DNA Repair Gene *XRCC1* and *XPD* Polymorphisms and Risk of Prostate Cancer

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Abstract

The X-ray repair cross-complementing group 1 (*XRCC1*) and xeroderma pigmentosum group D (*XPD*) genes are involved in base excision repair and nucleotide excision repair of DNA repair pathways, respectively. A growing body of evidence suggests that XRCC1 and XPD are important in environmentally induced cancers, and polymorphisms in both genes have been identified. To determine whether the *XRCC1* (codon *Arg*399*Gln*) and *XPD* (codon *Asp*312*Asn* and codon *Lys*751*Gln*) polymorphisms are associated with prostate cancer susceptibility, we genotyped these polymorphisms in a primarily Caucasian sample of 506 sibships ($n = 1,117$) ascertained through a brother with prostate cancer. Sibships were analyzed with a Cox proportional hazards model with age at prostate cancer diagnosis as the outcome. Of the three polymorphisms investigated, only the *XPD* codon 312 *Asn/Asn* genotype had an odds ratio (OR) significantly different from one (OR, 1.61; 95% CI, 1.03–2.53). Analyses stratified by the clinical characteristics of affected brothers in the sibship did not reveal any significant heterogeneity in risk. In exploring two-way gene interactions, we found a markedly elevated risk for the combination of the *XPD* codon 312 *Asn/Asn* and *XRCC1* codon 399 *Gln/Gln* genotypes (OR, 4.81; 95% CI, 1.66–13.97). In summary, our results suggest that the *XPD* codon 312 *Asn* allele may exert a modest positive effect on prostate cancer risk when two copies of the allele are present, and this effect is enhanced by the *XRCC* codon 399 *Gln* allele in its recessive state.

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Introduction

Prostate cancer is the most common noncutaneous malignancy in men in the United States. It is estimated that in 2002, 189,000 men in the United States were diagnosed with prostate cancer, and ~30,200 deaths were directly attributable to this disease (1). Risk of disease varies most prominently with age, ethnicity, family history, and diet (2). A strong family history indicative of a highly penetrant prostate cancer gene is believed to account for only 5–10% of prostate cancers, whereas a larger percentage of prostate cancers may be due to common polymorphisms in genes giving rise to a low penetrance risk of disease (3–5). Malignant transformation of prostate cells is accompanied by somatic genomic changes, including deletions, amplifications, and point mutations (6, 7). *In vitro* studies of human prostate tissue have demonstrated that DNA adducts form in prostate tissue after exposure to environmental toxins (8, 9). Moreover, intake of antioxidants via the diet or as supplements may decrease prostate cancer risk through the inactivation of reactive oxygen species, thereby protecting the DNA from oxidative damage (10). This evidence suggests that DNA repair capacity may play an important role in prostate carcinogenesis, but little is known about what direct effect DNA repair capacity has on prostate cancer risk.

To our knowledge, no studies have examined the relationship between DNA repair capacity phenotypes and prostate cancer susceptibility, and only a few published studies on DNA repair genotypes and prostate cancer exist. Xu *et al.* (11) found that two genetic variants in the DNA repair enzyme gene *hOGG1* were associated with prostate cancer in both sporadic and familial cases. Van Gils *et al.* (12) showed that the *XRCC1* codon *Arg*399*Arg* genotype was associated with elevated prostate cancer risk in those with low vitamin E or lycopene intake. In linking genotypic risk with environmental exposure, the results of this study were consistent with the theory that DNA repair genes are most relevant in situations of high mutagenic exposures.

There are currently over 100 known DNA repair genes, and most are known to have genetic variation in humans (13). For the present study, we focused on two well-studied DNA repair genes, X-ray repair cross-complementing group 1 (*XRCC1*) and xeroderma pigmentosum group D (*XPD*), which might have relevance in prostate carcinogenesis based on their known functions. XRCC1 is involved in DNA repair in the base excision repair pathway and appears to play a scaffolding role in bringing together a complex of DNA repair proteins, including poly(ADP-ribose) polymerase (PARP), DNA ligase 3 (LIG3), and DNA polymerase-β (14–16). Codons 194 and 399 contain polymorphisms that result in amino acid substitutions within evolutionarily conserved regions (17, 18). Several studies have linked *XRCC1* polymorphisms with biomarkers of DNA damage, including aflatoxin B1-DNA adducts and glycophorin A variants in erythrocytes (19), polyphenol-DNA adducts (20), and DNA repair capacity in lymphocytes (21).

