

Epidemiology

XPD common variants and their association with melanoma and breast cancer risk

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Summary

There are suggestions in the literature that common variants in the XPD gene may be associated with an altered risk of melanoma and breast cancer. To establish if the XPD common variants Asp312Asn and Lys751Gln are associated with an increased melanoma or breast cancer risk we performed an association study based on genotyping 426 unselected patients with malignant melanoma (MM) and 1830 consecutive breast cancer cases and compared the results to 1262 geographically matched newborns, 621 adults from the region of Szczecin (unselected for age and cancer family history), 421 healthy adults age- and sex-matched with the melanoma cases and 511 healthy controls matched with the breast cancer patients from the region of Szczecin. Additionally we examined the prevalence of three additional XPD variants, Gly156Gly, Leu485Pro and Arg112His amongst the 421 unselected melanoma patients. All of the variants when evaluated singularly were found not to be associated either with melanoma or breast cancer risk in younger or older patients. A modest association was observed with breast cancer risk when the Lys751Gln_CC/Asp312Asn_AA genotype (OR = 1.5, $p < 0.05$) segregated together. Individuals harboring the Lys751Gln_CC/Gly156Gly_CC genotype were significantly over-represented among late-onset melanoma cases (OR = 1.7, $p < 0.05$). The results of analyses of linkage disequilibrium and haplotype frequency support the thesis that a combination of at least two SNPs (Lys751Gln_CC/Gly156Gly_CC or Lys751Gln_CC/Asp312Asn_AA) inherited as a haplotype was associated with disease. These two pairs of SNPs could therefore be regarded as a single hereditary unit that would have a very small probability of being disrupted by recombination. Additional studies are required to determine whether these particular changes can be associated with an increased risk of other malignancies at different sites of origin.

Introduction

There is continuing interest in identifying low-penetrance genes, which are associated with an increased susceptibility to common types of cancer. There are several approaches to this problem, including the use of chip-based single nucleotide polymorphism (SNP) arrays to interrogate a large number of genes simultaneously or by pre-selecting candidate genes of interest and then undertake an in-depth analysis looking for genetic differences that segregate with disease. Candidate genes for cancers at a

particular site may be selected because they are known to predispose to malignancies in other organs, or because they are mutated somatically in the cells from the cancer of the interest.

Cutaneous and ocular malignant melanomas (MM) represent one of the most aggressive neoplasms and their frequency is increasing rapidly [1,2]. The genetic basis of MM is complex and appears to involve multiple genes. Individuals with the rare inherited syndrome xeroderma pigmentosum (XP) have an approximate 1000-fold increased incidence of skin malignancies,

including melanoma [3]. There are several genes associated with XP and these include ERCC2, ERCC3, XP-G and XP-F [3,4]. The *XPD (ERCC2)* gene product has a dual function in basal transcription and in nucleotide excision repair [5,6]. In the literature there are four reports evaluating *XPD* gene polymorphisms and melanoma risk. Two of them were performed on a very small number of cases [7,8], whereas the other two suggested a modest positive association of two single nucleotide polymorphisms in exons 10 (Asp312Asn) and 23 (Lys751Gln) with either melanoma or a subset of older onset cases of melanoma based on 219 and 176 melanoma cases, respectively [9,10].

Recently the two *XPD* variants (Asp312Asn and Lys751Gln) have also been linked with the occurrence of breast cancer [11,12]. Interestingly, the positive findings for Asp312Asn in the 223 Finnish unselected breast cancer patients were not repeated in 172 Polish familial cases [13]. To establish if the *XPD* common variants Asp312Asn and Lys751Gln are associated with increased melanoma or breast cancer risk we performed an association study based on genotyping 426 unselected patients with MM and 1830 consecutive breast cancer cases compared to 1262 geographically matched newborns, 621 adults unselected for age and cancer family history, 421 healthy adults age- and sex-matched with the melanoma cases and 511 healthy controls matched with the breast cancer patients from the region of Szczecin. Additionally we examined the prevalence of Gly156Gly, Leu485Pro and Arg112His variants in *XPD* among 421 unselected melanoma patients as it is unknown if these SNPs are associated with disease risk.

