ERCC5 p.Asp1104His and *ERCC2* p.Lys751Gln Polymorphisms Are Independent Prognostic Factors for the Clinical Course of Melanoma

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Genetic variants in DNA repair enzymes contribute to the susceptibility to cutaneous melanoma; consequently, we analyzed whether common nonsynonymous single-nucleotide polymorphisms in DNA repair enzyme genes might also influence the course of disease. To this end, we determined eight polymorphisms of seven different DNA repair enzymes in 742 patients with cutaneous melanoma, and correlated these with overall survival. Univariate Cox proportional hazards model analyses revealed that *ERCC5* (*XPG*) 1104 His/His was significantly associated with impaired survival. Indeed, the univariate hazard ratio (HR) was 2.8 times higher for patients with *ERCC5* 1104 His/His ($P<0.001$) compared with *ERCC5* 1104 Asp/Asp. Accordingly, the 5-year survival rate was 55% (95% confidence interval 43–71) for patients with *ERCC5* 1104 His/His, whereas 82% (95% confidence interval 78–86) of patients with *ERCC5* 1104 Asp/Asp were still alive at this time. Importantly, adjusted Cox regression analysis not only confirmed *ERCC5* 1104 His/His as an independent prognostic factor (multivariate HR = 4.5; $P<0.001$), but also revealed the significant impact of *ERCC2* (*XPD*) 751 Gln/Gln on prognosis, with a 2.2-fold increased HR compared with *ERCC2* 751 Lys/Lys ($P=0.009$). Thus, *ERCC5* codon 1104 and *ERCC2* codon 751 polymorphisms are independent prognostic factors in patients with cutaneous melanoma.

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INTRODUCTION

Malignant melanoma is an aggressive cancer with a high mortality rate once metastasized. One of the major risk factors for melanoma is sun exposure (Halpern and Altman, 1999). Notably, UV radiation causes various kinds of DNA damage. UVB, i.e., 280–315 nm, provokes DNA damage by cyclobutane pyrimidine dimers and pyrimidine photoproducts, whereas wavelengths of 315–400 nm, i.e., UVA, cause single-strand breaks, DNA–protein crosslinking, and generation of free oxidative radicals (Pfeifer *et al.*, 2005). As unrepaired DNA damage can either result in apoptosis or DNA aberrations leading to unregulated cell growth and cancer, cells are endued with various DNA repair pathways that are activated upon DNA damage.

In order to maintain the integrity of the genome, at least four different pathways of DNA repair operate on specific types of damaged DNA. For example, base excision repair (BER) corrects small DNA lesions such as oxidized or reduced bases, as well as fragmented or nonbulky adducts. The nucleotide excision repair (NER) pathway repairs bulky lesions such as pyrimidine dimers, larger chemical adducts, or DNA crosslinks. In addition, at least two pathways to repair double-strand breaks exist, i.e., the homologous recombination pathway and the nonhomologous end-joining repair pathway (reviewed in Goode *et al.*, 2002).

As maintenance of DNA integrity is important to prevent carcinogenesis, genes encoding DNA repair molecules are prime candidates for cancer-susceptibility genes (Bartsch *et al.*, 2007). Indeed, several single-nucleotide polymorphisms (SNPs) in these genes have been implicated in increased cancer susceptibility (Kiyohara and Yoshimasu, 2007; Naccarati *et al.*, 2007): e.g., *ERCC2* (*XPD*) 751 Gln and *XRCC3* 241 Met are both associated with an increased risk to develop cutaneous melanoma (Winsey *et al.*, 2000; Li *et al.*, 2006a). However, genetic variation in DNA repair enzymes might also affect the clinical course of cancer by, e.g., affecting genetic stability (Umar and Kunkel, 1996; Kloor *et al.*, 2010). Indeed, it has been recently reported that

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Abbreviations: BER, base excision repair; ERCC5, excision repair cross-complementing rodent repair deficiency, complementation group 5; HR, hazard ratio; NER, nucleotide excision repair; NBN, Nijmegen break syndrome mutated gene; SNP, single-nucleotide polymorphism; XP, xeroderma pigmentosum complementation group; XRCC, X-ray repair complementing defective repair in Chinese hamster cells

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primary melanomas overexpressing DNA repair genes are characterized by an impaired prognosis (Kauffmann *et al.*, 2008). The authors hypothesized that tumor cells in the process of metastasis try to replicate in a fast and error-free mode to maintain those genetic aberrations associated with a growth advantage. To further extent this notion, we scrutinized the prognostic impact of polymorphic variation in DNA repair genes on the course of melanoma by analyzing eight SNPs in a cohort of 742 melanoma patients. All of the addressed SNPs are nonsynonymous. The included SNPs were *xeroderma pigmentosum complementation group C* (*XPC*) p.Ala499Val and *XPC* p.Lys939Gln, *excision repair cross-complementing rodent repair deficiency, complementation group 2* (*ERCC2*; alias *XPD*) p.Lys751Gln and *ERCC5* (*XPG*) p.Asp1104His all involved in NER, *APEX1* (*APEX nuclease (multifunctional DNA repair enzyme) 1*) p.Asp148Glu and *X-ray repair complementing defective repair in Chinese hamster cells 1* (*XRCC1*) p.Arg399Gln both participating in BER and *XRCC3* p.Thr241Met and *Nijmegen break syndrome mutated gene* (*NBN*; *NBS1*) p.Glu185Gln, i.e., molecules of the homologous recombination repair pathway. This analysis revealed that *ERCC5* p.Asp1104His and *ERCC2* p.Lys751Gln have a significant and independent impact on the clinical course of melanoma.

RESULTS

Patient characteristics

Of the 742 patients, 328 were female (44. 2%) and 414 male (55.8%). Superficial spreading melanoma (346 patients) and nodular melanoma (182 patients) were the most common histological diagnoses. The median age at diagnosis of the patient cohort was 54.8 years and the median follow-up time was 74.2 months. A total of 226 patients died during the follow-up period. The detailed patient and tumor characteristics are presented in Table 1.

Genotyping frequencies of polymorphisms and survival

The allelic and genotypic frequencies of SNPs are given in Table 2. Notably, from the majority of samples the genotype could be determined; only 4 to 12.9%, with an average of ~7.6%, of samples did not give a distinct result. The observed genotype frequencies were in the range of the frequencies reported on the respective SNP database websites of the National Center for Biotechnology Information. From the analyzed SNPs only *XPC* p.Lys939Gln and *XPC* p.Ala499Val were statistically significant in linkage disequilibrium after Bonferroni–Holmes adjustment ($P<0.001$; $D'=0.906$; $r^2=0.19$). This linkage disequilibrium for the two *XPC* SNPs has been published previously (Huang *et al.*, 2006).

