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CANDIDATE'S THESIS

Radiation Response Genotype and Risk of Differentiated Thyroid Cancer: A Case-Control Analysis

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Background: **Radiation is the only clear etiologic agent for differentiated thyroid cancer (DTC). Understanding the factors affecting sensitivity to gamma radiation and susceptibility to DTC will be critical to early detection and prevention of DTC.** ***Hypothesis:*** **Germline variants of double-strand break repair genes are markers of DTC risk.** ***Objective:*** **Determine the frequency of common single nucleotide polymorphisms of genes of the double-strand break repair pathway in patients with DTC and cancer-free controls.** ***Study Design:*** **Case-control study.** ***Methods:*** **This study included 134 patients with DTC, 79 patients with benign thyroid lesions, and 166 cancer-free control subjects. To avoid ethnic confounding, all subjects were non-Hispanic whites. Genotype analyses were performed on DNA isolated from peripheral blood lymphocytes. Multivariate logistic regression analyses were performed to estimate the risk of DTC associated with each variant genotype.** ***Results:*** **The *XRCC3* 18067T polymorphic allele was found significantly more commonly among the DTC cases than for the control subjects ($P = .006$). After multivariate adjustment, having the *XRCC3* 18067T allele was associated with an increased risk of DTC (adjusted odds ratio [OR] = 2.1; 95% confidence interval [CI] = 1.3 to 3.4; $P = .004$). In addition, there was a suggestion that the *XRCC3* 18067T polymorphic allele was more common among the patients with benign thyroid disease ($P = .054$), and the homozygous polymorphic genotype was associated with risk for benign thyroid disease (adjusted OR = 2.1; 95% CI = 0.9–4.9; $P = .078$).** ***Conclusions:*** **In this case-control analysis, the *XRCC3* 18067T polymorphism is associated with DTC risk. However, such work needs confirmation in larger studies.** ***Key Words:*** **Thyroid cancer, DNA repair, genotype, case-control study.**

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INTRODUCTION

Thyroid cancer is the most common endocrine malignancy, with an estimated 25,690 new cases in the United States during the year 2005 (reflecting a doubling over 20 years).[1] Papillary thyroid carcinoma is the predominant type, and along with follicular and Hürthle cell carcinoma is termed differentiated thyroid carcinoma (DTC), accounting for almost all (approximately 90%) thyroid malignancies.[2] Although the exact etiology of DTC remains unknown, exposure to gamma radiation appears to be a predisposing factor. However, not everyone exposed to radiation will develop thyroid cancer, and most patients with thyroid cancer report no known history of radiation exposure. This suggests that potential predisposing genetic factors impact an individual's sensitivity to gamma radiation and his or her susceptibility to DTC.

Ionizing radiation, such as x-rays and gamma rays, causes a variety of DNA damage, including single- and double-strand DNA breaks and the generation of reactive oxygen species that cause DNA base damage.[3–5] This type of DNA damage, which causes relatively minor changes in the helical DNA structure, is efficiently removed by one of the most highly conserved DNA repair mechanisms, base-excision repair.[6] Unrepaired damage, however, replicates with DNA, causing originally one-strand lesions to spread to the second DNA strand, causing chromosomal instability.[7] Furthermore, the two-strand damage, also called "chromosomal damage," interferes with subsequent rounds of chromosomal

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replication and therefore needs chromosomal repair.[8] The major chromosomal repair pathway is homologous recombination.[9] Deficient repair of DNA double-strand breaks, resulting in an abnormally high frequency of chromosomal aberrations (including breaks, deletions, and reciprocal translocations) after exposure to radiation, appears to be associated with a predisposition to cancer.[10] Cytogenetic assays in peripheral blood lymphocytes have been used extensively to survey or evaluate human exposure and response to genotoxic agents, including ionizing radiation. We previously hypothesized that individual differences in sensitivity to gamma–radiation-induced chromatid breaks in peripheral blood lymphocytes may be a marker of susceptibility to thyroid disease, especially DTC.[11] To explore this hypothesis, we have conducted a pilot case-control study involving 106 patients with thyroid disease (57 patients with papillary thyroid carcinoma and 49 patients with benign thyroid disease) and 105 cancer-free control subjects. Multivariate logistic regression analyses identified that an elevated gamma–radiation-induced breaks per cell value as a significant risk factor for thyroid disease generally (odds ratio [OR] = 4.04; 95% confidence interval [CI], 2.21 to 7.41) and for both papillary thyroid carcinoma (OR = 4.43; 95% CI, 2.07–9.48) and benign thyroid disease (OR = 3.66; 95% CI, 1.69–7.92) when analyzed separately. We concluded that high levels of chromatid breaks induced by gamma radiation in lymphocytes may constitute an independent risk factor for both papillary thyroid carcinoma and benign thyroid disease, with the risk estimates appearing stronger for papillary thyroid carcinoma.

If, as we have demonstrated in this work, sensitivity to gamma radiation as measured by the number of chromosomal aberrations in lymphocytes is associated with risk of DTC, then a spectrum of susceptibility would appear to exist in the population. Such a finding may have implications for primary cancer prevention and for early cancer detection. The phenotypic assay used thus far is both cumbersome and expensive; therefore, an inexpensive and quick assay is needed to make screening of large populations feasible. The genotyping of genes responsible for phenotypic response to gamma radiation may offer such advantages. The objective of this study was to compare the frequency of single-nucleotide polymorphisms in genes (*XRCC3*, *XRCC7*, *RAD51*, *RAD52*, *BRCA1*, and *BRCA2*) contributing to the response to gamma radiation. The next step in exploring the observed sensitivity to gamma radiation, presumably a marker of susceptibility to DTC, would be to explore the genetic differences responsible for the observed phenotypic differences in chromosomal sensitivity. Therefore, we have performed a case-control analysis examining genes involved in the response to gamma radiation.