Material and methods

Melanoma patients

The unselected case group consisted of 471 MM patients (264 females, 207 males, mean age at onset 54.5 years, age range 20–85), comprising: (a) 301 unselected patients with MM (mean age 54.7 years, age range 26–78) diagnosed in north-western Poland between 2000 and 2003 (Szczecin, Gorzów Wlkp, Zielona Góra), 80 unselected consecutive MM cases (mean age 53.2 years, age range 29–74) diagnosed between 2002 and 2003 in north-eastern Poland (Białystok), and 90 unselected consecutively collected MM cases (mean age 54.1, age range 34–79) diagnosed between 2002 and 2003 in south-west Poland (Opole).

All MM cases were identified from cancer registries in the five cities mentioned above. Participation rates exceeded 75% for Białystok and Opole. In north-western Poland (301 cases regarded as unselected for age, sex nor cancer family history) the participation rate exceeded 75%; in Szczecin (214 cases regarded as unselected consecutive MM) between 50 and 75% of cases were recruited, in Gorzów Wlkp (47 cases regarded as unselected but non-consecutive) and Zielona Góra (40 cases regarded as unselected but non-consecutive).

Among 471 unselected cases, 56 patients (11.9%) had a first- or second-degree relative affected with MM of which 14 patients (3.0%) had a first-degree relative affected with MM (familial melanoma cases). In 45 unselected cases we were unable to perform molecular analyses due to the poor quality of DNA.

Breast cancer patients

This study population includes prospectively ascertained cases of consecutive invasive breast cancer diagnosed at 7 treatment centers throughout Poland from 1997 to 2003, unselected for age and family history. The patients were invited to participate in person during their hospital stays or by mailed invitation. During the interview the goals of the study were explained, informed consent was obtained, genetic counseling was given and a blood sample was taken for DNA analysis. A detailed family history of cancer was ascertained (first, and second-degree relatives included) and a risk factor questionnaire was completed. A total of 2500 incident cases of invasive breast cancer were identified at the 7 different centers during the study period. Of these, 1900 women accepted the invitation to participate in this genetic study (76%). The medical records and pathology reports of all participants were reviewed. From the total of 1900 enrollees 70 patients could not be genotyped because of poor quality DNA. This brought the total number of breast cancer cases studied to 1830, of them 511 were diagnosed in the city of Szczecin.

Controls

Three control groups were established.

The first group consisted of 1262 geographically matched newborn male and female children collected in the same hospitals from where the melanoma and breast cancer cases were collected. Samples of cord blood from unselected infants were forwarded to the study center in Szczecin.

The second control group consisted of 621 adults from the region of Szczecin unselected for cancer family history, sex or age. To ensure comparability of the control groups the allele frequencies of the *XPD* alleles were assessed separately for the adult and neonatal control groups, which also took into account their geographic origin.

The third control group consisted of 421 healthy adults matched for sex and age with the melanoma cases and 511 healthy adults from the region of Szczecin matched for sex and age with the breast cancer cases collected from the city of Szczecin.

Methods

DNA samples were obtained from peripheral blood of individuals or from umbilical chord blood of newborns according to the method of Miller et al. (1988).

The Asp312Asn variant was analyzed by restriction fragment length polymorphism PCR, using 936gaF (ATCAAAGAGACAGACGAGCAG) and 936gaR (GCTCACCTGCAGCACAACGT) primers. PCR products were digested with the Psp 14061 enzyme and separated in 2–3% agarose gels.

The Gly156Gly variant was analyzed by restriction fragment length polymorphism PCR, using tvilex6f (TGGAGTGCTATGGCAGCATCTCT) and tvilex6r (CCATGGGCATCAAATTCCTGGGA) primers. PCR products were digested with the *Tfi*I enzyme and separated in 2–3% agarose gels.