The *XPD* gene codes for a DNA helicase involved in transcription and nucleotide excision repair (22). Mutations in the *XPD* gene can completely prevent DNA opening and dual incision, steps that lead to the repair of DNA adducts (22). The DNA repair function of XPD is critical to reparation of genetic damage from tobacco and other carcinogens (23). Several common single bp substitution polymorphisms in the *XPD* gene have been identified. Lunn *et al.* (24) showed that individuals with the *XPD* codon 751 *Lys*/*Lys* genotype had a 7-fold increased risk of suboptimal DNA repair. The *XPD* codon 312 polymorphism has been associated with increased adduct levels in breast tumor tissue (25).

On the basis of the key role the *XRCC1* and *XPD* genes play in DNA repair mechanisms, we propose that variation in these genes may be associated with prostate cancer susceptibility. We test this hypothesis in a large sample of brothers ascertained through a prostate cancer case. The strength of the family-based design is that it controls for genetic background unrelated to the disease of interest that can potentially confound traditional case–control designs (26).

Materials and Methods

Subjects. We recruited a study population of brothers (n = 1117; 637 cases, 480 controls) between January 1998 and January 2001 from the major medical institutions in the greater Cleveland, Ohio area and from the Henry Ford Health System, Detroit, Michigan. Institutional Review Board approval was obtained from the participating institutions, and all of the study participants gave informed consent. This analysis used concordant and discordant sibling pairs and, hence, has a larger sample size than our previous report (27) on the discordant population only.

Sibling sets consisted of probands with prostate cancer diagnosed at age 73 or younger and at least one brother with or without prostate cancer. If unaffected, the brother "control" was no more than 8 years younger than the proband's age at diagnosis. This age restriction was used to increase the potential for genetic factors affecting disease and to lessen the probability that the controls were not unaffected because simply of being of a younger age. We further confirmed the disease status of unaffected brothers through prostate-specific antigen (PSA) testing (see "Discussion"). By using a sibling-based study design, we are assured that brothers within sibships have been ascertained from the same genetic source population, excluding the potential for bias due to population stratification (26). The composition of the sibling sets with regard to the distribution of cases and controls is described in Table 1. Of the 506 sibships studied, 90% were Caucasian, 9% were African American, and the remaining 1% were Asian or Hispanic. The age at diagnosis for cases ranged from 41 to 79 years and the mean age at diagnosis was 61.9 ± 6.8 years. The mean age at enrollment (for controls) was 62.7 ± 9.1 years. Clinical characteristics, including Gleason score, PSA, and tumor stage were obtained from the cases' medical records.

Table 1 Sibship configurations in a family-based study of prostate cancer (n = 1117)[a]

		Number of controls per sibship (n = 480)					
		0	1	2	3	4	5
Number of Cases per sibship (n = 637)[b]	1	0	347	39	4	2	0
	2	83	13	3	1	0	1
	3+	9	2	0	2	0	0

[a] One hundred ninety-seven cases have no brother controls and are, hence, from concordant sib-pairs; 440 cases have ≥1 brother control, and so are from discordant pairs.
[b] One sibship had 7 affected brothers.

Genotyping. With all study participants, standard venipuncture was used to collect blood samples in tubes with EDTA as an anticoagulant. Genomic DNA was extracted from buffy coats using the QIAmp DNA Blood kit (Qiagen Inc, Valencia, CA). All of the purified DNA samples were diluted to a constant DNA concentration of 10 ng/μl in 10 mM Tris, 5 mM EDTA buffer (pH 8).

