR = 34.3; 95% CI 7.48–157.2; $P < 0.0001$) in patients with any three unfavorable genotypes, compared with those without any unfavorable genotypes (Table 4 and Figure 1). Since there were only ten patients carrying three unfavorable genotypes, we next grouped all patients into a low-risk group (patients

with <=1 unfavorable genotypes) and a high-risk group (patients with 2 or 3 unfavorable genotypes) for further stratified analysis (Table 4).

Stratification analysis between the unfavorable genotypes and melanoma survival

We further performed stratified analysis to investigate if the combined effect of unfavorable genotypes on survival was modified by some important clinicopathological factors in Table 1. We found that only patients in the high-risk genotype group, but not the low-risk genotype group, showed substantially increased risk of death in the presence or absence of concomitant clinicopathological risk factors (e.g., thick tumor, involvement of lymph nodes, increased mitotic rate, advanced AJCC tumor stages, presence of tumor ulceration and tumor site in face and head and neck), except for the subgroups of thin tumor and without lymph node involvement (Figure 2).

DISCUSSION

In this relatively large melanoma patient cohort, we found that some variants of the NER genes (e.g., *XPE* rs28720291, *ERCC5* rs4150314, *XPC* rs2470458 and *ERCC2* rs50871) may independently or jointly modulate survival of melanoma patients. These genetic variants, in combination with clinicopathological factors, effectively predicted survival in subgroups of melanoma patients.

Previous studies demonstrated that some clinicopathological characteristics were associated with prognosis of melanoma patients, such as hair color, history of childhood freckling in the sun, tumor stage and ulceration status (Buettner *et al.* 2005). These results were also confirmed in the current analysis. However, we were interested in finding out some genetic variants of the NER genes that may play a role in modulating patient survival. This is because NER is an essential DNA repair mechanism that ensures genomic integrity. There is evidence that genomic instability increases not only in primary melanoma, compared with nevi, but also in metastases, compared with primary tumors (Chin *et al.* 2006). Hence, an increased NER capacity may reduce DNA mutations that may stimulate malignant progression and metastasis. More interestingly, previously reported two NER SNPs [*ERCC5* p.Asp1104His (rs17655) and *ERCC2* p.Lys751Gln (rs13181)] (Schrama *et al.* 2011) were replicated in our current analysis, because they are tagged by our selected tagging SNPs (*ERCC5* rs4150314 and *ERCC2* rs50871). However, we found that additional two NER SNPs (*XPE* rs28720291 and *XPC* rs2470458) also independently predicted the prognosis of melanoma. Therefore, our data further support the notion that genetic variants in the NER pathway may modulate clinical outcome of melanoma patients.

NER employs a relatively small number of essential repair proteins, including XPA, XPC, XPE/DDB1, ERCC1, ERCC2/XPD, ERCC3/XPB, ERCC4/XPF and ERCC5/XPG, to correct bulky DNA damage induced by chemical carcinogens or UV exposure. Briefly, repair of the damaged DNA strand includes making an incision at the 5′ and 3′ of the lesion, removing a 30-nucleotide section containing the damage, and ligating the gap by pairing DNA synthesis (Sancar 1995). XPC-hHR23B is a NER factor that detects DNA damage and recruits TFIIH to the damaged site (Yokoi *et al.* 2000); then proteins encoded by *XPA-G* genes, the ERCC1-hHR23B-RPA trimmers, and TFIIH are involved in the excision step (de

Boer and Hoeijmakers 1999). Two helicase subunits of TFIIH (i.e., XPB and XPD), together with XPA, RPA, and XPG, form a 30 base-pair bubble around the lesion for damage verification and correct positioning of two endonucleases (i.e., XPG and XPF-ERCC1) before incision (Missura *et al.* 2001). The incisions made by XPG and XPF-ERCC1 are at the double- and single-stranded DNA border in the incision complex (Sijbers *et al.* 1996). Observation of the mobility of various NER proteins in living cells suggests that NER proceeds by the sequential assembly of individual factors involved, rather than through the action of a preassembled repairosome (Houtsmuller *et al.* 1999).