The Lys751Gln variant was analyzed by restriction fragment length polymorphism PCR, using 2253F (CTGTTCTCTCCAGGAGGATCAG) and 2253R (GACAGTGAGAAATGTCACCTGAC) primers. PCR products were digested with the *Pst*I enzyme and separated in 2–3% agarose gels.

The Leu485Pro variant was analyzed by restriction fragment length polymorphism PCR, using 1455F (CCAGTCTGTCATCATCACATCTG) and 1455R (TAAAGCTCTCCTGCCTGAGCAG) primers. PCR products were digested with the *Hpa*II enzyme and separated in 2–3% agarose gels.

The Arg112His variant was analyzed by restriction fragment length polymorphism PCR, using 336gaF (TGTGCCTCCAATGAGCACAAGCT) and 336gaR (TGTGTGTAGCCACTGCATGCCT) primers. PCR products were digested with the *Fau*I enzyme and separated in 2–3% agarose gels.

DNA sequencing was undertaken on random samples to ensure that all PCR products represented the respective XPD alleles.

Statistics

To evaluate whether identified XPD alterations were associated with MM or breast cancer we compared the frequency of the detected variant in our patients to the control group from the general population, using a two-tailed Fisher exact test (with Bonferroni correction). The patient cohorts were further analyzed by dividing the cases into those persons under 50 years of age and comparing the frequency of the variants observed in this group to those patients over 50 years of age. The age determinant of 50 years was chosen since: (1) those cases under 50 years of age were more likely to be due to genetic influences compared to those patients over 50 years of age whose disease was more likely to be a result of environmental exposure and (2) XPD variants have been associated with an increased cancer risk in older (>50 years) subjects [10].

The average age at diagnosis among the different genotypes was assessed for significant differences using the *t*-test with Welsh-correction (heteroskedastic distributions).

Possible deviation of the allele frequencies from those expected under Hardy–Weinberg equilibrium (HWE) was assessed using the chi-square probability test [14].

Linkage disequilibrium analysis was performed on the basis of the EM-Algorithm using LDA 1.0 software [15], the *p*-value of the likelihood ratio test was based on 1000 iterations.

Haplotype frequency analysis was performed using SNP-HAP v.1.3.1 (open-source software provided by David Clayton <http://www-gene.cimr.cam.ac.uk/clayton/software/>).

Results

Haplotype analysis in the control populations

There were no significant differences in the allele frequencies of the XPD variants in all three control populations (Table 1). Since only the newborn group consisted of cases geographically matching the melanoma and breast cancer patients, and as it was larger than the adult control population further statistical calculations were performed only on this control group.

There were no significant differences in the 5 allele frequencies of the polymorphisms among males and females with a diagnosis of MM and among controls. There were no significant differences among newborns recruited from 7 treatment centers throughout Poland, including the regions of Szczecin/Gorzów/Zielona Góra, Białystok and Opole (data not shown).

The expected allelotype distributions for all polymorphisms were in Hardy–Weinberg equilibrium irrespective whether they were breast cancer cases, melanoma cases or controls.

Haplotype analysis in melanoma patient population

Arg112His, Leu485Pro

We were unable to evaluate an association between melanoma and Arg112His and Leu485Pro since neither of these polymorphisms were represented among the control and subject populations used in this study, suggesting that both changes are very rare in the Polish population (Table 1).

Lys751Gln

We found no association with the Lys751Gln genotype and melanoma or breast cancer risk (Table 1). There were no statistically significant differences among Lys751Gln genotype variant prevalence among early- and late-onset cases (data not shown). There were no major differences in the mean age of diagnosis between the subjects carrying the AA, AC or CC genotypes (*p* values 0.39, 0.30, 0.37, respectively).

Asp312Asn

We found no association with the Asp312Asn genotype and breast cancer or melanoma risk (Table 1). There were no statistically significant differences among Asp312Asn genotype prevalence among early- and late-onset cases (data not shown).

