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Genetic polymorphisms of multiple DNA repair pathways impact age at diagnosis and *TP53* mutations in breast cancer

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Defective DNA repair may contribute to early age and late stage at time of diagnosis and mutations in critical tumor suppressor genes, such as *TP53* in breast cancer. Using DNA samples from 436 breast cancer cases (374 Caucasians and 62 African-Americans), we tested these associations with 18 non-synonymous single-nucleotide polymorphisms (nsSNPs) in four DNA repair pathways: (i) base excision repair: *ADPRT V762A*, *APE1 D148E*, *XRCC1 R194W/R280H/R399Q* and *POLD1 R119H*; (ii) double-strand break repair: *NBS1 E185Q* and *XRCC3 T241M*; (iii) mismatch repair: *MLH1 I219V*, *MSH3 R940Q/T1036A* and *MSH6 G39E* and (iv) nucleotide excision repair: *ERCC2 D312N/K751Q*, *ERCC4 R415Q*, *ERCC5 D1104H* and *XPC A499V/K939Q*. Younger age at diagnosis (<50) was associated with *ERCC2 312 DN/NN* genotypes [odds ratio (OR) = 1.76; 95% confidence interval (CI) = 1.10, 2.81] and NBS1 185 QQ genotype (OR = 3.09; 95% CI = 1.47, 6.49). The *XPC 939 QQ* genotype was associated with *TP53* mutations (OR = 5.80; 95% CI = 2.23, 15.09). There was a significant trend associating younger age at diagnosis (<50) with increasing numbers of risk genotypes for *ERCC2 312 DN/NN*, *MSH6 39 EE* and *NBS1 185 QQ* (P_{trend} < 0.001). A similar significant trend was also observed associating *TP53* mutations with increasing numbers of risk genotypes for *XRCC1 399 QQ*, *XPC 939 QQ*, *ERCC4 415 QQ* and *XPC 499 AA* (P_{trend} < 0.001). Our pilot data suggest that nsSNPs of multiple DNA repair pathways are associated with younger age at diagnosis and *TP53* mutations in breast cancer and larger studies are warranted to further evaluate these associations.

Introduction

Breast cancer is the most frequently diagnosed cancer in women. In 2010, there were ~207 090 new breast cancer cases and 39 840 deaths from the disease in the USA (1). Heterogeneity in breast cancer diagnosis, treatment and survival may be partially explained by breast biology and malignant phenotype. Utilization of mammography screening has dramatically decreased the rates of advanced breast cancer, particularly in postmenopausal women and increased years of life after surgery or other treatment. In contrast, premenopausal women have not benefited from early detection to the extent of age ≥50 women (2–4). Increasing evidence suggests etiological and mechanistic differences in breast cancer development in young women as well as in African-Americans (5,6). A complex interplay of genetics, environmental exposures, hormones and behaviors may predispose subpopulations to breast carcinogenesis during specific life phases (7). Some young women may have a window of greater breast cancer susceptibility in premenopausal years as a result of interactions between carcinogen exposure and genetic predisposition in undifferentiated mammary epithelium. In older women, accumulation of DNA damage and resultant cellular growth deregulation may be stronger determinants for breast malignancy later in life. These phenomena create a bimodal distribution of peak frequencies for high-risk and low-risk breast tumors, with age at-onset of ~50 and 70 years, respectively (8). A crossover effect from high-risk to low-risk breast cancers by age has been well documented and provides evidence for two populations with divergent susceptibility factors (2,9).