Previous studies have extensively explored associations between NER and melanoma susceptibility, but few investigated the effect of NER on clinical outcomes of melanoma patients. In a study of 90 stage IV melanoma patients, an *ERCC1*-rs11615 SNP was found to be weakly associated with overall survival (Liu *et al.* 2005). In another study of 244 melanoma patients in Sweden, an *ERCC2*-rs13181 SNP was suggested to be a prognostic factor for melanoma progression (Kertat *et al.* 2008). More recently, both *ERCC5*-rs17655 and *ERCC2*-rs13181 SNPs were found to be independent prognostic factors in 742 melanoma patients (Schrama *et al.* 2010). However, none of the above SNPs were replicated or in linkage with the four positive SNPs (i.e., *XPE*-rs28720291, *ERCC5*-rs4150314, *XPC*-rs2470458 and *ERCC2*-rs50871) investigated in the present study. Several possible reasons may explain the discrepancies: first, the studies by Liu *et al.* and Kertat *et al.* were small sample-sized (90 cases and 244 cases, respectively), which could lead to chance findings or miss some SNPs with mild effects due to a limited study power; second, the majority of melanoma patients in these two studies had late-stage tumors (stages III/IV), while a large proportion of patients in our analysis and the study by Schrama *et al.* had early-stage tumors (866/1044 and 652/742, respectively).

Despite these discrepancies, there was some consistency among these published studies and ours. If excluding the most small-sized study by Liu *et al.*, it appears there are increasing levels of significance as well as increasing number of genes as the study patient population size increases, and the significant SNPs/genes identified in previous small-sized study could be confirmed by later larger-sized studies (*ERCC2* in 244 patients, *ERCC2* and *ERCC5* in 742 patients, and *ERCC2*, *ERCC5*, *XPC* and *XPE* in 1042 patients). Since SNPs may alter the related gene's function, our analysis, together with previous studies, suggested that the four genes, *ERCC2/XPD*, *ERCC5/XPG*, *XPC* and *XPE/ERCC3*, may play important roles in modulating melanoma patients' survival.

Although the effect of a single SNP on cancer risk or clinical outcomes, if any, may be limited, the combined effect of several SNPs in the same or different genes could be more significant. In the present analysis, we were also interested in whether there was an additive/synergistic effect in the association of the four SNPs (*XPE*-rs28720291, *ERCC5*-rs4150314, *XPC*-rs2470458 and *ERCC2*-rs50871) with melanoma survival. Indeed, patients with two or three unfavorable genotypes showed dramatically increased risk of death, compared with those with none or one unfavorable genotype. This is biologically plausible, because multiple variants may be more likely to have a substantial joint effect on the DNA repair capacity phenotype.

Through stratified analyses, we found that the genotype-survival association was most pronounced in the presence of clinicopathological risk factors, suggesting that suboptimal repair of DNA damage induced externally (UV-exposure) or internally (free radicals from metabolism) could aggregate the existing genomic instability of a fast-growing melanoma, promoting melanoma development and progression in the high-risk populations. Since these high-risk patients comprised 20.0% (198 out of 1042) of all the study subjects, our analysis identified a significant proportion of melanoma patients (such as those with unfavorable genotypes) that may require close clinical surveillance or alternative treatment to improve their survival.

The current analysis has some limitations. First, since we used a tagging SNP approach, we were not able to explore the mechanism by which the studied genetic polymorphisms influence melanoma patients' survival. Although the four identified SNPs and their tagged SNPs (LD ≥0.8) may have potential biological functions as predicted by software tools (http://snpinfo.niehs.nih.gov/snpfunc.htm), none of them have been reported or investigated as functional SNPs in the literature. Only four SNPs tagged by *XPC*-rs2470458 were found to be associated with risk of bladder cancer (rs2228000, rs2470352, and rs2470458) and lung cancer (rs2229090) in previous association studies (Shen *et al.* 2005; Stern *et al.* 2009). Further functional studies of these SNPs are required. Second, there were only 52 deaths out of 1042 patients at our last follow-up at a median of nearly 3 years. Therefore, the current study is, to a large extent, an interim survival analysis. We will report updated results after we have a longer follow-up time. Third, we did not adjust for multiple tests, simply because this was an exploratory study with a limited study power. We plan to confirm current findings in our ongoing prospective expansion studies in more stringent conditions with a larger study population. Fourth, since treatment of melanoma, advanced melanoma in particular, has not been standardized, patients included in the current analysis who developed advanced melanoma may have received a wide variety of systemic therapies, often sequentially. The systemic therapies available for the cohort of patients included in our analysis would only have been expected to be modestly effective in a minority of melanoma patients (the study period ended in 2008; vemurafenib was approved by the FDA for the treatment of advanced melanoma in 2011). Because of the variety of treatments administered (often multiple types of treatments to the same patient) and their very modest anticipated effect on overall survival, we did not evaluate the potential role of these therapies in the outcomes of the patients, or their potential relationship to the polymorphisms identified. While evaluation of the association between the polymorphisms investigated and response to a variety of melanoma systemic therapies is important, such an evaluation is beyond the scope of the current analysis.