Table 1. Frequencies of the examined alleles in controls and subjects populations

		Newborns (1141)	Unselected adults (596)	Melanoma-matched adults (421)	Breast-matched adults (511)	Melanoma (426)	Breast cancer (1830)
Lys751Gln	AA	432 (37.9%)	218 (36.6%)	161 (38.2%)	187 (36.5%)	146 (34.3%)	703 (38.4%)
	AC	547 (47.9%)	276 (46.3%)	196 (46.6%)	245 (47.9%)	207 (48.6%)	850 (46.5%)
	CC	162 (14.2%)	102 (17.1%)	64 (15.2%)	79 (15.5%)	73 (17.1%)	277 (15.1%)
Asp312Asn		Newborns (1262)	Unselected adults (621)	Melanoma-matched adults (421)	Breast-matched adults (511)	Melanoma (425)	Breast cancer (1726)
	GG	492 (39%)	228 (36.7%)	150 (35.6%)	180 (35.3%)	168 (39.5%)	672 (38.9%)
	AG	597 (47.3%)	300 (48.3%)	200 (47.5%)	252 (49.3%)	188 (44.2%)	785 (45.5%)
Gly156Gly		Newborns (1052)				Melanoma (421)	
	CC	332 (31.2%)	–	–	–	145 (35.3%)	–
	AC	536 (51%)	–	–	–	203 (49.4%)	–
Arg112His		Newborns (500)					
	AA	0 (0%)	–	–	–	0 (0%)	–
	AG	0 (0%)	–	–	–	0 (0%)	–
Leu485Pro	GG	500 (100%)	–	–	–	421 (100%)	–
	TT	0 (0%)	–	–	–	0 (0%)	–
	TC	0 (0%)	–	–	–	0 (0%)	–
	CC	500 (100%)	–	–	–	421 (100%)	–

There were no significant differences in the mean age of disease diagnosis observed for patients who harbored the AA, AC or CC genotypes (p values 0.16, 0.10, 0.31, respectively).

Gly156Gly

No association was observed between the Gly156Gly genotype and melanoma risk (Table 1), for either early- or late-onset melanoma (data not shown). There was no major difference in the mean age of diagnosis between the subjects carrying AA, AC or CC genotypes (p values 0.15, 0.18, 0.43, respectively).

There remained the possibility that any single polymorphism would not alter the risk of breast cancer or melanoma sufficiently to show an effect. The likelihood that two or more SNPs in XPD were associated with disease risk was investigated by assessing this more complex association.

Lys751Gln + Asp312Asn

We found an association of the Lys751Gln_CC/Asp312Asn_AA genotype with breast cancer risk (Table 2). The genotype was significantly over-represented among early-onset breast cancer patients (OR = 1.5, $p=0.0212$) and late onset cases (OR = 1.5, $p=0.0169$).

No association between compound heterozygous carriers of Lys751Gln and Asp312Asn variants and melanoma risk was observed. None of the compound heterozygous genotypes were significantly over-represented among melanomas compared to controls (Table 2). The highest OR was for subjects carrying Lys751Gln_CC/Asp312Asn_AA genotype (OR = 1.4 with $p=0.2$). The genotype Lys751Gln_CC/Asp312Asn_GG [12] was rare in our population with only 7 and 17 carriers among 423 melanoma patients and 1053 controls (OR = 1.1, $p=0.9$), respectively. When taking the age of diagnosis into

Table 2. Lys751Gln/Asp312Asn genotypes among cases and control groups

Lys751Gln	Asp312Asn	Newborns ($n=1017$)	Melanoma ($n=423$)	OR (95%CI), p	Breast ($n=1713$)
AA	GG	267 (26.3%)	115 (27.2%)	1.0 ns	494 (28.8%)
AA	AG	104 (10.2%)	28 (6.6%)	0.6 ns	153 (8.9%)
AA	AA	16 (1.6%)	2 (0.5%)	0.3 ns	17 (1%)
AC	GG	107 (10.5%)	46 (10.9%)	1.0 n.s	151 (8.8%)
AC	AG	334 (32.8%)	131 (31%)	1.0 ns	559 (32.6%)
AC	AA	49 (4.8%)	28 (6.6%)	1.4 n.s	77 (4.5%)
CC	GG	20 (2%)	7 (1.6%)	0.8 n.s	22 (1.3%)
CC	AG	51 (5%)	27 (6.4%)	1.3 n.s	67 (3.9%)
CC	AA	69 (6.8%)	39 (9.2%)	1.4, ns	173 (10.1%)