The effect of each polymorphism on overall survival was estimated by the Kaplan–Meier method, revealing that *ERCC5* p.Asp1104His has a significant impact on overall survival ($P<0.001$; log-rank test): *ERCC5* 1104 His/His was associated with a highly impaired prognosis compared with *ERCC5* 1104 His/Asp or Asp/Asp (Figure 1). This observation was confirmed by the 5-year survival rate (Table 3): 153 patients died during the first 5 years after diagnosis, and 469 patients had a follow-up time of >60 months. Validated prognostic factors such as age, gender, tumor classification, and clinical stage at diagnosis influenced the 5-year survival significantly, thereby demonstrating that our cohort is representative for the general melanoma population (Balch *et al.*, 2001b). Notably, from the analyzed DNA repair gene SNPs, only *ERCC5* 1104 polymorphisms significantly influenced the 5-year survival rate. Indeed, the survival impact was obvious in both the whole patient population and the gender subgroups. In this regard, the 5-year survival rate was 82% (95% confidence interval 78–86) for *ERCC5* 1104 Asp/Asp versus 56% (95% confidence interval 43–71) for His/His ($P<0.001$; log-rank test).

Hazard ratio (HR) for DNA repair polymorphisms

Next, we calculated the HRs for DNA repair SNPs by univariate and multivariate Cox regression analyses. The univariate analyses confirmed that gender, age, tumor classification, and stage at diagnosis as well as *ERCC5* p.Asp1104His polymorphisms influenced the risk to die from melanoma (Figure 2). The HR for *ERCC5* 1104 His/His was 2.8 times higher than for *ERCC5* 1104 Asp/Asp ($P<0.001$). Additionally, the *ERCC2* p.Lys751Gln polymorphism, for which we observed a trend in the 5-year survival analysis, significantly influenced prognosis: patients with *ERCC2* 751 Gln/Gln had a 1.7 times increased risk to die compared with *ERCC2* 751 Lys/Lys patients ($P=0.035$). To test whether *ERCC5* p.Asp1104His and *ERCC2* p.Lys751Gln are independent prognostic factors, we performed multivariate Cox regression analysis. For this analysis, we adjusted each SNP to each other as well as to the known prognostic parameters gender, age, and stage at diagnosis. In order to decrease the number of patient subgroups, stage IIB and IIC as well as stage IIIB and IIIC patients were grouped. These analyses confirmed *ERCC5* 1104 His/His (HR = 4.5; $P<0.001$) and *ERCC2* 751 Lys/Lys (HR = 2.2; $P=0.009$) as independent prognostic factors for overall survival of cutaneous melanoma patients (Figure 3). All results of the multivariate analyses were confirmed by bootstrapping, i.e., analysis of randomly selected patient cohorts.

DISCUSSION

DNA repair is essential to ensure integrity and stability of the genome. Genetic instability is responsible for the cellular changes that confer progressive transformation on cancerous cells; thus, defects in DNA repair can be expected to promote cancer. Indeed, patients with XP, an autosomal recessive disease associated with defective NER, have a 1,000-fold higher susceptibility for skin cancer (Kraemer *et al.*, 2007). The ability to repair DNA damages, however, also varies to a considerable extent within the normal human population. These variations could be a consequence of common polymorphic amino-acid substitutions in DNA repair genes (Mohrenweiser *et al.*, 2003). Consequently, many studies have been performed to reveal possible associations between SNPs in DNA repair genes and susceptibility to cancer (Berwick and Vineis, 2000; Goode *et al.*, 2002). Most studies, however, do not address whether such variations in DNA repair impact on the clinical course of cancer. Several lines of

Table 1. Patient characteristics

Variables	Total	Women	Men
Median (IQR) age at diagnosis	54.8 (42.9–66.0) (*n*=742)	53.7 (40.2–65.9) (*n*=328)	55.8 (44.8–66.1) (*n*=414)
Median (IQR) age at death	61.0 (49.0–71.9) (*n*=226)	58.4 (48.0–72.8) (*n*=94)	61.5 (50.6–70.8) (*n*=132)
Median (IQR) follow-up time (months)	74.2 (45.3–114.1) (*n*=742)	80.8 (51.8–116.7) (*n*=328)	70.7 (40.1–109.2) (*n*=414)
Death	226 (30.1%)	94 (28.7%)	132 (31.9%)
Therapy[1]			
Adjuvant IFN	154 (20.8%)	73 (22.3%)	81 (19.6%)
Chemotherapy	213 (28.7%)	89 (27.1%)	124 (30%)
Radiation	84 (11.3%)	37 (11.3)	47 (11.4%)
Immunotherapy	160 (21.6%)	67 (20.4%)	93 (22.5%)
T-classification[2]			
T1a	202 (28.7%)	90 (28.7%)	112 (28.7%)
T1b	13 (1.8%)	4 (1.3%)	9 (2.3%)
T2a	187 (26.6%)	84 (26.8%)	103 (26.4%)
T2b	27 (3.8%)	9 (2.9%)	18 (4.6%)
T3a	126 (17.9%)	66 (21.0%)	60 (15.4%)
T3b	51 (7.2%)	22 (7.0%)	29 (7.4%)
T4a	71 (10.1%)	30 (9.6%)	41 (10.5%)
T4b	27 (3.8%)	9 (2.9%)	18 (4.6%)
Stage at diagnosis[2]			
IA	198 (27.7%)	87 (27.6%)	111 (27.8%)
IB	183 (25.6%)	77 (24.4%)	106 (26.6%)
IIA	134 (18.8%)	65 (20.6%)	69 (17.3%)
IIB	96 (13.4%)	45 (14.3%)	51 (12.8%)
IIC	13 (1.8%)	2 (0.6%)	11 (2.8%)
IIIA	32 (4.5%)	15 (4.8%)	17 (4.3%)
IIIB	44 (6.2%)	18 (5.7%)	26 (6.5%)
IIIC	5 (0.7%)	3 (1.0%)	2 (0.5%)
IV	9 (1.3%)	3 (1.0%)	6 (1.5%)
Histological type			
ALM	44 (6.8%)	19 (6.8%)	25 (6.8%)
LMM	37 (5.7%)	15 (5.3%)	22 (6.0%)
NM	182 (28.2%)	77 (27.4%)	105 (28.8%)
SSM	346 (53.6%)	154 (54.8%)	192 (52.6%)
Other	12 (1.9%)	6 (2.1%)	6 (1.6%)
Nonclassifiable	25 (3.9%)	10 (3.6%)	15 (4.1%)

Abbreviations: ALM, acral lentiginous melanoma; IFN, interferon; IQR, interquartile range; LMM, lentigo maligna melanoma; NM, nodular melanoma; SSM, superficial spreading melanoma.
[1]Number of patients receiving the respective therapies.
[2]T-classification of primary tumor as well as pathological staging at diagnosis was performed according to the American Joint Committee on Cancer (AJCC) Classification from 2002 (Balch *et al.*, 2001a).

