Polymorphisms in the DNA Repair Gene *XPD*: Correlations with Risk and Age at Onset of Basal Cell Carcinoma[1]

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Abstract

The XPD protein has a dual function, both in nucleotide excision repair and in basal transcription. We have studied the role of two nucleotide substitutions in the *XPD* gene, one in exon 23 leading to an amino acid substitution (Lys751Gln) and one silent in exon 6 in relation to basal cell carcinoma (BCC). Both are two-allele polymorphisms, with the nucleobases A and C at the given positions. We genotyped psoriasis patients with and without BCC and nonpsoriatic persons with and without BCC (4 × 20 persons). The choice to study psoriasis patients was motivated by their high genotoxic exposure via treatment and their high relative rate of early BCC. Subjects carrying two *A* alleles (*AA* genotype) in exon 23 were at 4.3-fold higher risk of BCC than subjects with two *C* alleles (95% CI, 0.79–23.57). In addition, the mean age at first skin tumor for BCC cases with the *AA* genotype was significantly lower than the mean age for BCC cases with the *AC* or *CC* genotype ($P = 0.012$). Thus, the variant *C*-allele of exon 23 may be protective. The exon 6 genotype was associated with the risk of BCC among the psoriasis patients; psoriatics carrying two *A* alleles in exon 6 were at 5.3-fold higher risk of BCC than psoriatics with two *C* alleles (95% CI, 0.78–36.31). For the psoriatics, the mean age at onset of BCC for cases with the *AA* genotype was marginally lower than the mean age for cases with genotype *AC* or *CC* ($P = 0.060$). Our results raise the possibility that the polymorphisms in the *XPD* gene may be contributing factors in the risk of BCC development. They are, therefore, important candidates for future studies in susceptibility to cancer.

Introduction

A complex system of DNA repair enzymes has a vital role in protecting the genome of the cell from carcinogenic exposure. A considerable interindividual variation in DNA repair capacity has been observed in the general population, and it has been reported that individuals with a nucleotide excision repair capacity below the population mean are at increased risk of developing skin and lung cancer (1, 2). Like many other phenotypical traits, the variation in DNA repair capacity is probably genetically determined. Given the known association between DNA repair capacity and cancer (most clearly demonstrated by xeroderma pigmentosum patients that are defective in genes in the nucleotide excision pathway and have a 1000-fold increased risk of getting BCC[4]; Ref. 3), sequence variation of DNA repair genes has the potential to be cancer risk factors in the population.

The *XPD* gene encodes a helicase involved in the nucleotide excision repair pathway (4). In addition to repair, the *XPD* gene also has a function in basal transcription. Because *XPD* has been found to be a subunit of the transcription factor IIH required for all transcription by the RNA polymerase II, it is an essential gene (5). Consistent with this, it has recently been reported that inactivation of the *XPD* gene in mice leads to embryonic lethality in the preimplantation stage (6).

In a previous report, five different two-allele polymorphisms were found in the coding sequence of the *XPD* gene (7); and in a more recent study, several two-allele polymorphisms in five different DNA repair genes were reported (8). The variant alleles existed at frequencies ranging from 0.04 to 0.45 in a group of 12 healthy individuals. These DNA repair gene variations remain to be studied in cohorts of cancer cases and their controls in which the finding of a higher incidence of a certain allele in cancer patients than in healthy individuals could suggest that this allele is a contributing factor in an individual's risk of cancer.

Psoriasis patients are via their treatment exposed to a variety of genotoxic agents, including coal tar, psoralen, and methotrexate. Presumably as a consequence of the treatment psoriasis patients are at increased risk of getting BCC (9). The risk is particularly increased among young psoriasis patients (relative risk 12; age group, 30–39 years). Young psoriasis patients, therefore, offer an attractive study group for an attempt to elucidate the role of protective mechanisms, *i.e.*, DNA repair, in relation to cancer.