Double-strand breaks, the most harmful damage to DNA, can result from exogenous agents such as ionizing radiation and from endogenous agents such as reactive oxygen species.[9] Two double–strand-break repair pathways are responsible for the repair of strand breaks produced directly and indirectly by exposure to ionizing radiation or indirectly by incomplete repair of other types of damage.[12] Nonhomologous end joining is considered the major pathway for initial double–strand-break repair in humans and requires little or no sequence homology.[9] At least 15 genes are used to repair double-strand breaks by way of the relatively error prone nonhomologous end joining mechanism, which is operative primarily in noncycling cells.[13] Homologous recombination repair relies on extensive nucleotide sequence complementary between the intact homologous partner chromosome or sister chromatid and the damaged structure for strand exchange and repair.[14] More than 20 genes of the homologous recombination repair pathway are active in repairing double-strand breaks in replicating cells in S and G2 phase. Homologous recombination repair plays an important role in double–strand-break repair and therefore is essential for cell viability and genome stability.[9] Impairment of this repair mechanism may lead to loss of genomic integrity; consequently, such strand–break-repair pathways have a key role in preventing mutations, chromosomal instability, and cancer.[9]

In humans, several genes of the x-ray repair cross-complementing (XRCC) group participate in either nonhomologous end joining (*XRCC4, XRCC5, XRCC6,* and *XRCC7*) or homologous recombination repair (*XRCC2* and *XRCC3*) pathways.[15] Recent studies found that some of these genes are polymorphic; one, *XRCC3* located on chromosome 14q32.3, participates in homologous recombination repair of DNA double-strand breaks and cross-links.[16,17] XRCC3 is required for the assembly and stabilization of RAD51, to which it is structurally related. XRCC3-deficient cells do not form RAD51 foci after radiation damage and exhibit genetic instability and increased sensitivity to DNA-damaging agents.[18] *XRCC3* has a polymorphic C→T substitution at position 18067 in exon 7 that results in an amino acid change (Thr241Met),[19] which may affect the function of the enzyme or its interaction with other proteins involved in DNA damage repair. To our knowledge, no studies of the association between the *XRCC3* C18067T polymorphism and thyroid cancer have been reported. We have not identified other common exonic polymorphisms of *XRCC3* or of *XRCC2* and *XRCC6*, whereas *XRCC5* appears to have only some rare microsatellite polymorphisms.[20] *XRCC4* has one exonic variant (T401C) that causes an amino acid change of Ile to Thr, but the frequency of the variant allele is only approximately 0.05, and its functional impact is unknown.[21] *XRCC7* only has an intronic variant (T6721G).[22]

In addition to the *XRCC*, other genes such as *RAD51*, *RAD52*, *RAD54, BRCA1,* and *BRCA2* also participate in homologous recombination repair.[15] RAD51 acts with BRCA1 and BRCA2 in a common DNA damage response pathway implicated in double-strand repair and interacts with other DNA repair proteins in homologous recombination repair pathways to ensure chromosome stability.[14] No exonic polymorphism of *RAD51* that results in an amino acid change has been identified, suggesting the important role of this highly conserved DNA repair gene.[23] A single nucleotide polymorphism in the 5' untranslated region of *RAD51* (RAD51-G135C) may modify the risk of cancer in *BRCA1/2* mutation carriers.[24] RAD52 acts in conjunction with RAD51 in the homologous repair pathway, but unlike RAD51, homozygous deletion

of RAD52 is not an embryonic lethal event.[25] Although two nonsense (stop codon) mutations have been identified, they are relatively rare (allele frequency < 5%) and have not been shown to affect cancer risk.[25,26] Three variants that result in an amino acid changes have also been identified, but these appear to be very infrequent.[23] In addition, a more common polymorphism has been identified in the 3' untranslated region (C2259T). *RAD54* polymorphisms that result in amino acid change have been identified, but reported allele frequencies are less than 2%.[23] BRCA1 and BRCA2 also play a critical role in the early steps of homologous recombination repair, and germline mutations in *BRCA1* and *BRCA2* are associated with familial breast cancer.[9] Numerous common polymorphisms in *BRCA1* and *BRCA2* that result in amino acid changes have been identified, and at least one of these appears to be associated with cancer risk.[27–30] The role of these polymorphisms in DTC, however, is untested.

We hypothesized that genetic variants of genes of the recombination repair system are more frequent in DTC patients. To test this hypothesis, we conducted a case-control study to compare the frequencies of polymorphisms in genes of this system in patients with DTC with their frequencies in cancer-free control subjects. The frequencies of these polymorphisms have not been reported in DTC patients. Such work could facilitate the understanding of DTC development and ultimately help improve prevention efforts.