A RFLP assay for the XPD codon 751 variant was adapted from Lunn *et al.* (24). We detected the *XPD* codon *Lys*751*Gln* variant by amplifying genomic DNA with the forward primer 5′-CAG GTG AGG GGG ACA TCT G-3′ and the reverse primer 5′-CTC TCC CTT TCC TCT GTT C-3′. The PCR amplification parameters were a 2-min initial denaturation cycle at 94°C, and 30 cycles each of 30 s at 94°C, 30 s at 52°C, and 30 s at 72°C, followed by a 7-min final elongation cycle at 72°C. The 733-bp PCR product was digested with *Mbo*II (New England Biolabs, Beverly, MA) at 37°C for 90 min. Digested products were separated by electrophoresis on a 6% acrylamide gel and were visualized by ethidium bromide staining. Wild-type alleles resulted in 98-bp, 505-bp, and 131-bp fragments after restriction enzyme digestion. The variant alleles resulted in 603-bp, and 131-bp products after digestion.

New Amplification Refractory Mutation System (ARMS) assays (28) were developed for detection of the *XPD* codon 312 and *XRCC1* codon 399 variants. The ARMS assay is an allele-specific PCR method that uses two primers, each with the 3′ terminal base complementary to one of the alleles to be identified. A mismatch at the penultimate 3′ base is also introduced, to decrease nonspecific binding of the primer to the opposite allele (28). An internal control primer pair was included in each reaction (ARMSA: 5′-CCC ACC TTC CCC TCT CTC CAG GCA AAT GGG-3′; ARMSB: 5′-GGG CCT CAG TCC CAA CAT GGC TAA GAG GTG-3′) at a 1:5 dilution (0.1 μM) relative to the allele-specific primers (0.5 μM). All of the ARMS amplifications were performed using Platinum Taq (0.3 units; Invitrogen, Carlsbad, CA) on an MWG Biotech thermal cycler (High Point, NC).

For the *XPD* codon *Asp*312*Asn* variant (24), a 150-bp PCR fragment was generated with the following primers: reverse primer 5′-CAG GAT CAA AGA GAC AGA CGA GCA GCG C-3′; *G* allele forward specific primer 5′-GTC GGG GCT CAC CCT GCA GCA CTT CGG C-3′; *A* allele forward specific primer 5′-GTC GGG GCT CAC CCT GCA GCA CTT CGA T-3′.

For the *XRCC1* codon *Arg*399*Gln* variant (29), a 1161-bp PCR fragment was generated with the following primers: forward primer 5′-CAG GCC CCA GTC TGA CTC CCC TCC AGA TTC-3′; *A* allele-specific reverse primer 5′-AGC AGG GTT GGC GTG TGA GGC CTT ACC TAT-3′; G allele specific reverse primer 5′-AGC AGG GTT GGC GTG TGA GGC CTT ACC TAC-3′. To ensure quality control of both sets of ARMS genotyping results, 5% of samples were randomly selected and genotyped by a second investigator and 1% was sequenced using a 377 ABI automated sequencer. For the *XPD* codon *Asp*312*Asn* variant, the following forward primer was used with the common reverse primer for sequencing 5′-CTG CGA GGA GAC GCT ATC AGC GGC GAC G-3′. For the *XRCC1* codon *Arg*399*Gln* variant, the following reverse primer

was used with the common forward primer for sequencing 5′-ACT TCT GCC CCC CAC CAG CTG TGC CTT TGC-3′.

Statistical Analysis. We first calculated descriptive genotype frequencies, stratified by case-control status. Because this was a family-based study, to estimate the association between a genetic variant and prostate cancer, we used a Cox age-of-onset model, decomposing the variance of sib genotypes into within- and between-family components (30). This allowed for the simultaneous analysis of sibships either with only affected brothers or with affected and unaffected brothers. The Cox model assumed that a susceptibility allele would decrease the age at prostate cancer onset, and unaffected brother "controls" were censored at their age at study enrollment. For the genotype decomposition, the between-family component is simply the sibship's mean genotype score. As an example, assume that we have a single family with two brothers, and that one brother carries no variant alleles, whereas the other carries two variant alleles. Assuming a recessive model in which an individual must have two copies of the variant allele to have an increased disease risk, we could code the first brother as 0 (referent genotype) and the second brother as 1 (at-risk genotype). In this example, the mean genotype score for the two brothers is ½. The regression coefficient for this component measures potential genetic admixture; if statistically significant, it suggests that population stratification exists. The within-family component is the difference between an individual's genotype score and his sibship's mean genotype score. Looking again at the example, the first brother's within-family genotype score would be $0 - ½ = -½$, whereas the second brother's score would be $1 - ½ = ½$. The regression coefficient obtained from modeling these within-family scores reflects the linkage disequilibrium between the variant and prostate cancer and provides an estimate of genotypic risk. To allow for the familial correlation in siblings' genotypes, we used a robust estimator of the variance (31).