In the present study, we analyzed two known *XPD* polymorphisms: one silent nucleotide substitution and one amino

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[4] The abbreviations used are: BCC, basal cell carcinoma; OR, odds ratio; CI, confidence interval.

acid substitution (7, 8) in relation to BCC in psoriasis patients and apparently normal individuals (3). Because both polymorphisms alter a restriction site, we developed two PCR/RFLP based assays. We performed genotyping of psoriasis patients with and without BCC and nonpsoriatic persons with and without BCC (4 × 20 persons). For both polymorphisms, we found that the distribution of the genotypes differed between cases and controls, and that the genotype was associated with the age at which the skin cancer patients had their first tumor and possibly the risk of BCC.

Materials and Methods

Study Subjects. Four groups, each consisting of 20 persons, participated in the study (Table 1). Group 1 included persons with a diagnosis of both psoriasis and BCC, group 2 included persons with diagnosed psoriasis, group 3 included persons with a diagnosis of BCC, and group 4 included healthy persons.

All BCC subjects were identified from a population-based cohort of persons treated by Danish dermatologists in the year 1995. The index group consisted of those with both BCC and psoriasis who fulfilled these criteria: (*a*) age in 1995 < 50 years; and (*b*) clinically verified diagnosis of psoriasis. The diagnosis of BCC was clinically and histologically confirmed. The other study groups were matched to the index group by age and sex. The group of psoriasis patients without BCC was selected from among patients treated in the years 1992–1995 for psoriasis by dermatologists who participated in the national cohort study 1995. The group of control subjects was recruited from the participating institutes from among personnel and relatives. All of the control subjects were genetically independent. Persons who had received psoriatic treatments (*i.e.,* UV-radiation, psoralen and UV-A light, bucky rays, coal tar, and oral methotrexate) during the last 3 months before blood collection were excluded. The 40 patients with BCC differed from the average patient in the national cohort with BCC in that the ratio of male:female was 1:3 against 6:5 in the cohort, and the average age at first BCC was 38.3 years (± 5.7) against 56.5 years (± 14.0) in the cohort. Subjects completed an extensive questionnaire on risk factors for skin cancer and provided a blood sample. All of the study persons were Caucasians; the majority was blue- or gray-eyed. Fair skin was more frequent in group 3 (14 persons) than in the other groups (4–8 persons). There was a tendency that persons in group 1 had been treated for psoriasis for a longer time than those of group 2, and also that the treatments were more intense.

All of the subjects gave written informed consent. The study was conducted in accordance with the Helsinki declaration and was approved by the local medical ethical committee.

DNA Extraction and PCR Analysis. Genomic DNA was extracted from 3–5 × 10^6 granulocytes obtained from a blood specimen. The DNA was extracted with the Puregene DNA Isolation Kit (Gentra Systems, Minneapolis, MN).

The PCR primers were synthesized by TAG-Copenhagen Aps (Copenhagen, Denmark). The polymorphic site in exon 6 was amplified using forward primer 5′-TGG AGT GCT ATG GCA CGA TCT CT-3′ and reverse primer 5′-CCA TGG GCA TCA AAT TCC TGG GA-3′. The polymorphic site in exon 23 was amplified using forward primer 5′-ATC CTG TCC CTA CTG GCC ATT C-3′ and reverse primer 5′-TGT GGA CGT GAC AGT GAG AAA T-3′. The PCR reactions were initially optimized using the PCR Optimization Kit (Boehringer-Mannheim, Mannheim, Germany).

The PCR reactions were performed in a 25-μl reaction volume containing: 20 mM Tris-HCl, 50 mM KCl (pH 8.4), 1.0 mM $MgCl_2$, 0.2 mM each deoxynucleotide triphosphate, 1.0 μM each primer, 0.5 units of *Taq* DNA polymerase (Life Technologies, Denmark), and 50–200 ng of genomic DNA. The cycling conditions were: initial denaturation at 96°C for 1 min, 30 cycles of denaturation at 94°C for 30 s, primer annealing at 60°C for 30 s, primer extension at 72°C for 1 min, and finally an extension at 72°C for 2 min.