METHODS

This was a molecular epidemiologic case-control study of DTC to examine the frequency of single nucleotide polymorphisms of genes of the DNA double–strand-break repair pathway to further explore our preliminary findings[11] regarding the role of radiosensitivity phenotype in the etiology of DTC. The cases and controls were similar with respect to age and sex, and to eliminate potential confounding genetic by differences among differing ethnic groups, only non-Hispanic whites were included. Each subject completed a self-administered questionnaire regarding demographic and exposure information. Radiation exposure was defined as whole body or head and neck radiation exposure. Those subjects who had smoked more than 100 cigarettes in their lifetimes were defined as smokers, with those having quit more than 1 year before recruitment were classified as former smokers. Those who drank alcoholic beverages at least once a week for more than 1 year were defined as drinkers, with those having quit more than 1 year before recruitment were classified as former drinkers. After institutional review board-approved informed consent, each subject donated a 20 mL fresh blood sample.

Patients (n = 305) presenting to the head and neck surgery clinic between November 1999 and September 2004 with either a history of DTC (n = 19), recurrent DTC (n = 14), or with newly diagnosed previously untreated DTC or a mass suspicious for DTC (n = 272) were recruited. Seventeen Asian-American patients, 22 African-American patients, and 45 Hispanic-American patients were excluded from this genotyping analysis to reduce potential racial confounding. We restricted age to greater than 17 years. Eight additional patients were excluded because their final histologies were not DTC (anaplastic thyroid carcinoma [n = 3], medullary thyroid carcinoma [n = 4], and mucoepidermoid carcinoma of the thyroid [n = 1]). The 213 remaining patients were included in genotyping analyses. From this group, 134 had DTC (papillary carcinoma [n = 118], follicular carcinoma [n = 11], and Hurthle-cell carcinoma [n = 5]), whereas the remainder had benign pathology (n = 79) and were included as an intermediate group for comparison. In our previous work, these patients with benign thyroid pathology had intermediate levels of sensitivity to gamma radiation as measured by the phenotypic assays.[11,31] We also included patients with benign thyroid disease as a group for comparison because such lesions may be precursors for DTC.[7,8]

The control subjects (n = 166) were identified without knowledge of genotyping results from a cancer-free control group (n = 469) of a study of molecular epidemiology of squamous carcinoma of the head and neck ongoing during approximately same time period at our institution. These control subjects were recruited among people (spouses/visitors) who accompanied patients to our institution if they had no previous history of cancers (except nonmelanoma skin cancer).

The leukocyte cell pellet obtained from the buffy coat by centrifugation of 1 mL of whole blood was used for genomic DNA extraction with the QIAGEN DNA Blood Mini Kit (QIAGEN Inc., Valencia, CA) according to the manufacturer's instructions. Polymerase chain reaction-restriction fragment-length polymorphism assays were used for genotyping with primer and enzyme specifics listed in Table I.

Chi-square analyses (two-sided) were used to calculate the frequency differences between cases and controls in age, sex, radiation exposure history, smoking status, and alcohol status. Univariate analysis using chi-square analyses (two-sided) was first performed to compare the differences in genotype and allele frequencies between case and control subjects. The ORs and their 95% CIs for the genotypes were calculated by logistic regression analysis with adjustment for age, sex, radiation exposure, smoking, and alcohol use. All of the statistical analyses were performed with Statistical Analysis System software (Version 9.0; SAS Institute, Inc., Cary, NC) or STATA 7.0 software (Stata Corporation, College Station, TX). A *P* value of less than .05 was used as a cutoff for significance.

RESULTS

The mean age was 46.8 (median 45.5, range 18–78) years for the DTC case subjects, 49.3 (median 51, range 20–83) years for the benign case subjects, and 51.5 (median 51, range 20–77) years for the control subjects. No difference existed between the DTC cases and controls in age distribution (based on median of the controls), sex, radiation exposure, or smoking status (Table II). However, a higher percentage of DTC cases than controls reported a history of never drinking ($P = .030$) (Table II). The case subjects with benign thyroid disease were more commonly female than the control subjects ($P = .002$), but these groups did not differ significantly with respect to age, radiation exposure, smoking, or drinking (Table II). Although these findings suggest that, for the most part, the two groups were similar with respect to demographic and exposure characteristics, all logistic regression analyses included adjustment for age, sex, radiation exposure history, smoking, and drinking.

The genotyping results are presented in Table III. The genotype assays were successful in 97% or more of the samples tested with exception of *RAD52* T38207C 92.1%; furthermore, on repeat genotyping of more than 10% of each genotype, 100% concordance was found. The genotype distribution was significantly different between the DTC case subjects and the control subjects for the *XRCC3* C18067T genotype ($P = .008$) (Table III). Furthermore, the less-common polymorphic allele was significantly more frequent among the DTC case subjects than the control subjects for

TABLE I.
Genotyping Methods.