Complex exposures to different types of DNA damage require multiple repair pathways to maintain genomic integrity, including base excision repair (BER), nucleotide excision repair (NER), mismatch repair (MMR) and double-strand break repair (DSBR). The BER pathway removes DNA damage caused by ionizing radiation, reactive oxidative species and methylating agents. ADP-ribosyltransferase or Poly (ADP-ribose) polymerase (ADPRT/(PARP-1)) is a vital member of the BER pathway; it senses DNA strand breaks and initiates DNA damage signaling, which may play roles in breast cancer (10). Genetic variants of PARP-1 may contribute to breast carcinogenesis and PARP-1 htSNP c.852T > C may influence response to hormonal therapy for breast cancer (11). The NER pathway plays a critical role in repairing various forms of DNA damage: bulky adducts generated from genotoxic compounds, ultraviolet-induced photo lesions and intrastrand cross-links (12). MMR is a highly conserved repair pathway that functions in improving replication fidelity by correcting replication-associated base–base and insertion/deletion mispairs (13). High-frequency microsatellite instability is detected more frequently in bilateral but not in unilateral breast cancers (14). Losses of heterozygosity and/or microsatellite instability were detected in 83% of the skin samples from breast cancer patients, which suggest a potential role of MMR in breast cancer susceptibility (15). Double-strand breaks (DSBs) may result in cell death or genetic alterations, including deletions, loss of heterozygosity, translocations and chromosome loss (16). A number of proteins influence homologous recombination and non-homologous end-joining subpathways, including the MRE11–RAD50–NBS1 (MRN) complex, BRCA1, histone H2AX, PARP-1, RAD18, protein kinase catalytic subunit and ATM. Breast cancer risk is also associated with modifications of the genes encoding DSBR proteins (17,18).

High levels of DNA damage and deficient DNA repair are associated with breast cancer risk (19–21). Decreased DNA repair capacity in young breast cancer cases as compared with controls has been reported (22,23). Biomarkers of rapid tumor growth and genomic instability have been observed in young breast cancer patients (24). From midlife through advancing age, levels of these biologic indicators become more clinically relevant.

The *TP53* tumor suppressor gene is the most commonly mutated gene in human cancers. It is an essential homeostatic regulator, acting through the multiple pathways of cell cycle regulation, negative growth control, DNA repair and apoptosis (25). About 15–30% of breast tumors contain *TP53* mutations, with the majority occurring in the DNA-binding region encoded by exons 4–9 (26,27). Mutations may lead to decreased protein stability or function and deregulate normal p53 activities such as transactivation, thereby leading to cellular growth advantage (28). *TP53* mutation during breast carcinogenesis may be an early or late event, depending on the clinical presentation of the tumor (26,29). In a large study of 1794 breast cancer patients, *TP53* mutations have an independent prognostic value and could have potential uses in clinical practice (30).

Since mutations in *TP53* and other highly penetrant risk determinant genes, such as *BRCA1* and *BRCA2*, occur at low frequencies in

Abbreviations: BER, base excision repair; BMI, body mass index; CI, confidence interval; DSB, double-strand break; DSBR, double-strand break repair; FDR, false-discovery rate; MRN, MRE11-RAD50-NBS1; NER, nucleotide excision repair; nsSNP, non-synonymous single-nucleotide polymorphism; PCR, polymerase chain reaction; OR, odds ratio.

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the population, the majority of breast cancer risk may be associated with more common non-synonymous single-nucleotide polymorphisms (nsSNPs) in low penetrance genes. DNA repair genetic variations are common in the general population (31,32) and may attenuate repair capacity, thus contributing to elevated DNA damage and breast cancer susceptibility. Since genetic polymorphisms in DNA repair genes differ in frequency by race, studies addressing DNA repair and breast cancer risk may benefit from stratification of study populations, as gene function modifiers may vary by race.

Different DNA repair pathways play critical roles in maintaining genome stability and SNPs in multiple repair pathways may result in early age, late stage and *TP53* mutations in breast cancer. Based on the concept that nsSNPs lead to amino acid substitutions and may result in altered function, we hypothesize that nsSNPs from different repair pathways have additive or multiplicative effects on age, stage and *TP53* mutations in breast cancer. Therefore, this study evaluated 18 nsSNPs in four DNA repair pathways—(i) BER: *ADPRT V762A* (rs1136410), *APE1 D148E* (rs3136820), *XRCC1 R194W* (rs1799782)/*R280H* (rs25489)/*R399Q* (rs25487) and *POLD1 R119H* (rs1726801); (ii) DSBR: *NBS1 E185Q* (rs1805794) and *XRCC3 T241M* (rs861539); (iii) MMR: *MLH1 I219V* (rs1799977), *MSH3 R940Q* (rs184967)/*T1036A* (rs26279) and *MSH6 G39E* (rs1042821) and (iv) NER: *ERCC2 D312N* (rs1799793)/*K751Q* (rs13181), *ERCC4 R415Q* (rs1800067), *ERCC5 D1104H* (rs17655) and *XPC A499V* (rs2228000)/*K939Q* (rs2228001).