Any possible effect modification by age was also evaluated by stratifying by age at diagnosis (<63 *versus* ≥63). In addition, to investigate the potential effect of genotype on disease aggressiveness, we stratified the analyses by the cases' clinical characteristics at diagnosis. Aggressiveness was defined as "low" if a case's Gleason score was <7 and his tumor category was $<T_{2c}$, and "high" if his Gleason score was ≥7 or his tumor stage was $\geq T_{2c}$. The tumor stage reflects the Tumor-Node-Metastasis System (32). All *P*s are from two-sided tests, and all analyses were undertaken with S+ software (version 6.0, Insightful Corp.).

Results

In the 506 sibships studied, 637 brothers were diagnosed with prostate cancer. At the time of diagnosis, mean PSA levels were 14.4 with a range between 0.5 and 1000 and a median of 7.0. Of the 90% of cases in which biopsy Gleason grade was available, the most common grade was 6 (50%), followed by 7 (24%) and 5 (13%). Almost 8% of patients had a Gleason grade of 8 or higher. For those 92% of tumors with stage data, 51% were T_1, 42% were T_2, and 7% were T_3.

Table 2 shows the distribution of the *XRCC1* codon 399, and *XPD* codons 312 and 751 genotypes with respect to case or control status. For the *XRCC* codon 399 and *XPD* codon 751 variants, no discernible case/control differences existed. For the *XPD* codon 312 variant, a slightly higher percentage of cases had the less frequent *Asn/Asn* genotype (11.5 *versus* 8.1%). A formal test for Hardy-Weinberg equilibrium in the control population by ethnic group, showed that, of three polymorphisms of interest, only the *XPD* codon 312 genotype distribution in Caucasians significantly deviated from Hardy-Weinberg equilibrium ($P = 0.02$).

Table 3 gives the odds ratio (OR) estimates for the association between the three genetic variants and prostate cancer. On the basis of the genotypic distributions observed in Table 2, we tested only a recessive model (*i.e.*, two copies of the variant allele are required for a increased risk) for all three genetic variants. Carrying two copies of the *XPD* codon 312 *Asn* allele was positively associated with prostate cancer, giving an OR of 1.61 (95% CI, 1.03–2.53; $P = 0.04$). Restricting the analysis to Caucasians gave a similar positive association (Table 3). No other noteworthy associations were observed.

When stratifying our analyses by clinical characteristics of affected men within the sibships, the null findings for *XRCC* codon 399 and *XPD* codon 751 variants remained (data not shown). The *XPD* codon 312 *Asn/Asn* genotype exhibited slightly higher OR estimates in stratification subsets in which genetic risk would *a priori* be considered less (*i.e.*, cases greater than 62 years of age, cases with a negative family history, cases with a less aggressive tumor, and cases with a lower PSA at diagnosis). The largest OR differentials were observed for the family history and PSA at diagnosis stratifications, in which the OR for the *XPD* codon 312 *Asn/Asn* genotype was about twice as large in the "low risk" subset. For instance, the OR for the *XPD* codon 312 *Asn/Asn* genotype was 0.90 (95% CI, 0.54–1.52) in positive family history sibships, but 1.94 (95% CI, 0.65–5.75) in negative family history sibships. Although empirically different, the ORs for the *XPD* codon 312 *Asn/Asn*

Table 2 Frequency of the *XRCC1* codon *Arg*399*Gln*, *XPD* codon *Asp*312*Asn*, and codon *Lys*751*Gln* genotypes by affection status in 506 prostate cancer sibships