Table 1 Distribution of age and sex in the study groups

Group	*n*	Age[a] (years)	Males	Females
1 (BCC + psoriasis)	20	47.1 ± 4.0	5	15
2 (psoriasis)	20	46.9 ± 3.8	5	15
3 (BCC)	20	46.8 ± 3.5	5	15
4 (control)	20	46.1 ± 3.6	5	15

[a] Mean ± SD.

Restriction Enzyme Analysis. For both polymorphisms, only two alternative nucleotides (A and C) have been identified by DNA sequencing (7, 8); therefore, in the following, we will only consider *A* and *C* as possible allele types.

Exon 6. PCR product (10 μl) was digested with 3 units of *Tfi*I enzyme (New England Biolabs, Beverly, MA) in a 20-μl reaction mixture, as suggested by the manufacturer, for 1.5 h and separated on a 2.0% agarose gel. The *A* but not the *C* allele in exon 6 has a *Tfi*I restriction site within the 652-bp amplified PCR product. In addition, there is a second *Tfi*I restriction site within the amplified fragment that serves as an internal control for digestion. The three possible genotypes are defined by three distinct banding patterns: *CC* (56- and 596-bp fragments), *CA* (56-, 114-, 482-, and 596-bp fragments), and *AA* (56-, 114-, and 482-bp fragments).

Exon 23. PCR product (5 μl) was digested with 15 units of *Pst*I enzyme (Life Technologies) in a 20-μl reaction mixture as suggested by the manufacturer for 1 h and separated on a 2.0% agarose gel. The *A* but not the *C* allele in exon 23 has a *Pst*I restriction site within the 324-bp amplification product. In addition, there is a second *Pst*I restriction site within the amplified fragment that serves as an internal control for digestion. The three possible genotypes are defined by three distinct banding patterns: *AA* (100- and 224-bp fragments), *AC* (66-, 100-, 158-, and 224-bp fragments), and *CC* (66-, 100-, and 158-bp fragments).

Host Cell Reactivation Assay. The assay was performed basically as described by Athas *et al.* (10). Briefly, the assay measures the ability of host lymphocytes to repair a UV-damaged reporter gene inserted into a plasmid DNA that is transfected into the lymphocytes.

Statistical Methods. The ORs and 95% CIs were calculated to assess the relationship between each polymorphism and BCC. The χ^2 test was used to compare the distribution of the genotypes between BCC cases and controls. A one-sided heteroscedastic *t* test was used to compare the ages of first BCC between the genotypes. For both polymorphisms, the mean age of having two *A* alleles (*AA*) was compared with the mean age of having one *C* allele (*AC*), and to the mean age of having one or two *C* alleles (*AC* and *CC*).

Results

We have performed a study of BCC in relation to *XPD* gene polymorphisms using PCR/RFLP based assays in psoriasis patients with and without BCC and nonpsoriatic persons with and without BCC (4 × 20 persons). Table 1 summarizes the

Table 2 Distribution of *XPD* genotypes in the four study groups

	Total	Exon 6				Exon 23			
		AA (%)	*AC* (%)	*CC* (%)	*C*-allele frequency	*AA* (%)	*AC* (%)	*CC* (%)	*C*-allele frequency
All subjects	80	19 (23.7)	38 (47.5)	23 (28.8)	0.525	38 (47.5)	33 (41.3)	9 (11.2)	0.319
Psoriatics									
BCC cases	20	6 (30)	11 (55)	3 (15)	0.425	10 (50)	9 (45)	1 (5)	0.275
Controls	20	3 (15)	9 (45)	8 (40)	0.625	8 (40)	8 (40)	4 (20)	0.400
Nonpsoriatics									
BCC cases	20	5 (25)	9 (45)	6 (30)	0.525	11 (55)	8 (40)	1 (5)	0.250
Controls	20	5 (25)	9 (45)	6 (30)	0.525	9 (45)	8 (40)	3 (15)	0.350

Table 3 *XPD* polymorphisms in relation to risk of basal cell carcinoma

	Genotype			Total
	CC	*AC*	*AA*	
Exon 23				
All subjects				
BCC cases	2	17	21	40
Controls	7	16	17	40
OR (95% CI)[a]	1.00	3.72 (0.67–20.64)	4.32 (0.79–23.57)	
P^b		0.118	0.075	
Exon 6				
Psoriatics only				
BCC cases	3	11	6	20
Controls	8	9	3	20
OR (95% CI)[a]	1.00	3.26 (0.66–16.03)	5.33 (0.78–36.31)	
P^b		0.138	0.078	

[a] The *CC* genotype served as reference category.
[b] χ^2 test.