Materials and methods

Study population

The subjects in this study included 436 biopsy-proven breast cancer cases from a case–control study as described previously (33,34) Patients were enrolled in the trial after biopsy and before definitive surgery. The majority of breast cancer cases were enrolled at the Wake Forest University Breast Care Center, with additional participants from Moses H. Cone Memorial Hospital and the University of North Carolina-Chapel Hill Lineberger Comprehensive Cancer Center. Medical records and pathology reports were used to confirm age and stage at diagnosis. All study subjects provided their signed informed consent as approved by the Wake Forest University Medical Center's and appropriate institutional review boards. Study participants completed a self-administered baseline breast cancer risk questionnaire, including information on demographics, reproductive and medical histories, prescription use and familial cancer history. Age at diagnosis was designated as the age on the date of study consent and tumor stage was assigned by a medical pathologist. Positive family history of breast cancer was defined as a woman with a mother or sister with breast cancer. Ever-smoking history was classified as lifetime smoking history of at least 100 cigarettes.

Genotyping analysis and quality control

Genomic DNA was extracted from frozen whole blood using a QIAamp DNA Blood Mini kit (Qiagen, Valencia, CA). Three criteria were used for genotyping analysis: (i) the SNP resulted in an amino acid substitution; (ii) the variant allele frequency was approximately ≥5% in the general population and (iii) sequence information was available for accurate assay development. The selected DNA repair SNPs were: (i) BER: *ADPRT V762A* (rs1136410), *APE1 D148E* (rs3136820), *XRCC1 R194W* (rs1799782)/*R280H* (rs25489)/*R399Q* (rs25487) and *POLD1 R119H* (rs1726801); (ii) DSBR: *NBS1 E185Q* (rs1805794) and *XRCC3 T241M* (rs861539); (iii) MMR: *MLH1 I219V* (rs1799977), *MSH3 R940Q* (rs184967)/*T1036A* (rs26279) and *MSH6 G39E* (rs1042821) and (iv) NER: *ERCC2 D312N* (rs1799793)/*K751Q* (rs13181), *ERCC4 R415Q* (rs1800067), *ERCC5 D1104H* (rs17655) and *XPC A499V* (rs2228000)/*K939Q* (rs2228001). The MassARRAY system (Sequenom, San Diego, CA) was used to determine genotypes. Sequences of forward, reverse and extension primers used in the analysis of DNA repair nsSNPs were described previously (33).

Genotyping was first completed on a panel of 90 DNA samples from The Coriell Institute for Medical Research (Camden, NJ) and compared with

Table I. Demographic characteristics of study population by age, breast cancer stage and *TP53* mutation status