Gene (location)	Amino Acid (base change)	Primer Sequences (PCR profiles)	Method (enzyme)	Genotypes (band lengths)
XRCC3	Thr241Met	5′-GCTGTCTCGGGGCATGGCTC-3′ (S)	RFLP	CC (208 bp)
(exon 7)	(C18067T)	5′-ACGAGCTCAGGGGTGCAACC-3′ (AS)	(*NlaIII*)	CT (208, 120, 88 bp)
AF037222		(95°C5m; 95°C30s, 60°C30s, 72°C60s, 30 cycle; 72°C2m)		TT (120, 88 bp)
XRCC7	—	5′-CGGCTGCCAACGTTCTTTCC-3′ (S)	RFLP	TT (274, 94 bp)
(intron 8)	(T6721G)	5′-TGCCCTTAGTGGTTCCCTGG-3′ (AS)	(*PvuII*)	TG (368, 274, 94 bp)
L27425		(95°C5m; 95°C30s, 60°C35s, 72°C45s, 30 cycle; 72°C10m)		GG (368 bp)
RAD51	—	5′-TGGGAACTGCAACTCATCTGG-3′ (S)	RFLP	GG (86, 71 bp)
(5′ UTR)	(G135C)	5′-GCGCTCCTCTCTCCAGCAG-3′ (AS)	(*MvaI*)	GC (157, 86, 71 bp)
D14134	—	(95°C5m; 95°C30s, 60°C35s, 72°C45s, 30 cycle; 72°C10m)		CC (157 bp)
RAD52	—	5′-GGCCACTTGGTCTAGGAGAA-3′ (S)	RFLP	TT (186, 12 bp)
(intron 11)	(T38207C)	5′-CTTCATGTCCTGGCTCTTCC-3′ (AS)	(*BafI*)	TC (186, 98, 88, 12 bp)
AY527412		(95°C5m; 95°C30s, 62°C35s, 72°C40s, 35 cycle; 72°C10m)		CC (98, 88, 12 bp)
BRCA1	Pro871Leu	5′-GTTTCAAAGCGCCAGTCATTGGATC-3′ (S)	RFLP	CC (81, 23 bp)
(exon 11)	(C2731T)	5′-GGACTTTGTTTCTTTAAGGACCCAG-3′ (AS)	(*BamHI*)	CT (104, 81, 23 bp)
NM007294		(95°C5m; 95°C30s, 60°C30s, 72°C30s, 35 cycle; 72°C10m)		GG (85, 23 bp)
BRCA1	Glu1038Gly	5′-GAAAGAGAAATGGGAAATGAGAAC-3′ (S)	RFLP	AA (108 bp)
(exon 11)	(A3232G)	5′-TTCATTAATATTGCTTGAGCGAGCT-3′ (AS)	(*SacI*)	AG (108, 85, 23 bp)
NM007294		(95°C5m; 95°C30s, 55°C30s, 72°C30s, 35 cycle; 72°C10m)		GG (85, 23 bp)
BRCA1	Lys1183Arg	5′-AGAACAGCCTATGGGAAGTA-3′ (S)	RFLP	AA (233 bp)
(exon 11)	(A3667G)	5′-GAGGGGCCAAGAAATTAGAG-3′ (AS)	(*MnLI*)	AG (233, 143, 90 bp)
NM007294		(95°C5m; 95°C30s, 62°C35s, 72°C40s, 35 cycle; 72°C10m)		GG (143, 90 bp)
BRCA1	Ser1613Gly	5′-CCAGAGTCAGCTCGTGGTGGC-3′	RFLP	AA (234 bp)
(exon 16)	(A4956G)	5′-AATTCTTCTGGGGTCAGGCCAG-3′	(*AvaII*)	AG (234, 149, 85 bp)
NM007294		(95°C5m; 95°C30s, 60°C30s, 72°C30s, 35 cycle; 72°C10m)		GG (149, 85 bp)
BRCA2	Asn372His	5′-CGCTGATGAATGTGAAAAAT-3′	RFLP	AA (127 bp)
(exon 10)	(A1342C)	5′-TCCACTCTCAAAGGGCTTCATAT-3′	(*NdeI*)	AC (127, 106, 21 bp)
NM000059		(95°C5m; 95°C30s, 54°C35s, 72°C45s, 35 cycle; 72°C10m)		CC (106, 21 bp)
BRCA2	Asn991Asp	5′-TACCAGAAAAAAATAATGATTCCATG-3′ (S)	RFLP	AA (102 bp)
(exon 11)	(A3199G)	5′-CCTTATTTGAAGCTGTTCTGAAG-3′ (AS)	(*NcoI*)	AG (102, 80 bp)
NM000059		(95°C5m; 95°C30s, 51°C30s, 72°C30s, 40 cycle; 72°C10m)		GG (80, 22 bp)

PCR = polymerase chain reaction.

the *XRCC3* C18067T polymorphism ($P = .006$) (Table III). There was a borderline suggestion that the *BRCA1* 3667G polymorphic allele was less common among the DTC case subjects than the control subjects ($P = .089$).

When risk estimates were calculated (Table IV), the homozygous polymorphic genotype was associated with a borderline or statistically significant increased risk for DTC for the *XRCC3* 18067TT genotype ($P = .055$), and this association was similar after adjustment for age, sex, radiation exposure, smoking, and drinking ($P = .063$). Furthermore, the heterozygous genotype *XRCC3* 18067CT was also associated with an increased risk for DTC ($P = .003$), and this association was similar after multivariate adjustment ($P = .006$) (Table IV). Moreover, when the *XRCC3* C18067T genotype was dichotomized into those with or without the polymorphic allele, having the polymorphic allele was associated with a significant risk of DTC ($P = .002$) (Table IV), and this remained significant after adjustment for age, sex, radiation exposure, smoking, and drinking ($P = .004$) (Table IV). No polymorphic genotype of any of the polymorphisms tested was associated with a statistically significantly decreased risk of DTC as compared with the homozygous wild-type genotype (Table IV).

The *XRCC3* 18067T allele appeared to be more common in the benign cases than the controls ($P = .054$), although the genotype distribution was not significantly different between the benign cases and the controls (Table III). No polymorphic allele was significantly less common in the benign cases than in the control subjects (Table III). The homozygous *XRCC3* 18067TT polymorphic genotype was associated with a borderline increased risk for benign thyroid disease ($P = .052$), and this finding was relatively stable after multivariate adjustment ($P = .078$) (Table IV). When the genotypes were dichotomized into those with and without the polymorphic allele, having the *XRCC3* 18067 polymorphic T allele was not associated with a significant risk of benign thyroid disease (Table IV). No polymorphic genotype was associated with a decreased risk of benign thyroid disease as compared with the homozygous wild-type genotype (Table IV); however, after

TABLE II.
Demographic and Exposure Characteristics of Case and Control Subjects.