Polymorphism: genotype	Total sample				Caucasian sample			
	Cases ($n = 637$)		Controls ($n = 480$)		Cases ($n = 572$)		Controls ($n = 437$)	
	Frequency	(%)	Frequency	(%)	Frequency	(%)	Frequency	(%)
XRCC1 codon 399:								
Arg/Arg	291	(45.7)	216	(45.0)	245	(42.8)	179	(41.0)
Arg/Gln	274	(43.0)	208	(43.3)	257	(44.9)	203	(46.5)
Gln/Gln	72	(11.3)	56	(11.7)	70	(12.2)	55	(12.6)
XPD codon 312:								
Asp/Asp	277	(43.6)	210	(43.8)	230	(40.3)	180	(41.2)
Asp/Asn	286	(45.0)	231	(48.1)	269	(47.1)	218	(49.9)
Asn/Asn	73	(11.5)	39	(8.1)	72	(12.6)	39	(8.9)
XPD codon 751:								
Lys/Lys	257	(40.4)	196	(41.0)	223	(39.1)	178	(40.9)
Lys/Gln	297	(46.7)	224	(46.9)	273	(47.8)	205	(47.1)
Gln/Gln	82	(12.9)	58	(12.1)	75	(13.1)	52	(12.0)

Table 3 Odds ratio (OR) estimates of prostate cancer risk for two copies of the variant allele of the *XRCC1* codon *Arg*399*Gln*, *XPD* codon *Asp*312*Asn*, and codon *Lys*751*Gln* Polymorphisms

Polymorphism genotype	Total sample (n = 1117)			Caucasian sample (n = 1009)		
	OR	(95% CI)[a]	P	OR	(95% CI)	P
XRCC1 codon 399						
Arg/Arg + *Arg/Gln*	1	Referent Category		1	Referent Category	
Gln/Gln	0.88	(0.56–1.38)	0.57	0.86	(0.54–1.36)	0.52
XPD codon 312						
Asp/Asp + *Asp/Asn*	1	Referent Category		1	Referent Category	
Asn/Asn	1.61	(1.03–2.53)	0.04	1.59	(1.01–2.51)	0.05
XPD codon 751						
Lys/Lys + *Lys/Gln*	1	Referent Category		1	Referent Category	
Gln/Gln	1.14	(0.72–1.82)	0.57	1.20	(0.74–1.95)	0.46

[a] CI, confidence interval.

genotype in the "high" and "low" risk strata for family history and PSA at diagnosis were not statistically significantly different.

To evaluate the impact of the joint effect of two variants, we used a regression model with both main effects from each variant and an interaction term. This provides inference into the component parts and the additional effect of having both variants (*i.e.,* the interaction term) after conditioning on the main effect of each variant. For clarity, however, we report in Table 4 the risk estimates associated with having both variants in the homozygous state *versus* having no variant alleles. The OR for the combined effects of the *XPD* codon 312 *Asn/Asn* and *XRCC1* codon 399 *Gln/Gln* genotypes was 4.81 (95% CI, 1.66–13.97; $P = 0.004$). This OR estimate was larger than the individual variant OR estimates combined on a multiplicative scale. The OR for the combination of the two *XPD* variants was also statistically significant (OR, 1.66; 95% CI, 1.00–2.77; $P = 0.05$), but it was about the same as the OR of the *XPD* codon 312 *Asn/Asn* genotype alone, suggesting that the *XPD* codon 751 *Gln/Gln* genotype did not alter the risk associated with the *XPD* codon 312 *Asn/Asn* genotype. The results for these genotypic combinations were similar for the total sample and the Caucasian subset.

We next examined the distribution of the at-risk genotype(s) within sibships. The *XPD* codon 312 *Asn/Asn* genotype was found in 71 of the 414 study sibships that had at least one unaffected sib (*i.e.,* discordant sibships). Among these 71 sibships, in 51% only the affected sib(s) had the "at risk" genotype, in 31% only the unaffected sib(s) had the genotype, and in the remaining 18%, both an affected and an unaffected sib had the genotype. In the 114 sibships that had two or more affected brothers (*i.e.,* concordant sibships), 23 sibships had at least one brother with the XPD codon 312 *Asn/Asn* genotype, but only 6 of the 23 had an affected brother pair that shared this genotype. For the combination of the XPD codon 312 *Asn/Asn* and XRCC1 codon 399 *Gln/Gln* genotypes, only 31 of the 414 discordant sibships had sibs who carried both of these genotypes. In these 31 families, 16 (51.6%) had only affected sibs with both high risk XPD and XRCC1 genotypes, 8 (25.8%) had only unaffected sibs with both high risk genotypes, and the remaining 7 (22.6%) had both affected and unaffected sibs who shared the XPD-XRCC1 high risk genotype combination. Nine concordant sibships had one or more sib who had both the XPD codon 312 *Asn/Asn* and XRCC1 codon 399 *Gln/Gln* genotypes, with three of the nine having an affected brother pair who shared both of these genotypes.