The p -value corresponds to the unadjusted p -value of the Chi-square test. The adjusted p -value after Bonferroni correction follows in parenthesis.

account (i.e. under or over 50 years of age) no association was observed (data not shown).

Lys751Gln + Gly156Gly

Subjects carrying *Lys751Gln_CC* genotype and *Gly156Gly_CC* genotype were associated with modest increase of melanoma risk (OR = 1.4). In a subgroup of late-onset melanoma patients the genotype was more tightly associated with melanoma risk with an OR = 1.7, $p < 0.05$ (Table 3).

Asp312Asn + Gly156Gly

None of the compound heterozygotes were significantly over-represented among melanoma cases (data not shown). The highest OR (and lowest p value) reached was in the subgroup harboring the *Asp312Asn_AA/Gly156Gly_CC* genotype which demonstrated an OR = 1.4 and $p = 0.088$ and was detected in 64 cases (15.84%) and 109 controls (12.19%). The *Asp312Asn_GG/Gly156Gly_CC* genotype was present in 27 (6.68%) cases and 67 (6.38%) controls with OR = 1.1, $p = 0.9$. None of the genotypes were significantly over-represented in either of the melanoma age subgroups (data not shown).

Lys751Gln + Asp312Asn + Gly156Gly

None of the compound heterozygous genotypes were significantly over-represented among melanoma cases (Table 3). Carriers of the *Lys751Gln_CC/Asp312Asn_GG/Gly156Gly_CC* had OR = 1.8 with $p = 0.5$. Carriers of the *Lys751Gln_CC/Asp312Asn_AA/Gly156Gly_CC* genotype had OR = 1.3 with $p = 0.3$. The high p value is due to the small numbers of carriers with all three polymorphisms.

Linkage disequilibrium. *Lys751Gln*, *Asp312Asn* and *Gly156Gly* polymorphisms are in linkage disequilibrium with one another (LR-Test, 1000 iterations, $p < 0.0001$) (R square = 0.42–0.36; $D = 0.64–0.9$).

Haplotype frequency. A statistically significant difference in the genetic background of the breast cancer population was observed when compared to the newborn control population (Chi square; $df = 3$; $p = 0.0209$) (Table 4). Although there was no significant difference for a particular haplotype, a trend of over-representation of the haplotype *Lys751Gln_CC/Asp312Asn_AA* among cases (Chi square, $df = 1$, $p = 0.08$) was observed. We found no significant differences among haplotype composition among melanoma cases when compared with the newborn controls (Table 4).

Exon splice enhancer (ESE) analysis. We performed ESE analysis of *Gly156Gly* using an algorithm that predicts the presence of these regions within the coding sequence of genes (<http://genes.mit.edu/cgi-bin/rescue-ese/>). The results of the analysis suggests that the *Gly156Gly* polymorphism effects the ESE binding motif and thereby potentially alters the splicing efficiency of this region of the gene.

Discussion

The results of this study indicate that the *XPD* gene can be regarded as a low-risk susceptibility gene for both melanoma and breast cancer. The results revealed that the *Lys751Gln_CC/Asp312Asn_AA* genotype is associated with a modest increase in breast cancer risk (OR = 1.5, $p < 0.05$). This result is further supported by a significantly different distribution of haplotypes among breast cancer cases compared to the controls (i.e. the frequency of one or more of the haplotypes differs between cases and controls). The same genotype also appeared to be over-represented in the melanoma patients (OR = 1.4) however it did not reach significance. Individuals harboring the *Lys751Gln_CC/Gly156Gly_CC* genotype were significantly over-represented among late-onset melanoma cases suggesting an