Table 2. Results of SNP determination

SNP	Allele (AA)	Allele frequency	Genotypes	Patients (frequency)	Women (frequency)	Men (frequency)
APEX1 p.Asp148Glu	T (Asp)	0.52	TT	189 (28.0)	70 (23.4)	119 (31.5)
(rs1130409)	G (Glu)	0.48	TG	324 (47.9)	155 (51.8)	169 (44.7)
			GG	164 (24.2)	74 (24.7)	90 (23.8)
ERCC2 p.Lys751Gln	A (Lys)	0.63	AA	285 (40.2)	122 (38.4)	163 (41.4)
(rs13181)	C (Gln)	0.37	AC	329 (46.4)	148 (46.5)	181 (45.9)
			CC	98 (13.8)	48 (15.1)	50 (12.7)
ERCC5 p.Asp1104His	G (Asp)	0.78	GG	449 (63.1)	204 (63.9)	245 (62.3)
(rs17655)	C (His)	0.22	GC	206 (28.9)	91 (28.5)	115 (29.3)
			CC	57 (8.0)	24 (7.5)	33 (8.4)
NBN p.Glu185Gln	G (Glu)	0.69	GG	329 (48.5)	146 (48.0)	183 (48.8)
(rs1805794)	C (Gln)	0.31	GC	282 (41.5)	120 (39.5)	162 (43.2)
			CC	68 (10.0)	38 (12.5)	30 (8.0)
XPC p.Ala499Val	C (Ala)	0.74	CC	389 (57.1)	168 (55.3)	221 (58.6)
(rs2228000)	T (Val)	0.26	CT	236 (34.7)	104 (34.2)	132 (35.0)
			TT	56 (8.2)	32 (10.5)	24 (6.4)
XPC p.Lys939Gln	A (Lys)	0.6	AA	238 (36.8)	108 (37.4)	130 (36.4)
(rs2228001)	C (Gln)	0.4	AC	295 (45.7)	129 (44.6)	166 (46.5)
			CC	113 (17.5)	52 (18.0)	61 (17.1)
XRCC1 p.Arg399Gln	G (Arg)	0.66	GG	303 (44.1)	139 (44.8)	164 (43.5)
(rs25487)	A (Gln)	0.34	GA	302 (44.0)	133 (42.9)	169 (44.8)
			AA	82 (11.9)	38 (12.3)	44 (11.7)
XRCC3 p.Thr241Met	C (Thr)	0.62	CC	283 (40.7)	118 (37.6)	165 (43.2)
(rs861539)	T (Met)	0.38	CT	300 (43.1)	142 (45.2)	158 (41.4)
			TT	113 (16.2)	54 (17.2)	59 (15.4)

Abbreviations: AA, amino acid; APEX1, APEX nuclease (multifunctional DNA repair enzyme) 1; ERCC5, excision repair cross-complementing rodent repair deficiency, complementation group 5; NBN, Nijmegen break syndrome mutated gene; SNP, single-nucleotide polymorphism; XPC, xeroderma pigmentosum complementation group C; XRCC, X-ray repair complementing defective repair in Chinese hamster cells.

evidence suggest that this may be the case: for melanoma, genomic instability is not only increased in primary tumors when compared with nevi, but also in metastases when compared with primary tumors (Chin *et al.*, 2006); similarly, hypoxia, which is associated with an aggressive phenotype of cancer, mediates among other changes genetic instability (Bristow and Hill, 2008). Thus, we scrutinized whether common nonsynonomous SNPs in DNA repair genes are related to the progression of melanoma in a cohort of >700 melanoma patients for whom detailed information and a close follow-up was available. The majority of the analyzed SNPs were selected as they are among the most studied DNA repair gene SNPs for melanoma susceptibility (Li *et al.*, 2006a, b; Mocellin *et al.*, 2009). In addition, the *NBN* SNP was included as germline mutations have been described for melanoma (Steffen *et al.*, 2004). Our study revealed that *ERCC5* p.Asp1104His and *ERCC2* p.Lys751Gln are independent prognostic factors for the clinical course of melanoma. Interestingly, although *ERCC2* SNP has been described as melanoma susceptibility SNP in one study (Li et al., 2006a), a large study of almost 1,200 melanoma patients as well as a review of the literature could not reveal an impact of these two SNPs on the susceptibility for melanoma (Figl *et al.*, 2010).

The number of studies addressing the association between SNPs in DNA repair genes and the course of neoplastic diseases is surprisingly low, particularly if compared with those addressing cancer susceptibility. This is probably because of the fact that the diagnosis of cancer itself is more widely available than the detailed medical history. Consequently, studies addressing SNPs as prognostic factors are largely characterized by a low patient number. For example, in a recent study scrutinizing three DNA repair SNPs, i.e., *XRCC1* p.Arg399Gln, *ERCC2* p.Lys751Gln, and *ERCC1* p.Thr118Cys, and their association with survival in stage IV melanoma patients receiving biochemotherapy, only 90 patients were included (Liu *et al.*, 2005). This analysis, however, revealed that the *ERCC1* polymorphism was weakly associated with overall survival, and the Gln allele of the *ERCC2* polymorphism was unfavorable without reaching significance. In contrast to our study, Liu *et al.* (2005) searched for a predictive marker, whereas we addressed

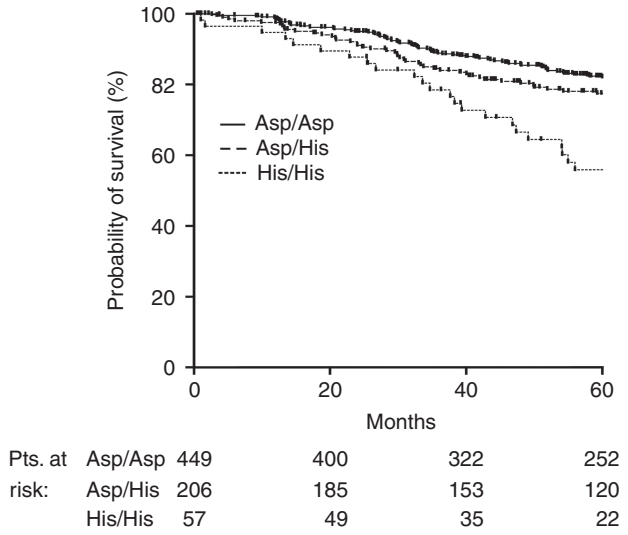

Figure 1. *ERCC5* (*excision repair cross-complementing rodent repair deficiency, complementation group 5*) p.Asp1104His polymorphism influences prognosis. Kaplan–Meier survival estimation for 712 melanoma patients stratified to *ERCC5* p.Asp1104His polymorphism ($P<0.0001$; log-rank test). The patients (Pts.) at risk are depicted in 20-month intervals.