In summary, we identified four SNPs of the NER genes (i.e., *XPE* rs28720291, *ERCC5* rs4150314, *XPC* rs2470458 and *ERCC2* rs50871) that may have independent or joint effects on survival of melanoma patients. These findings, once validated in future prospective studies with large sample sizes and better study designs, will provide some promising guidance for clinical management and tailored or personalized therapeutics in treating melanoma patients.

MATERIALS AND METHODS

Study Populations

Patients were accrued for an ongoing, hospital-based, case–control study of epidemiologic and genetic risk factors for melanoma. A total of 1042 histologically-confirmed patients with melanoma *in situ* and stage I to stage IV were enrolled between January, 2000 and September, 2008. Patients were enrolled into the study regardless of age, sex or disease stage. On entry into the study, each patient had a personal interview to elicit lifestyle factors, using a standardized questionnaire. Each patient also had a 30-mL sample of blood drawn for various biomarker studies, including genotyping. All patients were enrolled and diagnosed with staging system defined by the AJCC at The University of Texas M. D. Anderson Cancer Center, Houston, TX. Specifically, melanoma patients with melanoma *in situ*, stage I/II (primary tumor without evidence of regional or distant metastasis at diagnosis), stage III (locoregional disease, including in transit, satellite, and/or regional lymph node metastasis at diagnosis), and stage IV (distant metastasis at diagnosis) were classified according to the AJCC melanoma staging system (Balch *et al*., 2009). For patients with stage I/II disease, staging elements included Breslow primary tumor thickness, presence or absence of primary tumor ulceration, and mitotic rate (i.e., number of mitoses per square millimeter using dermal hotspot approach). All patients gave a written informed consent, and the protocol was approved by the M. D. Anderson Cancer Center Institutional Review Board. Patients were evaluated, staged, treated and followed using the standard guidelines, including the use of sentinel lymph node biopsy for high-risk primary melanoma (Gershenwald and Ross, 2011). Patients with high-risk local-regional, and those with recurrent and metastatic melanoma, received a variety of protocol-based and off-protocol systemic therapies, based on standard guidelines, physician recommendations, and patient preferences. The study protocol and informed consent were in compliance with Declaration of Helsinki Principles.

Polymorphism selection and genotyping

Genomic DNA was extracted from the buffy coat fraction of each blood sample by using a Blood Mini Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. DNA purity and concentrations were determined by spectrophotometric measurement of absorbance at 260 and 280 nm by a UV spectrophotometer (Nano Drop Technologies, Inc., Wilmington, DE). These 1042 patients complete set of selected SNPs was genotyped using the Illumina HumanOmni1-Quad_v1-0_B array and was called using the BeadStudio algorithm, at the John Hopkins University Center for Inherited Disease Research (CIDR). In this analysis, we selected the available 74 tagging SNPs in 8 core NER genes, including *XPA* (rs1800975 and rs2808667), *XPC* (rs1350344, rs2227999, rs2228000, rs2228001, rs2470458, rs2607772, rs2733533, rs2733537, rs3731062, rs3731125, rs3731127, rs3731146, rs3731149, and rs3731151), *XPE* (rs2230356, rs4939513, and rs28720291), *ERCC1* (rs11615, rs1007616, rs2298881, and rs3212955), *ERCC2* (rs13181, rs50871, rs171140, rs238406, rs238416, rs1052555, rs1618536, rs1799786, rs1799787, and rs1799793), *ERCC3* (rs1566823, rs1803541, rs4150403, rs4150436, rs4150496, rs4150523, rs4662718, and rs9282675), *ERCC4* (rs254942, rs1799801, rs1800067, rs1800124, rs2276464, rs2276465, rs2276466, rs3136146, rs3136166, rs3136187, rs3136189,