Table 3. *Lys751Gln/Gly156Gly* genotypes among melanoma and control groups

<i>Lys751Gln</i>	<i>Gly156Gly</i>	Melanoma ($n = 424$)	Newborns ($n = 1052$)	95% CI	p -value	OR
AA	AA	56 (13.2%)	157 (14.9%)		ns	0.9
	CA	70 (16.5%)	182 (17.3%)		ns	1.0
	CC	19 (4.5%)	62 (5.9%)		ns	0.7
AC	AA	7 (1.65%)	25 (2.4%)		ns	0.7
	CA	130 (30.66%)	318 (30.2%)		ns	1.0
	CC	66 (15.57%)	153 (14.6%)		ns	1.1
CC	AA	2 (0.50%)	2 (0.2%)		ns	2.5
	CA	9 (2.12%)	36 (3.4%)		ns	0.6
	CC	65 (15.3%) MM $\leq 50^a$ ($n = 172$)	117 (11.12%)	1.044–2.006	0.03 (0.18)	1.4
CC	CC	21 (12.2%) MM $> 50^a$ ($n = 250$)	117 (11.12%)		ns	1.1
CC	CC	44 (17.6%)		1.170–2.491	0.007 (0.042)	1.7

The p -value corresponds to the unadjusted p -value of the Chi-square test. The adjusted p -value after Bonferroni correction follows in parenthesis.
^aIn 2 melanoma cases age of diagnosis was not available.

Table 4. Haplotype frequencies calculated for breast cancer cases, melanoma cases and newborns

Haplotypes	Breast cancer cases (<i>n</i> = 1713)	Newborns (<i>n</i> = 1017)	<i>p</i> values
11*	0.533	0.518	0.44
22*	0.300	0.269	0.08
12*	0.084	0.110	0.026
			(0.104)
21*	0.083	0.103	0.078
All			0.0209
	Melanoma cases (<i>n</i> = 424)	Newborns (<i>n</i> = 815)	
112	0.359	0.372	0.65
221	0.304	0.273	0.25
111	0.143	0.141	0.85
121	0.069	0.083	0.38
211	0.084	0.072	0.44
212	0.024	0.029	0.60
122	0.010	0.020	0.19
222	0.005	0.010	0.36
2*1	0.385	0.345	0.13
All			0.59

Locus positions correspond to 11541 (Lys751Gln), 12591 (Asp312Asn) and 24931 (Gly156Gly) respectively. Each locus has two alleles; 1 = most abundant, 2 = less abundant. The third locus has not been studied for breast cancer cases. The haplotype “2*1” includes both “221” and “211” variants. The *p* values correspond to a simple Chi-square test (*df* = 1), except for the comparisons of the frequencies in the haplotype composition as whole (row “all”) where the *p* values refer to multiple Chi-square test (*df* = 3 and *df* = 7, respectively). Significant *p* values of repeated pairwise comparisons have been adjusted after Bonferroni correction (behind in parenthesis).

association that results in a modest increase of melanoma risk (OR = 1.7, *p* < 0.05). Interestingly, it confirms suggestions from the literature that Lys751Gln_CC SNP may be associated with a potential risk of malignancy [10,11]. The effect, however, is weaker in the Polish population as it appears that this variant alone is not associated with disease risk in younger or older patients. The genotype Lys751Gln_CC/Asp312Asn_GG described as being associated with breast cancer in the German population is a rare variant, which is only present in ~2% of both the German and Polish control populations. The difference in the frequency of these variants among German and Polish breast cancer patients may be a result of population stratification or the relatively small numbers of cases used for the respective studies.

Since the risk of disease is low but nevertheless greater than that of the wildtype alleles, for the Lys751Gln_CC/Gly156Gly_CC genotype and the Lys751Gln_CC/Asp312Asn_AA, respectively, then combined carriers of all of these polymorphisms should have a higher odds ratio of disease. However, sufficient statistical power to evaluate the three SNPs together would require thousands of cases and controls and is beyond the scope of this present study.