	DTC Cases (n = 134)			BTD Cases (n = 79)			Controls (n = 166)	
Variable	No.	(%)	*P* Value*	No.	(%)	*P* Value*	No.	(%)
Age			0.104			0.933		
≤50	78	(58.2)		39	(49.4)		81	(48.8)
>50	56	(41.8)		40	(50.6)		85	(51.2)
Sex			0.368			0.002		
Male	56	(41.8)		21	(26.6)		78	(47.0)
Female	78	(58.2)		58	(73.4)		88	(53.0)
Ethnicity			1.000			1.000		
Non-Hispanic white	134	(100)		79	(100)		167	(100)
Radiation exposure†			0.167			0.072		
No	129	(96.3)		73	(94.8)		157	(98.7)
Yes	5	(3.7)		4	(5.2)		2	(1.3)
Smoking status‡			0.234			0.736		
Current	17	(12.7)		11	(15.3)		31	(18.7)
Former	30	(22.4)		17	(23.6)		42	(25.3)
Never	87	(64.9)		44	(61.1)		93	(56.0)
Alcohol status‡			0.030			0.530		
Current	42	(31.4)		24	(33.3)		61	(36.7)
Former	18	(13.4)		13	(18.1)		37	(22.3)
Never	74	(55.2)		35	(48.6)		68	(41.0)

*Chi-squared analysis comparing case with control subjects.
†History of radiation exposure to the head and neck (data unavailable for 2 BTD cases and 7 control subjects).
‡Smoking and alcohol information unavailable for 7 BTD case subjects.
DTC = differentiated thyroid cancer; BTD = benign thyroid disease.

multivariate adjustment, the heterozygous *XRCC7* 6721 GT genotype was associated with a decreased risk of benign thyroid disease ($P = .030$) (Table IV).

DISCUSSION

This study represents a case-control study of the risk of DTC associated with polymorphisms of genes responsible for DNA repair in response to gamma radiation. We found that the *XRCC3* 18067T allele was associated with a twofold increased risk of DTC and that this risk was stable after adjustment for age, sex, radiation exposure, smoking, and drinking. Furthermore, this polymorphism was associated with benign thyroid disease, and these risk estimates of benign disease were in part intermediate in relation to that of DTC.

Although these findings support some of the criteria for causality, including strength of the association, temporality, biological coherence, and plausibility, they should be viewed with caution for several reasons. First, because this represents the first study to examine DNA repair genotypes in association with risk of DTC, independent confirmation with a larger sample is needed. Second, it is possible that the finding of association between the *XRCC3* C18067T genotype and risk of DTC was a chance occurrence. Additional confounders, both genetic and environmental, could exist that were not adjusted for in this analysis. Despite the study's relatively small size, the DTC and cancer-free controls had no significant differences with respect to age, sex, radiation exposures, or smoking use, suggesting that the two groups had similar demographic backgrounds lacking selection biases and that genetic comparisons in a case-control setting should be relatively stable. Likewise, selection biases could exist, leading to a group of cases or controls that do not reflect genetic characteristics of the DTC or general population at large. Although the frequency of the *XRCC3* 18067T variant allele in the control group is lower than some previous reports,[32–34] it is similar to the findings of others.[35,36] Furthermore, the observed genotype distribution of the *XRCC3* C18067T genotype among the controls was not in disagreement with that calculated from Hardy-Weinberg disequilibrium theory ($P = .165$).

The power of this study to detect a 15% elevated polymorphic allele frequency in the DTC cases (given an established allele frequency in a control population of between 5% and 15%) was 0.85 to 0.97 (alpha = 0.05, two-sided). However, the power drops to only 0.70 to 0.73 for detecting a similar difference in allele frequencies when the established allele frequency in a control population is between 30% and 50%. Obviously, further study with a larger sample size is needed to confirm these findings and to examine the interaction between genotypes. However, this study does provide preliminary case-control data on several polymorphisms in genes of the DNA repair pathways involved in the response to radiation.

Although these findings could be caused by chance, the *XRCC3* 18067T variant allele showed a relatively strong association with DTC risk. Furthermore, this polymorphism has been associated by others with both cancer risk and altered DNA repair.[32–35] In a study of healthy volunteers, Matullo et al.[34] found a significant association

TABLE III.
Genotyping of DNA Repair Genes.