Discussion

Polymorphisms in *XRCC1* and *XPD* genes have been studied in relation to risk for cancers of the lung (33, 34), head and neck (35, 36), breast (25, 37), and skin (38), but to our knowledge only one previous study has examined either of these genes in relation to prostate cancer risk (12). We examined the *XRCC1* codon 399 and *XPD* codons 312 and 751 polymorphisms in relation to prostate cancer risk in a large sample of primarily

Table 4 Odds ratio (OR) estimates of prostate cancer risk for combined effects of two copies of the variant alleles of the *XRCC1* codon *Arg*399*Gln*, *XPD* codon *Asp*312*Asn*, and codon *Lys*751*Gln* polymorphisms

Genotype combinations	Total sample (n = 1117)			Caucasian sample (n = 1009)		
	OR	(95% CI)[a]	P	OR	(95% CI)[a]	P
XRCC1 codon 399 *Arg/Arg* or *Arg/Gln* +						
XPD codon 312 *Asp/Asp* or *Asp/Asn*	1	Referent category		1	Referent category	
XRCC1 codon 399 *Gln/Gln*+						
XPD codon 312 *Asn/Asn*	4.81	(1.66–13.97)	0.004[b]	4.81	(1.64–14.10)	0.004[b]
XRCC1 codon 399 *Arg/Arg* or *Arg/Gln*+						
XPD codon 751 *Lys/Lys* or *Lys/Gln*	1	Referent category		1	Referent category	
XRCC1 codon 399 *Gln/Gln*+						
XPD codon 751 *Gln/Gln*	1.58	(0.46–5.42)	0.46	1.44	(0.39–5.34)	0.59
XPD codon 312 *Asp/Asp* or *Asp/Asn*+						
XPD codon 751 *Lys/Lys* or *Lys/Gln*	1	Referent category		1	Referent category	
XPD codon 312 *Asn/Asn*+						
XPD codon 751 *Gln/Gln*	1.66	(1.00–2.77)	0.05	1.63	(0.97–2.75)	0.06

[a] CI, confidence interval.
[b] P for the interaction term in the logistic regression model is 0.01.

Caucasian sibships. Only the XPD codon 312 *Asn* allele showed a modest association with increased prostate cancer risk, ~60%, when two copies of the allele were present. Perhaps more revealing, however, was the potential interaction between the *XPD* codon 312 *Asn* allele and the *XRCC1* codon 399 *Gln* allele. When both alleles were present in their homozygous states, the risk for prostate cancer increased 4.8-fold. Whereas mechanistically genetic interactions are thought to be more likely between genes involved in the same biological pathways, it is not unprecedented to find an increased joint effect between genes acting in different pathways. In the case of XRCC1 and XPD, because DNA damage caused by a mixture of environmental exposures may require either the base excision repair or nucleotide excision repair pathways, a reduction in the efficiencies of only one of these pathways may not increase disease risk to the same extent as when both pathways are compromised. Furthermore, our findings are not unprecedented because a report exists of an *XRCC1-XPD* interaction in relation to lung cancer risk (33).