rs3136195, rs3743538, and rs16963255), and *ERCC5* (rs17655, rs751402, rs873601, rs1047768, rs1047769, rs2227869, rs2296147, rs2296148, rs4150260, rs4150275, rs4150314, rs4150330, rs4150339, rs4150342, rs4150355, rs4150383, rs4771436, rs8002276, and rs11069498). Any SNP with a call rate lower than 95% was excluded from further analysis.

Statistical Analysis

We used the Cox proportional hazards regression model to evaluate the effect of genotypes and clinicopathological variables on overall survival (OS), calculated as hazard ratios (HRs) with their corresponding 95% confidence intervals (CIs). We performed a stepwise conditional logistic regression analysis to explore the best model to predict the survival outcome. The survival time was calculated from the first day of diagnosis until the date of event or the last-known follow-up. All HRs were adjusted for age, sex, tumor stage, tumor Breslow thickness, ulceration of tumor, tumor cell mitotic rate, involvement of lymph node, and primary tumor anatomic site. Kaplan-Meier analysis was used to evaluate the effect of clinicopathological and genetic variables on the cumulative probability of overall survival. All reported P values were two-sided, and $P < 0.05$ was considered to indicate statistical significance. All analyses were performed using SAS software (version 9.1; SAS Institute, Cary, NC).

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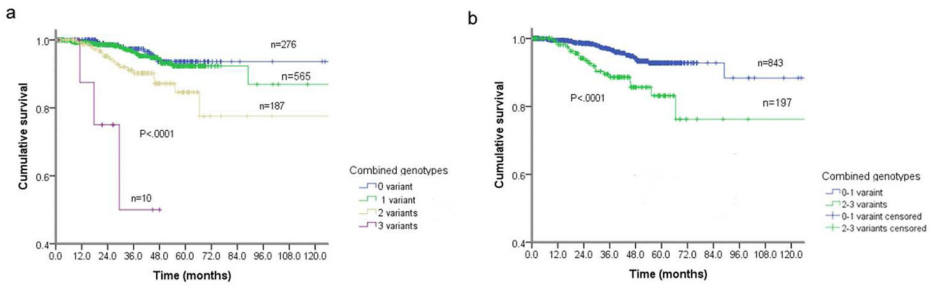

Fig. 1.

Kaplan-Meier overall survival for patients with primary melanoma by combined NER genotypes (i.e., rs28720291 GG, rs4150314 GG, rs2470458 AG+GG, and rs50871 AC+CC). (a) By 0, 1, 2,3 NER variant genotypes ($P < 0.0001$); and (b) By 0–1 and 2–3 NER variant genotypes ($P = 0.0001$).