	DTC Cases			BTD Cases			Controls	
Genotype	No.	(%)	*P* Value*	No.	(%)	*P* Value*	No.	(%)
XRCC3 C18067T (Thr241Met)								
CC	45	(33.6)	0.008	34	(43.0)	0.144	83	(51.5)
CT	69	(51.5)		29	(36.7)		60	(37.3)
TT	20	(14.9)		16	(20.3)		18	(11.2)
T allele frequency		(40.7)	0.006		(38.7)	0.054		(29.8)
XRCC7 T6721G (intron 8)								
TT	49	(36.6)	0.693	38	(48.1)	0.224	67	(40.4)
TG	68	(50.8)		27	(34.2)		76	(45.8)
GG	17	(12.7)		14	(17.7)		23	(13.9)
G allele frequency		(38.1)	0.741		(34.8)	0.677		(36.7)
RAD51 G135C (5′UTR)								
GG	110	(85.3)	0.716	66	(89.2)	0.597	144	(86.8)
GC	19	(14.7)		8	(10.8)		22	(13.2)
CC	0	(0.0)		0	(0.0)		0	(0.0)
C allele frequency		(7.4)	0.727		(5.4)	0.610		(6.6)
RAD52 T38207C (intron 11)								
TT	43	(37.1)	0.586	29	(42.7)	0.468	58	(35.1)
TC	68	(58.6)		36	(52.9)		95	(57.6)
CC	5	(4.3)		3	(4.4)		12	(7.3)
C allele frequency		(33.6)	0.551		(30.9)	0.285		(36.1)
BRCA1 C2731T (Pro871Leu)								
CC	68	(51.9)	0.521	39	(52.7)	0.376	75	(46.0)
CT	51	(38.9)		30	(40.5)		68	(41.7)
TT	12	(9.2)		5	(6.8)		20	(12.3)
T allele frequency		(28.6)	0.241		(27.0)	0.184		(33.1)
BRCA1 A3232G (Glu1038Gly)								
AA	69	(51.5)	0.449	39	(49.4)	0.600	77	(46.7)
AG	53	(39.5)		33	(41.8)		66	(40.0)
GG	12	(9.0)		7	(8.8)		22	(13.3)
G allele frequency		(28.7)	0.227		(29.7)	0.427		(33.3)
BRCA1 A3667G (Lys1183Arg)								
AA	68	(52.7)	0.172	40	(54.1)	0.315	74	(44.9)
AG	55	(42.6)		30	(40.5)		75	(45.4)
GG	6	(4.7)		4	(5.4)		16	(9.7)
G allele frequency		(26.0)	0.089		(25.7)	0.138		(32.4)
BRCA1 A4956G (Ser1613Gly)								
AA	70	(52.2)	0.184	41	(51.9)	0.532	78	(47.3)
AG	56	(41.8)		32	(40.5)		67	(40.6)
GG	8	(6.0)		6	(7.6)		20	(12.1)
G allele frequency		(26.9)	0.140		(27.8)	0.306		(32.4)
BRCA2 A1342C (Asn372His)								
AA	72	(55.8)	0.629	35	(47.9)	0.677	83	(50.3)
AC	53	(41.1)		37	(50.7)		77	(46.7)
CC	4	(3.1)		1	(1.4)		5	(3.0)
C allele frequency		(23.6)	0.451		(26.7)	0.937		(26.4)
BRCA2 A3199G (Asn991Asp)								
AA	114	(89.8)	0.370	68	(91.9)	0.821	153	(92.7)
AG	13	(10.2)		6	(8.1)		12	(7.3)
GG	0	(0.0)		0	(0.0)		0	(0.0)
G allele frequency		(5.1)	0.381		(4.1)	0.825		(3.6)

*Chi-squared analyses comparing genotype distributions and polymorphic allele frequencies between cases and controls.
DTC = differentiated thyroid cancer; BTD = benign thyroid disease.

TABLE IV.
DNA repair genotype risk estimates.