prognostic markers for melanoma. Moreover, the study of Liu *et al.* (2005) is likely to be underpowered to reach a significant result for *ERCC2*. Interestingly, *ERCC2* p.Lys751Gln was suggested to be a prognostic factor for melanoma progression, as in 244 Swedish patients the Lys/Gln phenotype was more frequent in patients with advanced melanoma (Kertat *et al.*, 2008). In a previous study of 400 melanoma patients, Figl *et al.* (2009) reported XRCC1 p.Arg399Gln to be associated with prognosis: patients with *XRCC1* Gln/Gln demonstrated a median overall survival of 24.4 years compared with 11.5 years for the two other genotypes. In the same study, however, this association was not detectable in an independent Spanish patient cohort selected by the same criteria, i.e., only patients first diagnosed at stage I or II. The authors concluded that this may have been because of the fact that only a few deaths were recorded for this cohort. In our study, although the independent patient cohort reflects the whole variety of tumor stages at diagnosis translating into a worse survival rate, we were not able to confirm the previous results. This observation demonstrates the importance of conducting multiple studies on potential biomarkers. Alternatively, supportive evidence might come from more frequent cancer entities analyzing the impact of variations in DNA repair on the course of disease. For example, in a large study of lung cancer patients, the authors detected 15 nonsynonomous SNPs associated with prognosis (Matakidou *et al.*, 2007); these primarily mapped to the NER and BER repair pathways, and *ERCC5* p.Asp1104His was among the most significant ones. Thus, *ERCC5* p.Asp1104His seems to be associated with prognosis in different cancer entities. Notably, in contrast to a previous report by Di Lucca *et al.* (2010), we did not find an association between *ERCC5* p.Asp1104His—nor any other of our analyzed—genotypes and Breslow thickness. Indeed, in multivariate analysis with tumor class instead of tumor stage, we obtained the same results (data not shown).

To date, all SNPs associated with prognosis belong to the NER and BER pathways. This notion is further corroborated by the observation that in early small cell lung cancer patients a higher expression of DNA synthesis and repair enzymes *RRM1* and *ERCC1* in the tumors was associated with a better overall survival (Zheng *et al.*, 2007). However, the picture is getting more complex as it has recently been demonstrated that primary melanomas with poor prognosis overexpress DNA repair genes (Kauffmann *et al.*, 2008). Indeed, the majority of these overexpressed genes code for proteins involved in rescuing stalled DNA replication forks, DNA double-strand break/interstrand crosslink repair, and telomere maintenance. Similar results were obtained in a larger study, in which overexpression of DNA repair genes and genes involved in cell cycle progression was observed in samples of relapsing compared with nonrelapsing patients (Jewell *et al.*, 2010). Thus, it seems that a defective or impaired DNA repair system can contribute to the initiation of tumors, whereas in the tumor stage patients whose tumors express larger amounts of DNA repair enzymes present with a faster relapse. This overexpression of DNA repair genes might allow the fast-growing cancer cells to replicate more correctly and thereby to prevent severe, i.e., deleterious, DNA damage. Somehow surprisingly, however, metastatic sites often demonstrate higher levels of genetic instability (Balazs *et al.*, 2001), and in mice the metastatic potential of tumor cells is associated with increasing genetic instability (Cifone and Fidler, 1981). As it is known that E2F integrates cell cycle progression with DNA repair (Ren *et al.*, 2002), overexpression of DNA repair genes can at least in part be ascribed to increased proliferation.

The precise mechanism of how the *ERCC5* and *ERCC2* SNPs influence overall survival in cutaneous melanoma patients remains elusive; as always for disease-associated SNPs, the cancer phenotype could be altered in two ways: either directly by altering the DNA repair capacity of the tumor cell or indirectly by being in linkage disequilibrium with other disease-modulating alleles. *ERCC5* and *ERCC2* are core proteins of the NER pathway and therefore play crucial roles in the correction of cyclobutane pyrimidine dimers, 6–4 photoproducts, and bulky adducts induced by chemical agents (Costa *et al.*, 2003). The potential of amino-acid substitutions encoded by SNPs to impact protein structure and activity can be predicted by two different algorithms: i.e., SIFT (Sorting Intolerant from Tolerant) and PolyPhen (Polymorphism Phenotyping). These algorithms are based on sequence conservation over evolutionary time, the physical and chemical properties of the exchanged residues, and/or protein structural domain information. Several benchmarking studies have demonstrated that these algorithms predict protein function very correctly (reviewed in Xi *et al.*, 2004). Both the SIFT and the PolyPhen algorithms estimate the His substitution of Asp at codon 1104 of *ERCC5* as intolerant or possible damaging, whereas the Lys substitution by Gln at codon 751 of *ERCC2* was predicted by both programs as benign. However, functional analysis of the respective

Table 3. The 5-year survival rates

Variables	Total			Women			Men		
	n (at risk/events)[1]	5-year survival rate (95% CI)[2]	*P*-value[3]	*n* (at risk/events)[1]	5-year survival rate (95% CI)[2]	*P*-value[3]	*n* (at risk/events)[1]	5-year survival rate (95% CI)[2]	*P*-value[3]
Gender									
Men	414 (246/98)	76 (72–80)							
Women	328 (223/55)	82 (78–87)	0.013						
Age at onset[4]									
≤45	219 (155/36)	84 (79–89)		113 (79/17)	85 (78–92)		106 (76/19)	83 (75–90)	
46–60	233 (158/44)	81 (76–86)		94 (69/14)	85 (78–93)		139 (89/30)	78 (71–85)	
>60	290 (156/73)	73 (68–78)	0.020	121 (75/24)	78 (70–86)	0.499	169 (81/49)	70 (62–77)	0.044
Tumor classification									
T1a	202 (147/14)	92 (89–96)		90 (65/4)	95 (90–100)		112 (82/10)	90 (85–96)	
T1b	13 (9/1)	92 (78–100)		4 (4/0)	100 (NA)		9 (5/1)	89 (68–100)	
T2a	187 (134/18)	90 (86–95)		84 (64/8)	90 (83–96)		103 (70/10)	91 (85–96)	
T2b	27 (13/12)	56 (37–76)		9 (5/2)	71 (38–100)		18 (8/10)	50 (27–73)	
T3a	126 (84/23)	81 (74–88)		66 (46/12)	82 (72–92)		60 (38/11)	80 (70–91)	
T3b	51 (26/13)	72 (60–85)		22 (15/4)	80 (62–98)		29 (11/9)	67 (49–85)	
T4a	71 (29/37)	50 (38–61)		30 (12/16)	49 (31–67)		41 (17/21)	50 (34–66)	
T4b	27 (8/16)	36 (17–55)	< 0.001	9 (3/4)	47 (10–83)	< 0.001	18 (5/12)	32 (10–54)	< 0.001
Pathological staging									
IA	198 (146/11)	94 (90–97)		87 (64/2)	97 (94–100)		111 (82/9)	91 (86–97)	
IB	183 (132/16)	91 (87–95)		77 (61/6)	91 (85–98)		106 (71/10)	91 (85–97)	
IIA	134 (87/26)	79 (72–86)		65 (46/9)	85 (75–94)		69 (41/17)	74 (64–85)	
IIB	96 (51/30)	69 (60–79)		45 (26/14)	70 (56–84)		51 (25/16)	69 (56–82)	
IIC	13 (6/5)	57 (28–86)		2 (1/0)	100		11 (5/5)	52 (21–83)	
IIIA	32 (16/16)	55 (38–73)		15 (9/6)	67 (43–91)		17 (7/10)	44 (20–68)	
IIIB	44 (11/28)	33 (19–48)		18 (6/10)	40 (16–64)		26 (5/18)	28 (9–44)	
IIIC	5 (0/4)	0 (NA)		3 (0/2)	0 (NA)		2 (0/2)	0 (NA)	
IV	9 (1/8)	11 (0–32)	< 0.001	3 (1/2)	33 (0–87)	<0.001	6 (0/6)	0 (NA)	< 0.001
ERCC5 p.Asp1104His									
GG	449 (296/77)	82 (79–86)		204 (146/27)	86 (81–91)		244 (150/48)	79 (74–85)	
GC	206 (130/46)	77 (72–83)		91 (59/16)	81 (72–89)		115 (71/28)	75 (67–83)	
CC	57 (26/23)	56 (42–70)	<0.001	23 (12/9)	57 (38–80)	0.005	33 (14/14)	53 (35–72)	0.024
ERCC2 p.Lys751Gln									
AA	285 (190/52)	82 (77–86)		122 (81/19)	84 (77–91)		163 (109/33)	80 (74–86)	
AC	329 (203/67)	79 (74–83)		148 (104/22)	85 (79–91)		181 (99/45)	74 (68–81)	
CC	98 (59/27)	72 (62–81)	0.068	48 (32/11)	76 (64–89)	0.468	50 (27/16)	67 (54–80)	0.220
XPC p.Lys939Gln									
AA	238 (150/48)	79 (74–85)		108 (73/19)	82 (75–90)		130 (77/29)	77 (69–84)	
CA	295 (186/64)	78 (73–83)		129 (88/22)	82 (76–89)		166 (98/42)	74 (67–81)	
CC	113 (64/29)	73 (65–82)	0.528	52 (30/11)	73 (61–87)	0.703	61 (34/18)	71 (60–83)	0.725