Although DNA repair capacity in breast cancer is a major area of research, primarily because of elucidation of the important role that the *BRCA1* and *BRCA2* gene products have in double-stranded DNA repair, most investigations of prostate cancer susceptibility genes have focused on the androgen biosynthesis pathway (4). Interestingly, recent studies have found that men with mutations in either the *BRCA1* (39, 40) or *BRCA2* (41, 42) genes are at increased risk for prostate cancer. Only a few studies of more common DNA repair genetic variants and prostate cancer risk exist in the literature. Xu *et al.* (11) studied 18 different genetic variants of the DNA repair enzyme gene *hOGG1*, involved in base excision repair, and found the genotype frequency of two sequence variants (11657*A/G* and *Ser*326*Cys*) was significantly different between prostate cancer cases and controls. They also confirmed the association with the 11657*A/G* variant in a family-based association study. Van Gils *et al.* (12) studied three genetic variants in another base excision repair enzyme gene, *XRCC1*, and found no associations between *XRCC1* polymorphisms and prostate cancer when only comparing genotype frequencies in cases and controls. However, when they stratified the study population by intake of several different dietary antioxidants, the more common *XRCC1* codon 399 *Arg/Arg* genotype was associated with prostate cancer in those with low vitamin E or lycopene intake. We, on the other hand, found the less common *XRCC1* codon 399 *Gln*/*Gln* genotype to be a potential modifying factor for prostate cancer risk associated with the *XPD* codon 312 *Asn*/*Asn* genotype. It is not inconceivable that interactions at the *XRCC1* codon 399 locus are dependent on genotype, with some genetic or environmental risk factors preferentially interacting with the *Arg/Arg* genotype and others more likely to interact with the *Gln/Gln* genotype. It should be noted that the unadjusted ORs for the *XRCC1* codon 399 *Gln/Gln* genotype in the study of van Gils *et al.* (12) was 0.77, which is comparable with our OR estimate of 0.88. Previous studies of the *XRCC1* codon 399 polymorphism are equivocal with some finding increased risk for the *Gln* allele (35, 37, 43) but others finding an increased risk for the *Arg* allele (44, 45).

Our family-based study had several strengths, which include the size of the study population, the elimination of potential bias due to population genetic substructure, and full utilization of sibship data (without parental genotypes) that were composed of numerous configurations including sibships with only affected brothers. In our analyses, we used a robust genotype score test that decomposed the variance of sib genotypes into between- and within-family components (46, 47). The latter reflects the linkage disequilibrium between marker and disease status and was our measure of genotypic risk. The between-family component measures potential genetic admixture; for all analyses, the covariate for this variable never approached statistical significance (data not shown). To increase statistical power and use disease discordant and concordant sibships, we used a Cox modeling framework of the genotype score test (30) that relied on the reasonable assumption that a susceptibility genotype decreases age at disease onset. Here, the unaffected brother "controls" were censored at age at study enrollment. To further ensure the disease status of unaffected brothers, we tested their serum PSA levels. Participants with PSA levels above 4 ng/ml were informed and advised to investigate their disease status with their physician. They were retained in the study as controls unless a subsequent diagnosis of prostate cancer was made; at which time they were reclassified as cases. Keeping them in the study is important because excluding men with elevated PSA levels regardless of their ultimate prostate cancer status can lead to biased risk estimates (48, 49).

In addition to controlling for potential population stratification, our family-based study was likely more homogeneous with respect to prostate cancer genetic risk factors because a large portion of the sibships (23%) had two or more affected individuals. The incorporation of genotypic information of unaffected brothers for a common disease such as prostate cancer can also significantly increase statistical power (50). Another advantage of a family-based design concerns the absence of Hardy-Weinberg equilibrium in a control population that we found for the *XPD* codon 312 polymorphism. Although this would be troublesome in a case-control population, it was less of a concern in our family-based study because it could be due to the association of this genotype with prostate cancer-affected brothers of these controls. Despite the many advantages of our family-based population, several potential disadvantages also exist including the potential for selection bias due to difficulty of enrolling multiple family members (51) and decreased statistical power compared with case-control populations under some circumstances (52, 53). In fact, we found, when stratifying on family history, that the risk associated with the *XPD* codon 312 *Asn/Asn* genotype was greater in sibships with a negative family history, which would suggest that the population risk for this genotype may be greater than we report.

In summary, we found a modest increased risk of prostate cancer in individuals with two copies of the *XPD* codon 312 *Asn* allele. This risk was increased 3-fold when two copies of the *XRCC1* codon 399 *Gln* were also present. These results suggest reduced DNA repair capacity may play a role in prostate cancer, particularly when the function of two genes involved in different DNA repair pathways are both compromised. Additional studies of DNA repair polymorphisms are required to bring our knowledge of this pathogenic pathway in prostate carcinogenesis up to the level of understanding currently existing in smoking-related and breast cancers.

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