The XPD 751Gln variant modifies the amino-acid configuration in a domain important for the interaction with the helicase activator p44 and is thought to produce the most significant change in XPD function [16,17]. Interestingly, its combination with the silent Gly156Gly change appears to result in an increased melanoma risk than with the Asp312Asn polymorphism. There are several possible explanations for this: (1) the Gly156Gly is in linkage disequilibrium with another change that does alter the function of the protein product, (2) the Gly156Gly polymorphism alters an exon splice suppressor sequence, and (3) the base change alters the exon splice enhancer property within the exon. According to the results of the ESE algorithm evaluation this last explanation appears to be likely.

The results of our investigation indicate that all the SNPs studied are in Hardy–Weinberg equilibrium, and in linkage disequilibrium with one another. Thus the combination of at least two SNPs (Lys751Gln_CC/Gly156Gly_CC or Lys751Gln_CC/Asp312Asn_AA) could be regarded as a single hereditary unit with a very low probability of being disrupted by recombination.

The genotyping results of our newborn control population in conjunction with findings published previously by our group [18,19] suggest that the Polish population is homogeneous. However, it is necessary to determine the distribution of genotypes at a number of other unrelated loci throughout Poland to evaluate likelihood of population stratification. Results published previously indicate that population stratification is not a problem in Poland and furthermore indicates that it is ideal for association studies as it does not suffer from local population variance. Since there is no apparent genetic drift between the newborn population and the test population any associations identified are likely to be a result of a true susceptibility.

XPD can be added to the list of genes, which are believed to be common breast cancer and malignant melanoma susceptibility genes. CDKN2A or p16 was the first of the melanoma genes to be associated with an increased risk of breast cancer [20] and we have reported an association of a common variant in p16, the A148T change, which has been associated with an increased melanoma risk [21] and breast cancer risk [22]. There are some reports suggesting an association of XPD variants with other malignancies, such as bladder cancer [23], basal cell carcinoma of the skin [24,25] or lung cancer [26].

In conclusion, we report herein that the Lys751Gln_CC/Asp312Asn_AA genotype is modestly associated with breast cancer risk, while the Lys751Gln_CC/Gly156Gly_CC genotype is modestly associated with melanoma in patients diagnosed above 50 years of age. Our study does not imply that this XPD variant is associated with increased disease risk alone but rather suggests there are interactive modifiers that when taken together result in an increased likelihood of malignancy. Additional studies are required to determine whether these particular changes can be associated with an increased risk of other malignancies at different sites of origin.