Table 3 continued on the following page

Table 3. Continued

Variables	Total			Women			Men		
	n (at risk/events)[1]	5-year survival rate (95% CI)[2]	*P*-value[3]	*n* (at risk/events)[1]	5-year survival rate (95% CI)[2]	*P*-value[3]	*n* (at risk/events)[1]	5-year survival rate (95% CI)[2]	*P*-value[3]
XPC p.Ala499Val									
CC	389 (238/83)	78 (73–82)		168 (109/28)	82 (76–88)		221 (129/55)	75 (69–80)	
CT	236 (148/50)	78 (73–84)		104 (75/15)	85 (78–92)		132 (73/35)	73 (65–81)	
TT	56 (38/11)	81 (71–92)	0.970	32 (22/7)	80 (66–94)	0.601	24 (16/4)	83 (67–98)	0.601
XRCC1 p.Arg399Gln									
GG	303 (188/59)	80 (75–84)		139 (87/25)	80 (73–87)		164 (101/34)	79 (73–85)	
GA	302 (188/67)	77 (73–82)		133 (98/21)	85 (79–91)		169 (90/46)	71 (64–78)	
AA	82 (56/13)	83 (74–91)	0.499	38 (27/5)	86 (74–97)	0.622	44 (29/8)	81 (69–93)	0.329
XRCC3 p.Thr241Met									
CC	283 (178/64)	77 (72–82)		118 (79/24)	79 (71–87)		165 (99/40)	75 (69–82)	
CT	300 (191/61)	79 (74–83)		142 (96/24)	82 (76–89)		158 (95/37)	75 (68–82)	
TT	113 (71/21)	82 (74–89)	0.642	54 (40/5)	90 (81–98)	0.193	59 (31/16)	75 (63–86)	0.839
APEX1 p.Asp148Glu									
TT	189 (117/35)	80 (74–86)		70 (43/10)	83 (73–93)		119 (74/25)	78 (70–86)	
TG	324 (209/75)	77 (73–82)		155 (111/29)	82 (75–88)		169 (98/46)	73 (67–80)	
GG	164 (100/31)	80 (74–86)	0.420	74 (49/9)	87 (79–95)	0.549	90 (51/22)	74 (65–83)	0.362
NBN p.Glu185Gln									
GG	329 (204/70)	78 (73–83)		146 (96/27)	80 (74–87)		183 (108/43)	76 (70–82)	
CG	282 (178/57)	79 (74–84)		120 (80/19)	83 (76–90)		162 (98/38)	76 (69–83)	
CC	68 (44/16)	79 (69–89)	0.727	38 (29/5)	89 (78–99)	0.774	30 (15/11)	66 (49–83)	0.139

Abbreviations: AA, amino acid; APEX1, APEX nuclease (multifunctional DNA repair enzyme) 1; CI, confidence interval; ERCC5, excision repair cross-complementing rodent repair deficiency, complementation group 5; NBN, Nijmegen break syndrome mutated gene; SNP, single-nucleotide polymorphism; XPC, xeroderma pigmentosum complementation group C; XRCC, X-ray repair complementing defective repair in Chinese hamster cells.
[1]Given is the total number of patients (*N*) as well as those who were observed for >5 years (at risk) and those who died within 5 years after diagnosis (events).
[2]The 5-year survival rates were determined by Kaplan–Meier estimations that comprise also those patients censored within 5 years.
[3]*P*-values were calculated by the log-rank test.
[4]The patient cohort was divided into three groups according to age at onset in such a way that almost equipollent groups resulted.

amino-acid substitutions is still the gold standard. To this end, we recently demonstrated for XPG Asp1104His that TT = T dimer repair was nonsignificantly lower in heterozygotes compared with the homozygous wild type (Kumar *et al.*, 2003); furthermore, we demonstrated an association between this SNP and the level of single-strand breaks (Vodicka *et al.*, 2004). In contrast, for *ERCC2* 751 variant proteins no difference in NER activity was detectable (Laine *et al.*, 2007). This observation, on one hand, confirms the results of the prediction algorithms and, on the other hand, argues that there is no causal relationship between *ERCC2* p.Lys751Gln polymorphism and reduced DNA repair (Clarkson and Wood, 2005). However, Wolfe *et al.* (2007) recently demonstrated that the minor allele of *ERCC2* 751 is associated with decreased constitutive *ERCC2* mRNA levels caused by an altered mRNA secondary structure. This reduced expression of the mRNA may explain the increased number of DNA adducts in individuals carrying Gln alleles suggesting lower DNA repair capacity in these individuals (reviewed in Benhamou and Sarasin, 2005).

It should be further noted that besides their function in NER, *ERCC2* is involved in transcriptional regulation (Schaeffer *et al.*, 1993), and *ERCC5* acts as a cofactor for a DNA glycosylase; an enzyme that removes oxidized pyrimidines from DNA. Moreover, *ERCC5* has also been involved in both transcription-coupled repair and RNA transcription itself (Clarkson, 2003). The SNPs in these genes may therefore affect not only NER, but also any of these functions.