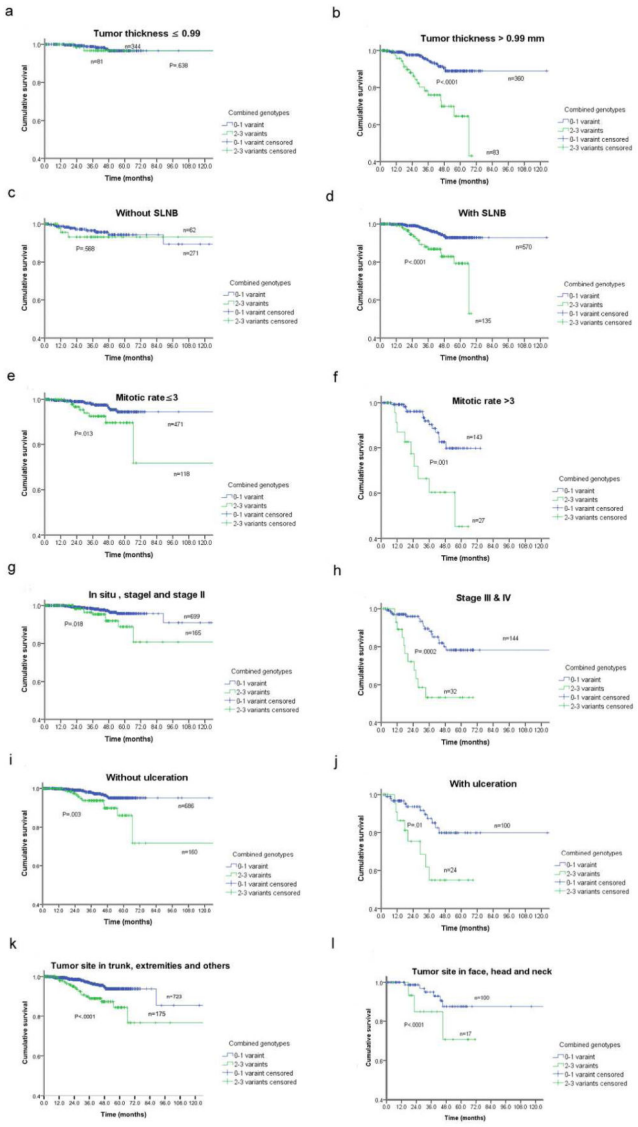

Fig. 2.

Kaplan-Meier overall survival curves for patients with primary melanoma of 0–1 and 2–3 NER variant genotypes (i.e., rs28720291 GG, rs4150314 GG, rs2470458 AG+GG, and rs50871 AC+CC) by tumor related characters: (a) and (b) By tumor Breslow thickness ($P < 0.0001$); (c) and (d) By SLNB ($P < 0.0001$); (e) and (f) By mitotic rate ($P < 0.0001$); (g) and (h) By AJCC stages ($P < 0.0001$); (i) and (j). By ulceration ($P < 0.0001$); and (k) and (l). By primary tumor anatomic site ($P < 0.0001$).

Table 1

Associations of patient demographics and tumor related characteristics with overall survival

Parameter[1]	Patient		Death		Univariate Analysis			Multivariate Analysis[2]		
	No.	%	No.	%	HR	95% CI	*P*-value	HR	95% CI	*P-VALUE*
Age										
<=50	481	46.2	14	26.9	1.00			1.00		
>50	561	55.7	38	73.1	2.65	1.43 – 4.89	0.002	1.90	0.86 – 4.18	0.110
Sex										
Female	613	58.8	36	69.2	1.00			1.00		
Male	429	41.2	16	30.8	0.56	0.31 – 1.01	0.053	0.90	0.41 – 2.04	0.825
Skin color										
Fair	938	90.1	47	9.6	1.00			1.00		
Dark and brown	103	9.9	5	90.4	0.98	0.39 – 2.46	0.961	4.55	1.28 – 16.70	0.019
Hair color										
Blond or red	355	34.1	10	19.2	1.00			1.00		
Black or brown	686	65.9	42	80.8	2.13	1.08 – 4.35	0.030	0.86	0.37 – 2.00	0.735
Eye color										
Not blue	604	58.0	29	55.8	1.00			1.00		
Blue	437	42.0	23	44.2	1.08	0.63 – 1.87	0.781	0.62	0.30 – 1.27	0.192
Tanning ability after prolonged sun exposure										
Good (high)	651	63.0	36	69.2	1.00			1.00		
Poor (low)	382	37.0	16	30.8	0.77	0.43 – 1.39	0.39	1.26	0.58 – 2.77	0.560
Life time sunburns with blistering										
0	302	29.0	18	35.3	1.00			1.00		
>=1	738	71.0	33	64.7	0.65	0.37 – 1.15	0.140	0.97	0.47 – 2.01	0.927
Freckling in the sun as a child										
No	600	57.6	17	32.7	1.00			1.00		
Yes	441	42.4	35	67.3	3.33	1.89 – 5.88	<0.0001	2.90	1.33 – 6.33	0.008
Moles										
No	242	23.2	11	21.1	1.00			1.00		
Yes	800	76.8	41	78.9	1.03	0.53 – 2.01	0.925	1.22	0.53 – 2.83	0.639