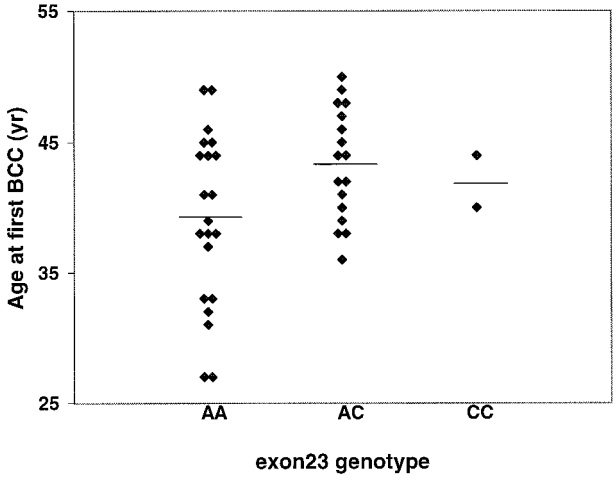

Fig. 1. The correlation between *XPD* exon 23 genotype and age at the first occurrence of BCC. All of the BCC cases ($n = 40$) are depicted. *Bars*, the mean ages (39, 43, and 42 years).

distribution of age and sex for the study persons. We analyzed two polymorphisms: one amino acid substitution and one silent nucleotide substitution; the distribution of the genotypes in the study groups is shown in Table 2.

Exon 23. The *A*→*C* polymorphism in exon 23 at nucleotide position 35931 gives rise to the amino acid substitution Lys→Gln. In our study population, the variant allele *C* had an average frequency of 0.32, which agrees with a previous study (7). The distribution of genotype by case-control status was comparable between psoriasis and nonpsoriasis patients (Table 2). Among all of the BCC cases, 95% carried at least one *A* allele, and 53% were homozygotes (genotype *AA*). Among all of the controls, 83% carried at least one *A* allele, and 43% were *AA*. The association between exon 23 genotype and risk of BCC is shown in Table 3, and all ORs are calculated relative to subjects with the *CC* genotype. Subjects with the *AA* or *AC* genotypes were at higher risk of skin cancer; the ORs were 4.3 (95% CI, 0.79–23.57) and 3.7 (95% CI, 0.67–20.64), respectively.

We also found an association between the exon 23 genotype and the age at which the BCC cases had their first skin tumor (Fig. 1). The mean ages at first tumor for the genotypes *AA*, *AC*, and *CC* were 39, 43, and 42 years, respectively, indicating that subjects with two *A* alleles may have a higher risk of early BCC than subjects with one or two *C* alleles. The mean age at first tumor for cases with the *AA* genotype was significantly lower than the mean age for the *AC* and *CC* genotypes combined ($P = 0.012$). Comparing the mean ages for the *AA* and *AC* genotypes gave a similar result. The small number of cases with the *CC* genotype ($n = 2$) limits the ability to compare the *AA* or *AC* genotype with the *CC* genotype.

Exon 6. The *A*→*C* polymorphism in exon 6 at nucleotide position 22541 does not result in an amino acid change. The average frequency of the variant allele *A* in exon 6 was 0.48 in this study population, which is in agreement with a previous report (7). Among only the psoriasis patients did we observe a difference in the distribution of genotypes between the cases and controls (Table 2). Among psoriasis patients with BCC, 85% carried at least one *A* allele and 30% were homozygotes (*AA*). Among psoriasis patients without BCC, 60% carried at least one *A* allele and only 15% were *AA*. The association between exon 6 genotype and risk of BCC is shown in Table 3, and the ORs are calculated relative to subjects with the *CC* genotype. Subjects with the *AA* or *AC* genotype were at higher risk of skin cancer; the ORs were 5.3 (95% CI, 0.78–36.31) and 3.3 (95% CI, 0.66–16.03), respectively.