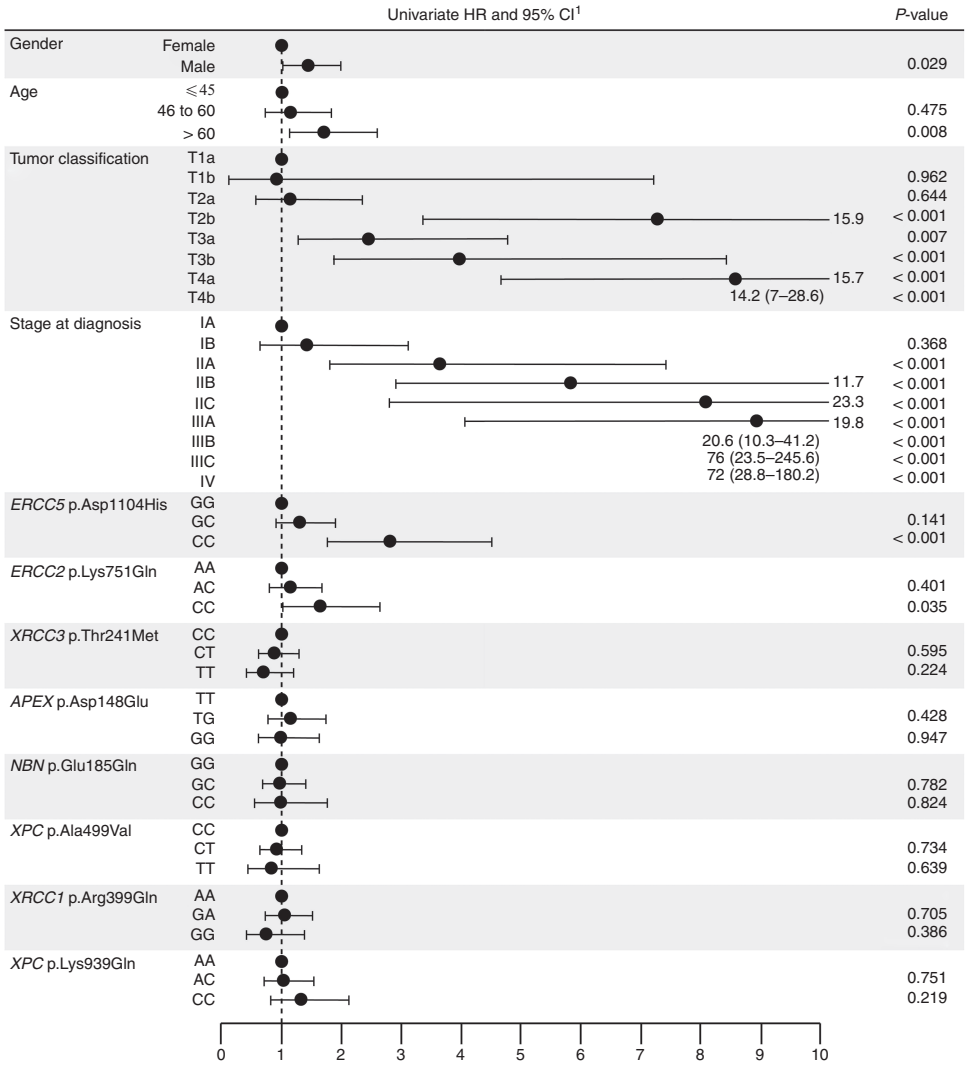

Figure 2. Univariate hazard ratios (HRs) for overall survival. All factors analyzed are depicted. Dots represent the HRs, lines the 95% confidence intervals (CIs). In cases where the HRs and/or the CIs are beyond the depicted range, the values are given. A polymorphism is relevant when the respective CI does not cross the value 1. [1]Patient numbers for each analysis are given in Table 1 and Table 2, respectively.

In conclusion, in this study we identified *ERCC5* p.Asp1104His and *ERCC2* p.Lys751Gln as independent prognostic factors for the clinical course of melanoma. Whether these SNPs impact the prognosis of melanoma directly via alteration of DNA repair efficacy or by other, possibly indirect, mechanisms has to be addressed by future studies. In any case, identification of prognostic factors will help to individualize and optimize medical care of patients; i.e., high-risk patients should receive a closer follow-up and should be considered for adjuvant therapy.

PATIENTS AND METHODS

Patients and sera

Serum samples were selected from frozen serum banks hosted by Skin Cancer Unit, Mannheim and the Department of Dermatology, Wuerzburg. Serum samples to be included in the analysis were chosen randomly but selected to meet the following criteria: (1) histological confirmation of primary cutaneous melanoma, (2) patient of Caucasian origin, and (3) availability of extended information of the medical history, primary tumor characteristics, as well as patient follow-up. The only exclusion criteria were sera from patients with secondary malignancies or *in situ* melanoma. The detailed patient characteristics are given in Table 1. All serum samples were obtained and processed following a standardized protocol. Briefly, blood was drawn into gel-coated serum tubes (Sarstedt, Nuembrecht, Germany) and allowed to clot at room temperature for 30–60 minutes. After centrifugation, the serum phase was harvested and subsequently frozen without any additives at −20 °C. All measures were performed with the approval of the institutional review board after patients' informed consent and in adherence to the Declaration of Helsinki Principles.

Genotyping

DNA was extracted from 200 µl serum of each cryopreserved sample with the QIAamp DNA mini kit (Qiagen, Hilden, Germany) as described by the manufacturer and genotyped for eight different