Parameter[1]	Patient		Death		Univariate Analysis			Multivariate Analysis[2]		
	No.	%	No.	%	HR	95% CI	*P*-value	HR	95% CI	*P-VALUE*
Dysplastic nevi										
No	939	90.1	51	98.1	1.00			1.00		
Yes	103	9.9	1	1.9	0.18	0.02 – 1.27	0.085	0.56	0.07 – 4.24	0.575
Family history of skin cancer										
No	385	36.9	19	36.5	1.00			1.00		
Yes	657	63.1	33	63.5	1.10	0.62 – 1.93	0.745	1.15	0.56 – 2.37	0.704
AJCC stages										
IN SITU, I and II	866	83.1	24	46.1	1.00			1.00		
III and IV	176	16.9	28	53.9	6.87	3.97 – 11.9	<0.0001	5.60	2.69 – 11.64	<0.0001
Primary tumor thickness										
<1 mm	425	49.0	8	17.0	1.00			1.00		
>0.99 mm	443	51.0	39	83.0	5.17	2.42 – 11.1	<0.0001	2.14	0.72 – 6.35	0.169
Ulceration										
No	846	87.2	28	59.6	1.00			1.00		
Yes	124	12.8	19	40.3	5.71	3.19 – 10.2	<.00001	2.72	1.28 – 5.78	0.0009
SLNB										
No	333	32.1	13	25.5	1.00			1.00		
Yes	705	67.9	38	74.5	1.31	0.69 – 2.49	0.417	0.44	0.19 – 1.03	0.058
Mitotic rate (mitoses/mm^2)										
≤ 3	589	78.6	20	47.6	1.00			1.00		
> 3	160	21.4	22	52.4	4.70	2.56 – 8.63	<0.0001	1.54	0.74 – 3.23	0.251
Primary tumor anatomic site										
Face, head, and neck	117	11.5	9	17.6	1.00			1.00		
Trunk, extremities, and others	902	88.5	42	82.4	1.75	0.85 – 3.61	0.152	0.52	0.17 – 1.57	0.244

Abbreviations: HR, hazard ratio; CI, Confidence Interval.

[1]The numbers of subjects in some of the strata were less than the total number of subjects included in our study, because some subjects did not provide complete information in their screening questionnaires

[2]Multivariate Cox regression analyses were adjusted for all factors listed in Table 1.

Table 2

Predictors of overall survival in melanoma patients obtained from stepwise multivariate cox regression analysis of selected variables[1]

Selected variables	*P*-value	HR	95% CI
Age (≤50 vs. >50)	0.003	1.05	1.01 – 1.07
Stage (*IN SITU*, I, II vs. III, IV)	<0.0001	6.34	3.11 – 11.9
Ulceration (no vs. yes)	0.013	2.52	1.25 – 5.35
Mitotic rate (≤3 vs. >3)	0.012	2.55	1.06 – 4.61
rs28720291 (GG vs. AG)	0.004	6.69	1.83 – 23.7
rs4150314 (GG vs. AG)	0.017	6.15	1.46 – 28.5
rs2470458 (AG+GG vs. AA)	0.025	2.42	1.08 – 4.76
rs50871 (AC+CC vs. AA)	0.020	2.23	1.18 – 4.50

Abbreviations: HR, hazard ratio; CI, Confidence Interval.

[1] Age, sex, tumor Breslow thickness, tumor stages, ulceration of the tumor, tumor cell mitotic rate, involvement of lymph nodes, primary tumor anatomic site and the 74 selected SNPs of the 8 NER core genes [i.e., *XPA* (rs1800975 and rs2808667), *XPC* (rs1350344, rs2227999, rs2228000, rs2228001, rs2470458, rs2607772, rs2733533, rs2733537, rs3731062, rs3731125, rs3731127, rs3731146, rs3731149, and rs3731151), *XPE* (rs2230356, rs4939513, and rs28720291), *ERCC1* (rs11615, rs1007616, rs2298881, and rs3212955), *ERCC2* (rs13181, rs50871, rs171140, rs238406, rs238416, rs1052555, rs1618536, rs1799786, rs1799787, and rs1799793), *ERCC3* (rs1566823, rs1803541, rs4150403, rs4150436, rs4150496, rs4150523, rs4662718, rs9282675), *ERCC4* (rs254942, rs1799801, rs1800067, rs1800124, rs2276464, rs2276465, and rs2276466, rs3136146, rs3136166, rs3136187, rs3136189, rs3136195, rs3743538, and rs16963255), and *ERCC5* (rs17655, rs751402, rs873601, rs1047768, rs1047769, rs2227869, rs2296147, rs2296148, and rs4150260, rs4150275, rs4150314, rs4150330, rs4150339, rs4150342, rs4150355, rs4150383, rs4771436, rs8002276, and rs11069498)] genotypes were included in the stepwise multivariate Cox proportional hazards regression analysis.