Characteristic	Categories	Age		*P* Value	Tumor stage		*P* Value	*TP53* mutation		*P* Value
		Age ≥ 50 (*N* = 296)	Age < 50 (*N*=140)		Early (*N* = 235)	Advanced[a] (*N* = 147)		No (*N* = 279)	Yes (*N* = 32)	
Race	African-American	38 (13%)	24 (17%)		31 (13%)	29 (20%)	0.09	18 (7%)	2 (6%)	0.97
	White	258 (87%)	116 (83%)	0.23	204 (87%)	118 (80%)		261 (93%)	30 (94%)	
Age	Mean ± SD	64.3 ± 9.0	42.5 ± 5.8		58.4 ± 12.9	55.1 ± 13.2	0.02	58.8 ± 12.4	52.8 ± 13.2	0.01
	<40		35 (25%)		18 (8%)	16 (11%)		15 (5%)	5 (16%)	
	40–49		105 (75%)		48 (20%)	39 (26%)		56 (20%)	12 (37%)	
	50–59	104 (35%)			57 (24%)	39 (26%)		72 (26%)	6 (19%)	
	60–69	106 (36%)			64 (27%)	26 (18%)		72 (26%)	4 (12%)	
	≥70	86 (29%)			48 (20%)	27 (19%)	0.17	64 (23%)	5 (16%)	0.02
Family history	None	233 (79%)	116 (83%)		184 (78.3)	119 (81%)		219 (78.5)	25 (78%)	
	Mother and/ or sister	63 (21%)	24 (17%)	0.31	51 (21.7)	28 (19%)	0.53	60 (21.5)	7 (22%)	0.96
Smoking history	Never	170 (59%)	73 (56%)		135 (58%)	82 (59%)		162 (61%)	14 (47%)	
	Former	85 (29%)	35 (27%)		69 (30%)	34 (25%)		69 (26%)	10 (33%)	
	Current	35 (12%)	23 (17%)	0.31	28 (12%)	23 (16%)	0.34	33 (13%)	6 (20%)	0.27
	Missing	6	9		3	8		15	2	
Age at menarche	≤12	130 (46%)	58 (43%)		98 (43%)	68 (48%)		130 (50%)	10 (32%)	
	13–14	109 (38%)	58 (43%)		99 (43%)	51 (36%)		95 (36%)	15 (48%)	
	≥15	45 (16%)	19 (14%)	0.66	31 (14%)	24 (17%)	0.31	37 (14%)	6 (20%)	0.19
	Missing	12	5		7	4		17	1	
BMI	Mean ± SD	28.36 ± 5.94	27.15 ± 7.23	0.09	27.56 ± 6.18	28.81 ± 6.68	0.06	27.6 ± 6.1	26.1 ± 4.1	0.18
	<25 kg/m^2	100 (35%)	65 (48%)		97 (42%)	46 (32%)		98 (38%)	13 (45%)	
	25–29.9 kg/m^2	100 (35%)	43 (32%)		78 (33%)	49 (34%)		95 (36%)	10 (34%)	
	≥30 kg/m^2	90 (30%)	27 (20%)	0.01	59 (25%)	48 (34%)	0.12	68 (26%)	6 (20%)	0.71
	Missing	6	5		1	4		18	3	
Age at first live birth	≤24	186 (63%)	56 (41%)		135 (58%)	83 (58%)		144 (54%)	13 (42%)	
	25–29	62 (21%)	25 (18%)		48 (20%)	28 (19%)		60 (23%)	8 (26%)	
	≥30	17 (6%)	23 (17%)		20 (9%)	12 (8%)		26 (10%)	5 (16%)	
	Nulliparous	28 (10%)	33 (24%)	<0.001	31 (13%)	21 (15%)	0.98	34 (13%)	5 (16%)	0.53
	Missing	3	3		1	3		15	1	

[a]Early = Stages 0–I, Advanced = Stages II–IV.

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Table II. Association of BER genetic variants with age, breast cancer stage and *TP53* mutation status