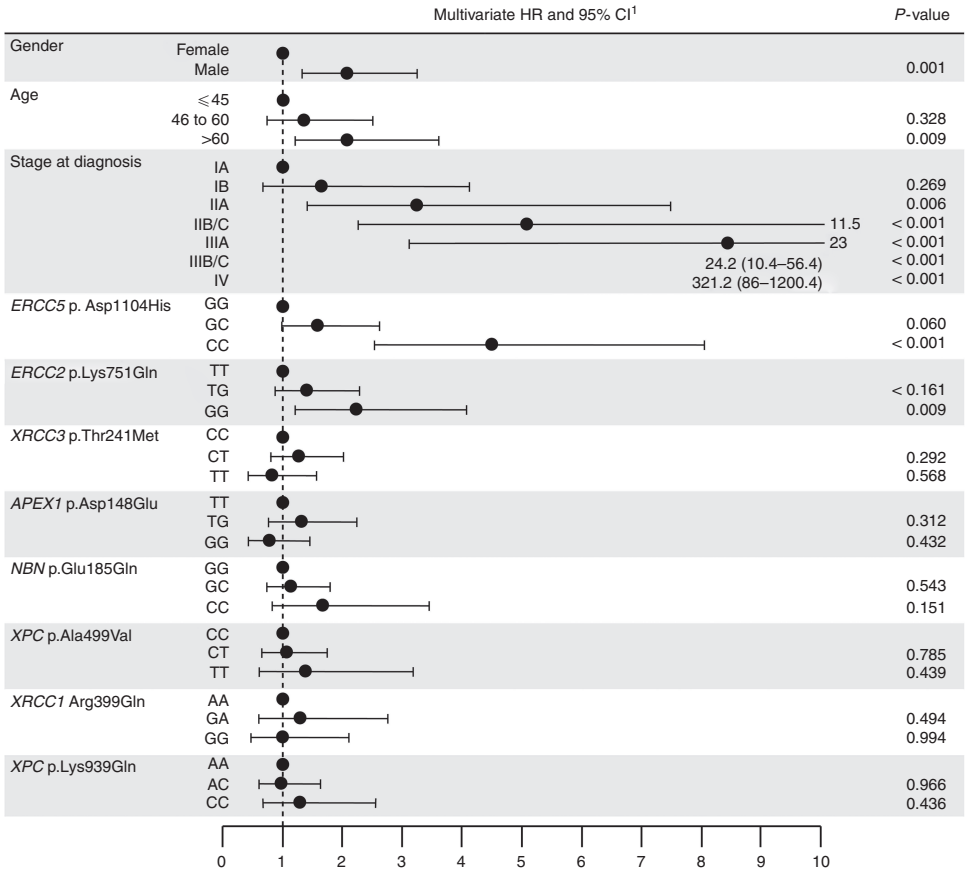

Figure 3. Multivariate hazard ratios (HRs) for overall survival. Besides the eight DNA repair single-nucleotide polymorphisms (SNPs), gender, categorized age, and stage at diagnosis were included. Dots represent the HRs, lines the 95% confidence intervals (CIs). In cases where the HRs and/or the CIs are beyond the depicted range, the values are given. A polymorphism is relevant when the respective CI does not cross the value 1. [1]This analysis is based on 498 patients since 244 patients had to be omitted due to absence of information on primary tumor or missing genotype of at least one of the analyzed SNPs.

SNPs in DNA repair genes including the NER genes *XPC* (A>C; p.Lys939Gln; rs2228001), *XPC* (C>T; p.Ala499Val; rs2228000), *ERCC2* (A>C; p.Lys751Gln; rs13181), and *ERCC5* (G>C; p.Asp1104His; rs17655); the BER genes *APEX1* (T>G; p.Asp148Glu; rs1130409) and *XRCC1* (G>A; p.Arg399Gln; rs25487); and the double-strand break repair genes *XRCC3* (C>T; p.Thr241Met; rs861539) and *NBN* (G>C; p.Glu185Gln; rs1805794). All these polymorphisms included in the study were nonsynonymous and have minor allele frequencies >0.2 (Table 2). Genotyping was performed by the 5′ nuclease allelic discrimination assay in TaqMan technology (Applied Biosystems, Foster City, CA). TaqMan primers and probes were purchased from Applied Biosystems as assays on demand (C_16018061_10 for rs2228001 and C_622564_10 for rs861539) or by own design for all others (Supplementary Table S1 online). PCR was performed in 5 µl volume reaction using 5 ng DNA as template, master mix (Applied Biosystems), and 0.5 × probe/primer mix. The initial temperature conditions for PCR were set at 50 °C for 2 minutes and 95 °C for 10 minutes followed by 40–45 cycles at 92 °C for 15 seconds and 60 °C for 1 minute. Genotyping on amplified PCR products was scored by differences in VIC and 5-carboxyfluorescein, succinimidyl ester (FAM) fluorescent level in plate read operation on ABI PRISM 7900HT sequence detection system (Applied Biosystems) using SDS 1.2 software (Applied Biosystems). Genotyping results from allelic discrimination assays were randomly verified by DNA sequencing. The sequencing reactions were performed using BigDyeR Terminator Cycle Sequencing Kit (Applied Biosystems) in a 10 µl volume containing PCR product pre-treated with ExoSapIT (USB Corporation, Cleveland, OH), 5 × Sequencing buffer (Applied Biosystems), and a sequencing primer. The temperature conditions set for sequencing reactions were 96 °C for 2 minutes followed by 27 cycles at 96 °C for 30 seconds, 54 °C for 10 seconds, and 60 °C for 4 minutes. Sequencing reaction products were precipitated with 2-propanol, washed with 75% ethanol, resuspended in 25 µl water, and loaded onto ABI prism 3100 Genetic analyzer (Applied Biosystems). Primary sequencing data were analyzed using the accompanied sequence analysis program (Applied Biosystems).

Statistical methods

Data were analyzed by the statistic software R (version 2.8) available at http://www.r-project.org using the libraries ''survival'' and ''genetics.'' Age was categorized into three groups (age ⩽45, 46–60, and >60 years). Each genotype-variable was dichotomized into three individual variables. For univariate analyses, the Kaplan–Meier method was used to compare survival times between groups. Differences in 5-year survival rates and survival times were assessed by the log-rank test. In addition, for univariate as well as

multivariate analyses, Cox's proportional hazards regression model was applied with dichotomized variables. As the majority of the melanoma-associated events happen within the first 5 years after diagnosis, close follow-up according to the German guidelines is only recommended for this time period (Garbe *et al.*, 2007). Consequently, we censored our patient cohort after this period. To scrutinize and validate relevant variables, multiple analyses including backward and forward stepwise Cox regressions were performed. Linkage disequilibrium was calculated with the function linkage disequilibrium of the library ''genetics.'' All presented multivariate regression models were evaluated by bootstrap methods according to the recommendations of Altman and Andersen (1989).

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

REFERENCES

Altman DG, Andersen PK (1989) Bootstrap investigation of the stability of a Cox regression model. *Stat Med* 8:771–83

Balazs M, Adam Z, Treszl A *et al.* (2001) Chromosomal imbalances in primary and metastatic melanomas revealed by comparative genomic hybridization. *Cytometry* 46:222–32

Balch CM, Buzaid AC, Soong SJ *et al.* (2001a) Final version of the American Joint Committee on Cancer staging system for cutaneous melanoma. *J Clin Oncol* 19:3635–48

Balch CM, Soong SJ, Gershenwald JE *et al.* (2001b) Prognostic factors analysis of 17,600 melanoma patients: validation of the American Joint Committee on Cancer melanoma staging system. *J Clin Oncol* 19:3622–34

Bartsch H, Dally H, Popanda O *et al.* (2007) Genetic risk profiles for cancer susceptibility and therapy response. *Recent Results Cancer Res* 174:19–36

Benhamou S, Sarasin A (2005) ERCC2 /XPD gene polymorphisms and lung cancer: a HuGE review. *Am J Epidemiol* 161:1–14

Berwick M, Vineis P (2000) Markers of DNA repair and susceptibility to cancer in humans: an epidemiologic review. *J Natl Cancer Inst* 92:874–97

Bristow RG, Hill RP (2008) Hypoxia and metabolism. Hypoxia, DNA repair and genetic instability. *Nat Rev Cancer* 8:180–92

Chin L, Garraway LA, Fisher DE (2006) Malignant melanoma: genetics and therapeutics in the genomic era. *Genes Dev* 20:2149–82

Cifone MA, Fidler IJ (1981) Increasing metastatic potential is associated with increasing genetic instability of clones isolated from murine neoplasms. *Proc Natl Acad Sci USA* 78:6949–52