Table 3

Association between selected NER genetic variants[1] and overall survival of melanoma patients

Genotypes[1]	Patient		Death		Univariate Analysis			Multivariate Analysis[†]		
	No.	%	No.	%	HR	95% CI	*P*-value	HR	95% CI	*P*-value
XPE										
rs28720291										
GG	1019	97.8	48	92.3	1.00			1.00		
AG	23	2.2	4	7.7	4.92	1.77 – 13.7	0.002	11.2	3.04 – 40.9	0.0003
ERCC5										
rs4150314										
GG	1021	98.2	50	96.1	1.00			1.00		
AG	19	1.8	2	3.9	2.39	0.58 – 9.83	0.227	4.76	1.09 – 20.8	0.038
XPC										
rs2470458										
AA	646	62.1	37	71.1	1.00			1.00		
AG	345	33.1	12	23.1	0.60	0.31 – 1.15	0.126	0.42	0.19 – 0.92	0.031
GG	50	4.8	3	5.8	1.06	0.33 – 3.43	0.927	0.89	0.21 – 3.78	0.879
AG+GG	395	37.9	15	28.9	0.66	0.36 – 1.20	0.175	0.47	0.23 – 0.97	0.040
AG+GG	395	37.9	15	28.9	1.00			1.00		
AA	646	62.1	37	71.1	1.52	0.83 – 2.77	0.172	2.11	1.03 – 4.33	0.040
ERCC2										
rs50871										
AA	286	27.4	23	44.2	1.00			1.00		
AC	529	50.8	18	34.6	0.41	0.22 – 0.76	0.005	0.45	0.22 – 0.90	0.024
CC	227	21.8	11	21.2	0.57	0.28 – 1.17	0.125	0.44	0.17 – 1.15	0.093
AC+CC	756	72.6	29	55.8	0.46	0.27 – 0.79	0.005	0.44	0.23 – 0.85	0.015
AC+CC	756	72.6	29	55.8	1.00			1.00		
AA	286	27.4	23	44.2	2.18	1.26 – 3.77	0.005	2.27	1.18 – 4.35	0.015

Abbreviations: HR, hazard ratio; CI, Confidence Interval.

[*] Only listed the four SNPs from the stepwise multivariate Cox proportional hazards regression analysis model shown in Table 2.

† Adjusted by age, sex, tumor Breslow thickness, tumor stage, ulceration of the tumor, tumor cell mitotic rate, involvement of lymph nodes, and primary tumor anatomic site.

Table 4

Association between combined NER variants and overall survival of melanoma patients

No. of variant genotypes[1]	Patient		Death		Univariate Analysis			Multivariate Analysis[2]		
	No.	%	No.	%	HR	95% CI	*P*-value	HR	95% CI	*P*-value
No. of variant genotypes[1]							0.0005			<0.00001
0	276	26.5	9	17.3	1.00			1.00		
1	566	54.4	23	44.2	1.28	0.59 – 2.78	0.525	1.26	0.51 – 3.10	0.061
2	188	18.1	17	32.7	2.94	1.31 – 6.59	0.009	3.90	1.50 – 10.1	0.005
3	10	1.0	3	5.7	17.8	4.77 – 66.3	<0.0001	34.3	7.48 – 157.2	<0.0001
								P_{trend} <0.0001		
Combined group										
0–1	844	81.0	32	61.5	1.00			1.00		
2–3	198	19.0	20	38.5	2.10	1.42 – 3.12	0.0002	4..01	2.04 – 7.86	<0.0001

Abbreviations: HR, hazard ratio; CI, Confidence Interval.

[1] rs28720291 AG, rs4150314 AG, rs2470458 AA, and rs50871AA.

[2] Multivariate Cox proportional Hazards regression analysis with adjustment for age, sex, tumor Breslow thickness, tumor stage, ulceration of the tumor, tumor cell mitotic rate, involvement of lymph nodes, and primary tumor anatomic site.