The association between the genotype of exon 6 and the age at onset of skin cancer was seen only in psoriasis patients and is presented in Fig. 2. For the psoriasis patients, the mean ages at first tumor for the genotypes *AA*, *AC*, and *CC* were 37, 43, and 46 years, respectively, indicating that subjects with two *A* alleles may have a higher risk of early BCC than subjects with one or two *C* alleles. The mean age at first cancer for cases with the *AA* genotype was marginally lower than the mean age for cases with the genotypes *AC* and *CC* combined ($P = 0.060$). Comparing the mean ages for the *AA* and *AC* genotypes gave a similar result. The small number of cases with the *CC* genotype ($n = 3$) precludes statistical significance when comparing the

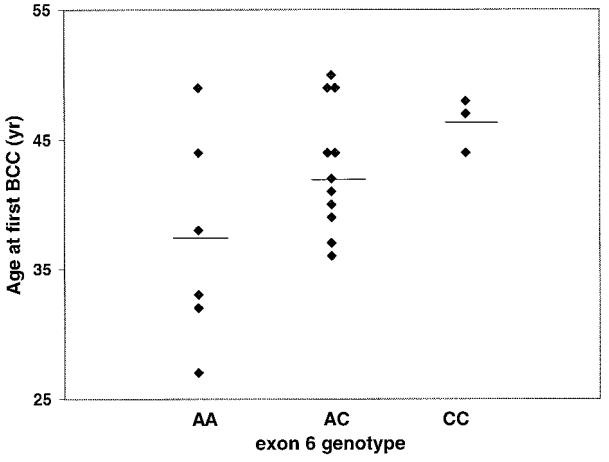

Fig. 2. The correlation between *XPD* exon 6 genotype and age at the first occurrence of BCC. Only the BCC cases with psoriasis ($n = 20$) are depicted. *Bars*, the mean ages (37, 43, and 46 years).

AA or *AC* genotype with the *CC* genotype. For the nonpsoriatics, the mean ages at first tumor for the genotypes *AA*, *AC*, and *CC* were 42, 39, and 41 years, respectively.

The PCR/RFLP assay did not allow us to identify the haplotype, but the combinations of the genotypes in exon 6 and exon 23 are summarized in Table 4. Interestingly, a person being homozygous *CC* in exon 23 (11%) was always homozygous *CC* in exon 6, and a person being homozygous *AA* in exon 6 (24%) was always homozygous *AA* in exon 23. There was a large proportion of individuals being heterozygous for the nucleotide variations in both exon 6 and exon 23 (28%). These data indicate strong linkage disequilibrium in the material and would be consistent with an absence of the exon6^Aexon23^C haplotype.

Previously we have measured the DNA repair capacity in this study population by use of a host cell reactivation assay in lymphocytes,[5] but we found no relationship of the DNA repair capacity with any of the two polymorphisms studied. In another study of the same population, we have reported the level of DNA damage by single cell gel electrophoresis (comet-assay) and the DNA repair capacity by unscheduled DNA synthesis in lymphocytes (11). Neither of these two parameters correlated with any of the polymorphisms.

Table 4 Combinations of the genotypes in exon 6 and exon 23[a]

		Exon 23			
		AA	*AC*	*CC*	Total
Exon 6	*AA*	19	0	0	19
	AC	16	22	0	38
	CC	3	11	9	23
	Total	38	33	9	

[a] The polymorphisms are linked χ^2 test, $P < 4 \times 10^{-10}$).

Discussion

Here we report from a study of two polymorphisms in the DNA repair gene *XPD* (7, 8) in relation to a cancer disorder. For both polymorphisms, we found that the distribution of genotypes differed between the study groups, and that the genotype was associated with the age at which the skin cancer patients had their first tumor and possibly the risk of BCC.