Clarkson SG (2003) The XPG story. *Biochimie* 85:1113–21

Clarkson SG, Wood RD (2005) Polymorphisms in the human XPD (ERCC2) gene, DNA repair capacity and cancer susceptibility: an appraisal. *DNA Repair (Amst)* 4:1068–74

Costa RM, Chigancas V, Galhardo RS *et al.* (2003) The eukaryotic nucleotide excision repair pathway. *Biochimie* 85:1083–99

Di Lucca J, Guedj M, Descamps V *et al.* (2010) Interactions between ultraviolet light exposure and DNA repair gene polymorphisms may increase melanoma risk. *Br J Dermatol* 162:891–3

Figl A, Scherer D, Nagore E *et al.* (2010) Single-nucleotide polymorphisms in DNA-repair genes and cutaneous melanoma. *Mutat Res* 702:8–16

Figl A, Scherer D, Nagore E *et al.* (2009) Single nucleotide polymorphisms in DNA repair genes XRCC1 and APEX1 in progression and survival of primary cutaneous melanoma patients. *Mutat Res* 661:78–84

Garbe C, Hauschild A, Volkenandt M *et al.* (2007) Evidence and interdisciplinary consense-based German guidelines: diagnosis and surveillance of melanoma. *Melanoma Res* 17:393–9

Goode EL, Ulrich CM, Potter JD (2002) Polymorphisms in DNA repair genes and associations with cancer risk. *Cancer Epidemiol Biomarkers Prev* 11:1513–30

Halpern AC, Altman JF (1999) Genetic predisposition to skin cancer. *Curr Opin Oncol* 11:132–8

Huang WY, Berndt SI, Kang D *et al.* (2006) Nucleotide excision repair gene polymorphisms and risk of advanced colorectal adenoma: XPC polymorphisms modify smoking-related risk. *Cancer Epidemiol Biomarkers Prev* 15:306–11

Jewell R, Conway C, Mitra A *et al.* (2010) Patterns of expression of DNA repair genes and relapse from melanoma. *Clin Cancer Res* 16:5211–21

Kauffmann A, Rosselli F, Lazar V *et al.* (2008) High expression of DNA repair pathways is associated with metastasis in melanoma patients. *Oncogene* 27:565–73

Kertat K, Rosdahl I, Sun XF *et al.* (2008) The Gln/Gln genotype of XPD codon 751 as a genetic marker for melanoma risk and Lys/Gln as an important predictor for melanoma progression: a case control study in the Swedish population. *Oncol Rep* 20:179–83

Kiyohara C, Yoshimasu K (2007) Genetic polymorphisms in the nucleotide excision repair pathway and lung cancer risk: a meta-analysis. *Int J Med Sci* 4:59–71

Kloor M, Michel S, von Knebel DM (2010) Immune evasion of microsatellite unstable colorectal cancers. *Int J Cancer* 127:1001–10

Kraemer KH, Patronas NJ, Schiffmann R *et al.* (2007) Xeroderma pigmentosum, trichothiodystrophy and Cockayne syndrome: a complex genotype-phenotype relationship. *Neuroscience* 145:1388–96

Kumar R, Hoglund L, Zhao C *et al.* (2003) Single nucleotide polymorphisms in the XPG gene: determination of role in DNA repair and breast cancer risk. *Int J Cancer* 103:671–5

Laine JP, Mocquet V, Bonfanti M *et al.* (2007) Common XPD (ERCC2) polymorphisms have no measurable effect on nucleotide excision repair and basal transcription. *DNA Repair (Amst)* 6:1264–70

Li C, Hu Z, Liu Z *et al.* (2006a) Polymorphisms in the DNA repair genes XPC, XPD, and XPG and risk of cutaneous melanoma: a case-control analysis. *Cancer Epidemiol Biomarkers Prev* 15:2526–32

Li C, Liu Z, Wang LE *et al.* (2006b) Genetic variants of the ADPRT, XRCC1 and APE1 genes and risk of cutaneous melanoma. *Carcinogenesis* 27:1894–901

Liu D, O'Day SJ, Yang D *et al.* (2005) Impact of gene polymorphisms on clinical outcome for stage IV melanoma patients treated with biochemotherapy: an exploratory study. *Clin Cancer Res* 11:1237–46

Matakidou A, el Galta R, Webb EL *et al.* (2007) Genetic variation in the DNA repair genes is predictive of outcome in lung cancer. *Hum Mol Genet* 16:2333–40

Mocellin S, Verdi D, Nitti D (2009) DNA repair gene polymorphisms and risk of cutaneous melanoma: a systematic review and meta-analysis. *Carcinogenesis* 30:1735–43

Mohrenweiser HW, Wilson DM III, Jones IM (2003) Challenges and complexities in estimating both the functional impact and the disease risk associated with the extensive genetic variation in human DNA repair genes. *Mutat Res* 526:93–125

Naccarati A, Pardini B, Hemminki K *et al.* (2007) Sporadic colorectal cancer and individual susceptibility: a review of the association studies investigating the role of DNA repair genetic polymorphisms. *Mutat Res* 635:118–45

Pfeifer GP, You YH, Besaratinia A (2005) Mutations induced by ultraviolet light. *Mutat Res* 571:19–31

Ren B, Cam H, Takahashi Y *et al.* (2002) E2F integrates cell cycle progression with DNA repair, replication, and G(2)/M checkpoints. *Genes Dev* 16:245–56

Schaeffer L, Roy R, Humbert S *et al.* (1993) DNA repair helicase: a component of BTF2 (TFIIH) basic transcription factor. *Science* 260: 58–63

Steffen J, Varon R, Mosor M *et al.* (2004) Increased cancer risk of heterozygotes with NBS1 germline mutations in Poland. *Int J Cancer* 111:67–71

Umar A, Kunkel TA (1996) DNA-replication fidelity, mismatch repair and genome instability in cancer cells. *Eur J Biochem* 238:297–307

Vodicka P, Kumar R, Stetina R *et al.* (2004) Genetic polymorphisms in DNA repair genes and possible links with DNA repair rates, chromosomal aberrations and single-strand breaks in DNA. *Carcinogenesis* 25:757–63

Winsey SL, Haldar NA, Marsh HP *et al.* (2000) A variant within the DNA repair gene XRCC3 is associated with the development of melanoma skin cancer. *Cancer Res* 60:5612–6

Wolfe KJ, Wickliffe JK, Hill CE *et al.* (2007) Single nucleotide polymorphisms of the DNA repair gene XPD/ERCC2 alter mRNA expression. *Pharmacogenet Genomics* 17:897–905

Xi T, Jones IM, Mohrenweiser HW (2004) Many amino acid substitution variants identified in DNA repair genes during human population screenings are predicted to impact protein function. *Genomics* 83:970–9

Zheng Z, Chen T, Li X *et al.* (2007) DNA synthesis and repair genes RRM1 and ERCC1 in lung cancer. *N Engl J Med* 356:800–8