References

1. Goldstein AM, Tucker MA: Genetic epidemiology of familial melanoma. *Dermatol Clin* 13: 605–612, 1995
2. Greene MH: The genetics of hereditary melanoma and nevi. 1998 update. *Cancer* 86: 2464–2477, 1999
3. Tsao H: Update on familial cancer syndromes and the skin. *J Am Acad Dermatol* 42: 939–970, 2000
4. Cleaver JE, Thompson LH, Richardson AS, States JC: A summary of mutations in the UV-sensitive disorders: xeroderma pigmentosum, Cockayne syndrome, and trichothiodystrophy. *Hum Mutat* 14: 9–22, 1999
5. Berneburg M, Lehmann AR: Xeroderma pigmentosum and related disorders: defects in DNA repair and transcription. *Adv Genet* 43: 71–102, 2001
6. Broughton BC, Steingrimsdottir H, Weber CA, Lehmann AR: Mutations in the xeroderma pigmentosum group D DNA repair/transcription gene in patients with trichothiodystrophy. *Nat Genet* 7: 189–194, 1994
7. Tomescu D, Kavanagh G, Ha T, Campbell H, Melton DW: Nucleotide excision repair gene XPD polymorphisms and genetic predisposition to melanoma. *Carcinogenesis* 22: 403–408, 2001
8. Winsey SL, Haldar NA, Marsh HP, et al.: A variant within the DNA repair gene XRCC3 is associated with the development of melanoma skin cancer. *Cancer Res* 60: 5612–5616, 2000
9. Han J, Colditz GA, Liu JS, Hunter DJ: Genetic variation in XPD, sun exposure, and risk of skin cancer. *Cancer Epidemiol Biomarkers Prev* 14: 1539–1544, 2005
10. Baccarelli A, Calista D, Minghetti P, et al.: XPD gene polymorphism and host characteristics in the association with cutaneous malignant melanoma risk. *Br J Cancer* 90: 497–502, 2004
11. Justenhoven C, Hamann U, Pesch B, et al.: ERCC2 genotypes and a corresponding haplotype are linked with breast cancer risk in a German population. *Cancer Epidemiol Biomarkers Prev* 13: 2059–2064, 2004
12. Zhang L, Zhang Z, Yan W: Single nucleotide polymorphisms for DNA repair genes in breast cancer patients. *Clin Chim Acta* 359: 150–155, 2005
13. Forsti A, Angelini S, Festa F, et al.: Single nucleotide polymorphisms in breast cancer. *Oncol Rep* 11: 917–922, 2004
14. Ott J: Utility programs for analysis of genetic linkage; Program HWE, URL: <http://www.hgmp.mrc.ac.uk/Registered/Help/link-util/>, 1988
15. Ding K, Zhou K, He F, Shen Y: LDA – A java-based linkage disequilibrium analyzer. *Bioinformatics* 19: 2147–2148, 2003
16. Coin F, Marinoni JC, Rodolfo C, Fribourg S, Pedrini AM, Egly JM: Mutations in the XPD helicase gene result in XP and TTD phenotypes, preventing interaction between XPD and the p44 subunit of TFIIH. *Nat Genet* 20: 184–188, 1998
17. Benhamou S, Sarasin A: ERCC2/XPD gene polymorphisms and cancer risk. *Mutagenesis* 17: 463–469, 2002
18. Gorski B, Jakubowska A, Huzarski T, et al.: A high proportion of founder BRCA1 mutations in Polish breast cancer families. *Int J Cancer* 110: 683–686, 2004
19. Cybulski C, Huzarski T, Gorski B, et al.: A novel founder CHEK2 mutation is associated with increased prostate cancer risk. *Cancer Res* 64: 2677–2679, 2004
20. Hashemi J, Platz A, Ueno T, Stierner U, Ringborg U, Hansson J: CDKN2A germ-line mutations in individuals with multiple cutaneous melanomas. *Cancer Res* 60: 6864–6867, 2000
21. Debniak T, Scott RJ, Huzarski T, et al.: CDKN2A common variants and their association with melanoma risk: a population-based study. *Cancer Res* 65: –835–839, 2005
22. Debniak T, Gorski B, Huzarski T, et al.: A common variant of CDKN2A (p16) predisposes to breast cancer. *J Med Genet*, 2005 (in press)
23. Schabath MB, Delclos GL, Grossman HB, et al.: Polymorphisms in XPD exons 10 and 23 and bladder cancer risk. *Cancer Epidemiol Biomarkers Prev* 14: 878–884, 2005
24. Brewster AM, Alberg AJ, Strickland PT, Hoffman SC, Helzlsouer K: XPD polymorphism and risk of subsequent cancer in individuals with nonmelanoma skin cancer. *Cancer Epidemiol Biomarkers Prev* 13: 1271–1275, 2004
25. Lovatt T, Aldersea J, Lear JT, et al.: Polymorphism in the nuclear excision repair gene ERCC2/XPD: association between an exon 6-exon 10 haplotype and susceptibility to cutaneous basal cell carcinoma. *Hum Mutat* 25: 353–359, 2005
26. Yin J, Li J, Ma Y, Guo L, Wang H, Vogel U: The DNA repair gene ERCC2/XPD polymorphism Arg 156Arg (A22541C) and risk of lung cancer in a Chinese population. *Cancer Lett* 223: 219–226, 2005

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