SNP	Genotype	Age ≥50/<50	OR[a] (95% CI)	Stage I–II/III–IV	OR[b] (95% CI)	*TP53* mutation no/yes	OR[c] (95% CI)
ADPRT V762A	*VV*	190/94	Referent	176/108	Referent	161/20	Referent
	VA	55/22	0.83 (0.47, 1.45)	47/30	1.13 (0.67, 1.90)	35/3	0.71 (0.19, 2.58)
	AA	6/1	0.37 (0.04, 3.15)	5/2	0.78 (0.15, 4.14)	9/1	0.90 (0.11, 7.66)
	VV	190/94	Referent	176/108	Referent	161/20	Referent
	VA/AA	61/23	0.79 (0.45, 1.36)	52/32	1.09 (0.66, 1.82)	44/4	0.75 (0.24, 2.36)
APE1 D148E	*DD*	86/39	Referent	75/50	Referent	69/8	Referent
	DE	122/47	0.86 (0.52, 1.44)	104/65	0.95 (0.59, 1.53)	90/9	0.94 (0.34, 2.63)
	EE	51/31	1.43 (0.79, 2.59)	51/31	0.90 (0.51, 1.62)	47/7	1.22 (0.40, 3.66)
	DD	86/39	Referent	75/50	Referent	69/8	Referent
	DE/EE	173/78	1.03 (0.64, 1.64)	155/96	0.93 (0.60, 1.46)	137/16	1.05 (0.42, 2.62)
XRCC1 R194W	*RR*	229/101	Referent	207/123	Referent	180/23	Referent
	RW	26/17	1.45 (0.75, 2.81)	23/20	1.39 (0.72, 2.65)	24/1	0.30 (0.04, 2.35)
	WW	5/1	0.45 (0.05, 3.97)	4/2	0.92 (0.16, 5.19)	5/0	N/A
	RR	229/101	Referent	207/123	Referent	180/23	Referent
	RW/WW	31/18	1.29 (0.69, 2.43)	27/22	1.32 (0.72, 2.44)	29/1	0.26 (0.03, 2.02)
XRCC1 R280H	*RR*	240/112	Referent	220/132	Referent	191/23	Referent
	RH	20/9	0.93 (0.41, 2.13)	14/15	1.86 (0.87, 4.01)	18/0	N/A
	HH	0/0	N/A	0/0	N/A	0/0	N/A
	RR	240/112	Referent	220/132	Referent	191/23	Referent
	RH/HH	20/9	0.93 (0.41, 2.13)	14/15	1.86 (0.87, 4.01)	18/0	N/A
XRCC1 R399Q	*RR*	117/58	Referent	107/68	Referent	100/10	Referent
	RQ	107/47	0.95 (0.59, 1.53)	95/59	1.07 (0.68, 1.70)	83/9	1.17 (0.44, 3.14)
	QQ	27/10	0.83 (0.37, 1.87)	24/13	0.94 (0.44, 2.01)	21/5	2.49 (0.74, 8.41)
	RR/RQ	224/105	Referent	202/127	Referent	183/19	Referent
	QQ	27/10	0.86 (0.40, 1.85)	24/13	0.91 (0.44, 1.87)	21/5	2.30 (0.76, 6.99)
POLD1 R119H	*RR*	198/92	Referent	178/112	Referent	163/21	Referent
	RH	46/24	0.97 (0.54, 1.75)	42/28	0.93 (0.53, 1.63)	32/2	0.44 (0.09, 2.09)
	HH	8/3	0.57 (0.14, 2.37)	6/5	1.07 (0.30, 3.91)	6/0	N/A
	RR	198/92	Referent	178/112	Referent	163/21	Referent
	RH/HH	54/27	0.92 (0.52, 1.63)	48/33	0.94 (0.55, 1.62)	38/2	0.38 (0.08, 1.83)

[a]Adjusted for stage (early/late) and race (Caucasian/African-American).
[b]Adjusted for race (Caucasian/African-American) and age (continuous).
[c]Adjusted for stage (early/late) and race (Caucasian/African-American) and age (continuous).

genotype data reported in two publicly accessible databases: http://www.ncbi.nlm.nih.gov and http://egp.gs.washington.edu. The quality control protocol included four control samples genotyped with 92 patient samples on each 96-well plate. Study cases and controls were loaded on each plate to minimize systematic bias. The average call rate for the assay was >95%. The concordance rate for the quality control samples was 100% and the concordance rate for the Coriell samples ranged from 91 to 100%. For each genotype, there was a 100% concordance rate for the four internal control samples on each plate. Each nsSNP was also tested for Hardy–Weinberg equilibrium.

TP53 mutational analysis

TP53 mutation analysis in breast tumor tissue was completed as described previously (34). In brief, paraffin-embedded tissue was deparaffinized and tumor cells laser capture microdissected. Cells were prepared for two rounds of tumor DNA polymerase chain reaction (PCR) amplification of exons 5–9 of the *TP53* gene. Primers and PCR amplification conditions were as described previously (34). A primary PCR round was completed on a matched blood sample from each patient. Single-stranded conformation polymorphism with the GenePhor flatbed electrophoresis system (Amersham Biosciences, Piscataway, NJ) was used to screen for *TP53* mutations in PCR-amplified genomic DNA. Tumor DNA samples producing single-stranded conformation polymorphism band shifts were reamplified and repeated band shifts were sequenced by either Wake Forest University School of Medicine Biomolecular Resources Facility DNA Sequencing Core or MWG-Biotech. To verify the presence of a heterozygous insertion or deletion, PCR products were cloned using a TOPO TA® Cloning Kit (Invitrogen, Carlsbad, CA) and sequenced. With the increasing availability of commercial high-throughput methods, a portion of samples were examined through a contract service with SpectruMedix LLC (State College, PA) by Reveal™ Temperature Gradient Capillary Electrophoresis and analyzed by SpectruMedix Analytical Software. As part of the *TP53* mutation analysis quality control procedures, preliminary studies were completed using cell lines with known *TP53* mutations or patient samples.