Genotype	DTC		BTD	
	Crude OR (95% CI)	Adj.* OR (95% CI)	Crude OR (95% CI)	Adj.* OR (95% CI)
XRCC3 C18067T (Thr241Met)				
CC	Reference	Reference	Reference	Reference
CT	2.1 (1.3–3.5)	2.1 (1.2–3.5)	1.2 (0.7–2.1)	1.2 (0.6–2.3)
TT	2.0 (1.0–4.3)	2.1 (1.0–4.4)	2.2 (1.0–4.7)	2.1 (0.9–4.9)
CT or TT	2.1 (1.3–3.4)	2.1 (1.3–3.4)	1.4 (0.8–2.4)	1.4 (0.8–2.6)
XRCC7 T6721G (intron 8)				
TT	Reference	Reference	Reference	Reference
TG	1.2 (0.7–2.0)	1.2 (0.7–2.0)	0.6 (0.3–1.1)	0.5 (0.3–0.9)
GG	1.0 (0.5–2.1)	1.0 (0.5–2.2)	1.1 (0.5–2.3)	0.9 (0.4–2.0)
TG or GG	1.2 (0.7–1.9)	1.2 (0.7–1.9)	0.7 (0.4–1.3)	0.6 (0.3–1.0)
RAD51 G135C (5′UTR)				
GG	Reference	Reference	Reference	Reference
GC or CC	1.1 (0.6–2.2)	1.5 (0.7–3.0)	0.8 (0.3–1.9)	0.8 (0.3–2.1)
RAD52 T38207C (intron 11)				
TT	Reference	Reference	Reference	Reference
TC	1.0 (0.6–1.6)	1.0 (0.6–1.7)	0.8 (0.4–1.4)	0.8 (0.4–1.4)
CC	0.6 (0.2–1.7)	0.5 (0.2–1.6)	0.5 (0.1–1.9)	0.4 (0.1–1.6)
TC or CC	0.9 (0.6–1.5)	1.0 (0.6–1.6)	0.7 (0.4–1.3)	0.7 (0.4–1.3)
BRCA1 C2731T (Pro871Leu)				
CC	Reference	Reference	Reference	Reference
CT	0.8 (0.5–1.3)	0.9 (0.5–1.5)	0.8 (0.5–1.5)	0.9 (0.5–1.7)
TT	0.7 (0.3–1.5)	0.9 (0.4–2.0)	0.5 (0.2–1.4)	0.5 (0.2–1.6)
CT or TT	0.8 (0.5–1.3)	0.9 (0.5–1.4)	0.8 (0.4–1.3)	0.8 (0.5–1.5)
BRCA1 A3232G (Glu1038Gly)				
AA	Reference	Reference	Reference	Reference
AG	0.9 (0.6–1.5)	1.0 (0.6–1.6)	1.0 (0.6–1.7)	1.1 (0.6–2.1)
GG	0.6 (0.3–1.3)	0.9 (0.4–2.0)	0.6 (0.2–1.6)	0.8 (0.3–2.1)
AG or GG	0.8 (0.5–1.3)	0.9 (0.6–1.5)	0.9 (0.5–1.5)	1.0 (0.6–1.9)
BRCA1 A3667G (Lys1183Arg)				
AA	Reference	Reference	Reference	Reference
AG	0.8 (0.5–1.3)	0.8 (0.5–1.4)	0.7 (0.4–1.3)	0.8 (0.4–1.4)
GG	0.4 (0.2–1.1)	0.6 (0.2–1.8)	0.5 (0.1–1.5)	0.5 (0.2–1.9)
AG or GG	0.7 (0.5–1.2)	0.8 (0.5–1.3)	0.7 (0.4–1.2)	0.7 (0.4–1.3)
BRCA1 A4956G (Ser1613Gly)				
AA	Reference	Reference	Reference	Reference
AG	0.9 (0.6–1.5)	1.0 (0.6–1.6)	0.9 (0.5–1.6)	0.9 (0.5–1.7)
GG	0.4 (0.2–1.1)	0.6 (0.2–1.5)	0.6 (0.2–1.5)	0.7 (0.2–1.9)
AG or GG	0.8 (0.5–1.3)	0.9 (0.6–1.5)	0.8 (0.5–1.4)	0.9 (0.5–1.6)
BRCA2 A1342C (Asn372His)				
AA	Reference	Reference	Reference	Reference
AC	0.8 (0.5–1.3)	0.9 (0.6–1.5)	1.1 (0.7–2.0)	1.2 (0.6–2.2)
CC	0.9 (0.2–3.6)	1.0 (0.3–4.2)	0.5 (0.1–4.2)	na
AC or CC	0.8 (0.5–1.3)	0.9 (0.6–1.5)	1.1 (0.6–1.9)	1.1 (0.6–2.0)
BRCA2 A3199G (Asn991Asp)				
AA	Reference	Reference	Reference	Reference
AG or GG	1.5 (0.6–3.3)	1.3 (0.5–3.0)	1.1 (0.4–3.1)	1.3 (0.5–3.8)

*Adjusted for age, sex, smoking, alcohol, and radiation exposure.
DTC = differentiated thyroid cancer; BTD = benign thyroid disease; na = unable to accurately estimate because of missing smoking, alcohol, and radiation exposure information in this subgroup.

between DNA benzo[a]pyrene adduct levels in peripheral blood lymphocytes and the *XRCC3* 18067T variant, and this finding was consistent after multivariant adjustment models including smoking status. Furthermore, they found a dose-response effect of increasing adduct levels with number of variant alleles present, and they hypothesize that this Thr241Met substitution in the *XRCC3* protein affects its enzymatic function or binding abilities.[34] These phenotypic findings and the other case-control studies linking the *XRCC3* 18067T polymorphisms to risk of melanoma,[35] breast cancer,[32,33] and carcinoma of the bladder[36] support our results.

CONCLUSIONS

We hypothesized that polymorphisms of genes responsible for response to gamma radiation may be associated with risk of DTC. Of the 10 polymorphisms screened, we identified *XRCC3* C18067T, which may be of importance to this disease process. Obviously, confirmatory studies with larger sample sizes will be needed, but these findings represent a step toward the understanding of genetic susceptibility to DTC. If these findings are confirmed, then genotypes could be incorporated into future genetic profiles of DTC risk and may serve in future cancer prevention efforts or public health responses to widespread radiation exposures.