The frequencies of the *A* and *C* alleles in exon 23 were 0.68 and 0.32, respectively, in our study population. We found that the *A* allele was associated with an increased risk of BCC. Forty-eight percent of our population was homozygous *AA*, and they had a more than 4-fold higher risk of BCC than individuals being homozygous *CC* (11%). Also the heterozygotes (genotype *AC*) had a higher risk of BCC that was almost 4-fold increased. In addition, we found that BCC cases with the *AA* genotype developed their first tumors at an earlier age than cases with the *AC* or *CC* genotype. The data suggest that the *C* allele has a protective influence against the cancer.

The *A*→*C* variation in exon 23 gives rise to the amino acid substitution Lys→Gln, which is a change from a basic to a polar amino acid. The nucleotide variation is located about 50 bases upstream from the poly(A) signal, and could possibly improve the function of the XPD protein.

The nucleotide substitution in exon 6 appears to be very common in the population, inasmuch as the frequency of the least common allele *A* in our study was 0.48. Only among the psoriasis patients did we find an association of the genotype with the risk of BCC. Twenty-three percent of the psoriasis patients had the genotype *AA* in exon 6, and they had a more than 5-fold higher risk of BCC than the psoriasis patients with the *CC* genotype (28%). The heterozygotes (genotype *AC*) had a more than 3-fold higher BCC risk. We also observed that the psoriasis patients with BCC who carryed at least one *A* allele developed their first skin tumor earlier in life than the psoriasis patients with BCC carrying two *C* alleles.

The nucleotide substitution in exon 6 does not change an amino acid. It could conceivably affect the stability of the mRNA or disturb protein synthesis by converting a high-usage codon to a low-usage codon in the 5′ proximal region of the gene (12–14). It is, however, important to emphasize that the *XPD* polymorphisms studied need not be directly responsible for the differences in cancer risk. Another possible explanation is that the polymorphisms may cosegregate with another difference in *XPD*, whose function (or lack of function) contributes to the development of malignancy. Finally, *ERCC1* is located close to *XPD* on chromosome region 19q13.2–13.3, along with *DNA ligase* and *XRCC1* (15, 16), and these genes may also cosegregate with the polymorphisms. All of the four genes are important elements in repairing DNA damage.

We do not understand why the exon 6 polymorphism had an effect only in the psoriasis groups. It is possible, that the nucleotide variation in exon 6 has no detectable consequences under ordinary circumstances but only becomes important under excessive genotoxic stress. Psoriasis patients are via their treatment exposed to a variety of genotoxic agents including coal tar, psoralen, and methotrexate, and eventually the DNA damage induced by these treatments may exceed the capacity of individuals who are homozygous for the variant *A* allele in exon 6.

Interpretation of our data is limited by the lack of knowledge about the functional significance of the polymorphisms on the *XPD* gene. None of the nucleotide variations in *XPD* are located in any known or hypothesized helicase/ATPase domains (17), which might be expected given that inactivation of these domains causes loss of function as well as disease or

[5] M. Dybdahl *et al.* Low DNA repair is a risk factor in skin carcinogenesis: a study of basal cell carcinoma in psoriasis patients, submitted for publication, 1998.

preimplantation lethality (6, 18). The XPD protein is a subunit of transcription factor TFIIH, which contains at least nine subunits. It is possible, that variations in the XPD subunit may cause minor structural changes that could modulate its interactions with other subunits, thereby modifying the overall transcriptional activity of the complex.

This preliminary study raises the possibility of an association of BCC development with two polymorphisms in the *XPD* gene, which makes this gene an important candidate for studies in susceptibility to commonly occurring forms of cancer. However, additional studies with larger sample sizes are required to detect the small effects observed. Future case-control studies of these two and other sequence variants identified in the *XPD* gene (8) and characterization of the functional significance of these variants will help to an understanding of the role of the *XPD* gene in cancer etiology.

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