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BIBLIOGRAPHY

1. Jemal A, Murray T, Ward E. Cancer Statistics 2005. *CA Cancer J Clin* 2004;54:8–29.
2. Hundahl SA, Fleming ID, Fremgen AM, Menck HR. A national cancer database report on 53,856 cases of thyroid carcinoma treated in the U. S., 1985–1995. *Cancer* 1998;83:2638–2648.
3. Little JB. Radiation carcinogenesis. *Carcinogenesis* 2000;21: 397–404.
4. Lindahl T, Wood RD. Quality control by DNA repair. *Science* 1999;286:897–905.
5. Lindahl T. Suppression of spontaneous mutagenesis in human cells by DNA base excision-repair. *Mutat Res* 2000;462: 129–135.
6. Krokan HE, Nilsen H, Skorpen F, et al. Base excision repair of DNA in mammalian cells. *FEBS Lett* 2000;476(1–2):73–77.
7. Van Gent DC, Hoeijmakers JH, Kanaar R. Chromosomal stability and the DNA double-stranded break connection. *Nat Rev Genet* 2001;2:196–206.
8. Kuzminov A. DNA replication meets genetic exchange: chromosomal damage and its repair by homologous recombination. *Proc Natl Acad Sci U S A* 2001;98:8461–8468.
9. Khanna KK, Jackson SP. DNA double-strand breaks: signaling, repair and the cancer connection. *Nat Genet* 2001;27:247–254.
10. Parshad R, Sanford KK. Radiation-induced chromatid breaks and deficient DNA repair in cancer predisposition. *Crit Rev Oncol Hematol* 2001;37:87–96.
11. Xiong P, Zheng R, Wang LE, et al. A pilot case-control study of gamma radiation sensitivity and risk of papillary thyroid cancer. *Thyroid* 2005;15:94–99.
12. Blaisdell JO, Wallace SS. Abortive base-excision repair of radiation-induced clustered DNA lesions in Escherichia coli. *Proc Natl Acad Sci U S A* 2001;98:7426–7430.
13. Critchlow SE, Jackson SP. DNA end-joining: from yeast to man. *Trends Biochem Sci* 1998;23:394–398.
14. Thompson LH, Schild D. Homologous recombinational repair of DNA ensures mammalian chromosome stability. *Mutat Res* 2001;477(1–2):131–153.
15. Karran P. DNA double-strand break repair in mammalian cells. *Curr Opin Genet Dev* 2000;10:144–150.
16. Tebbs RS, Zhao Y, Tucker JD, et al. Correction of chromosomal instability and sensitivity to diverse mutagens by a cloned cDNA of the XRCC3 DNA repair gene. *Proc Natl Acad Sci U S A* 1995;92:6354–6358.
17. Liu N, Lamerdin JE, Tebbs RS, et al. XRCC2 and XRCC3, new human Rad51-family members, promote chromosome stability and protect against DNA cross-links and other damages. *Mol Cell* 1998;1:783–793.
18. Masson JY, Stasiak AZ, Stasiak A, et al. Complex formation by the human RAD51C and XRCC3 recombination repair proteins. *Proc Natl Acad Sci U S A* 2001;98:8440–8446.
19. Shen MR, Jones IM, Mohrenweiser H. Nonconservative amino acid substitution variants exist at polymorphic frequency in DNA repair genes in healthy humans. *Cancer Res* 1998;58:604–608.
20. Price EA, Bourne SL, Radbourne R, et al. Rare microsatellite polymorphisms in the DNA repair genes XRCC1, XRCC3 and XRCC5 associated with cancer in patients of varying radiosensitivity. *Somat Cell Mol Genet* 1997;23:237–247.
21. Ford BN, Ruttan CC, Kyle VL, et al. Identification of single nucleotide polymorphisms in human DNA repair genes. *Carcinogenesis* 2000;21:1977–1981.
22. Sipley JD, Menninger JC, Hartley KO, et al. Gene for the catalytic subunit of the human DNA-activated protein kinase maps to the site of the XRCC7 gene on chromosome 8. *Proc Natl Acad Sci U S A* 1995;92:7515–7519.
23. Mohrenweiser HW, Xi T, Vazquez-Matias J, Jones IM. Identification of 127 amino acid substitution variants in screening 37 DNA repair genes in humans. *Cancer Epidemiol Biomarkers Prev* 2002;11:1054–1064.
24. Wang WW, Spurdle AB, Kolachana P, et al. A single nucleotide polymorphism in the 5' untranslated region of RAD51 and risk of cancer among BRCA1/2 mutation carriers. *Cancer Epidemiol Biomarkers Prev* 2001;10:955–960.
25. Bell DW, Wahrer DCR, Kang DH, et al. Common nonsense mutations in RAD52. *Cancer Res* 1999;59:3883–3888.
26. Han J, Hankinson SE, De Vivo I, et al. No association between a stop codon polymorphism in RAD52 and breast cancer risk. *Cancer Epidemiol Biomarkers Prev* 2002;11:1138–1139.
27. Healey CS, Dunning AM, Teare MD, et al. A common variant in BRCA2 is associated with both breast cancer risk and prenatal viability. *Nat Genet* 2000;26:362–364.
28. Goode EL, Dunning AM, Kuschel B, et al. Effect of germ-line genetic variation on breast cancer survival in a population-based study. *Cancer Res* 2002;62:3052–3057.
29. Goode EL, Ulrich CM, Potter JD. Polymorphisms in DNA repair genes and associations with cancer risk. *Cancer Epidemiol Biomarkers Prev* 2002;11:1513–1530.
30. Kerr P, Ashworth A. New complexities for BRCA1 and BRCA2. *Curr Biol* 2001;11:R668–R676.
31. Zheng R, Dahlstrom KR, Wei Q, Sturgis EM. Gamma radiation-induced apoptosis, G2 delay and the risk of salivary and thyroid carcinomas: a preliminary report. *Head Neck* 2004;26:612–618.
32. Kuschel B, Auranen A, McBride S, et al. Variants in DNA double-strand break repair genes and breast cancer susceptibility. *Hum Mol Gen* 2002;11:1399–1407.
33. Smith TR, Miller MS, Lohman K, et al. Polymorphisms of XRCC1 and XRCC3 genes and susceptibility to breast cancer. *Cancer Lett* 2003;190:183–190.
34. Matullo G, Palli D, Peluso M, et al. XRCC1, XRCC3, XPD gene polymorphisms, smoking and (32)P-DNA adducts in a sample of healthy subjects. *Carcinogenesis* 2001;22:1437–1445.
35. 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* 2000;60:5612–5616.
36. Matullo G, Guarrera S, Carturan S, et al. DNA repair gene polymorphisms, bulky adducts in white blood cells and bladder cancer in a case-control study. *Int J Cancer* 2001;92:562–567.