Statistical analyses

Student's *t*-tests and chi-square tests were used to compare case demographic characteristics. Distribution of *TP53* mutations by age and stage at breast cancer diagnosis were assessed with chi-square tests or Fisher's exact tests. Multivariate logistic regression was used to assess the association between DNA repair genetic variants and age (<50 versus ≥50), tumor stage (II–IV versus 0–I) or *TP53 mutation* (yes versus no) as main covariates. Odds ratios (OR) and 95% confidence intervals (95% CI) are reported. For the association between DNA repair genetic variants and age, models were adjusted for stage and race (African-American versus Caucasian). The associations with tumor stage were adjusted for race and age (continuous). For the association between DNA repair genetic variants and *TP53* mutation status, models were adjusted for age (continuous), race and tumor stage. In addition, we constructed polygenic models using logistic regression of combined DNA repair genotypes that demonstrated putative risk associations with individual OR ≥1.6. False-discovery rate (FDR) analysis, which controls for multiple comparisons, was used to confirm significant findings from these logistic models for each covariate of interest (age, stage, TP53). Statistical analysis was carried out in SAS® version 9.2 (SAS Institute, Cary, NC).

Results

Characteristics of the study population

Demographic characteristics of the study population are summarized in Table I by age at diagnosis, tumor stage and *TP53* mutation status. Detailed distribution of the *TP53* mutation was described previously (34). Of the 323 (301 Caucasian, 22 African-American) women analyzed for *TP53* mutations in this study, 34 (11%) exhibited mutations in exons 5–9 of *TP53*. As some of the women exhibited identical mutations, there are 30 different loci involved. Twenty-six of the 30 mutations were single-nucleotide mutations, three were small deletions and one was a complex mutation. Of the 26 single-nucleotide mutations, 19 were missense mutations, four were nonsense mutations and three were intronic/splice site mutations. Eight of the women in this study had a mutation at codon 248, which is a known mutation hotspot in breast cancer. Distributions of body mass index (BMI) and age at first live birth were different by age at diagnosis. Younger age at

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diagnosis (<50) was observed in cases with lower BMI ($P = 0.01$) and older age at first live birth or nulliparous ($P < 0.001$). As reported previously with a smaller subset of these patients (34), cases with stages II–IV breast cancer ($n = 147$) were diagnosed at significantly younger age (55.1 ± 13.2 versus 58.4 ± 12.9; $P = 0.02$), whereas cases with *TP53* mutations ($n = 32$) were diagnosed at significantly younger age (52.8 ± 13.2 versus 58.8 ± 12.4; $P = 0.01$).

DNA repair nsSNPs and age, stage and TP53 mutations

Table II summarizes the association between BER nsSNPs and age, stage and *TP53* mutations adjusted by race. At the single SNP level, none of the BER SNPs under study showed significant association with age, stage or *TP53* mutations. As shown in Table III for NER SNPs, the *ERCC2 312 DN/NN* genotypes were associated with early age at diagnosis (OR = 1.76; 95% CI = 1.10, 2.81) and the *XPC 939 QQ* genotype was associated with *TP53* mutations (OR = 5.80; 95% CI = 2.23, 15.09). Table IV summarizes the data on MMR/DSBR SNPs. The *NBS1 185 QQ* genotype was associated with early age at diagnosis (OR = 3.09; 95% CI = 1.47, 6.49). The association between *XPC K939Q QQ* genotype and *TP53* mutation remained significant after FDR analysis (FDR = 0.0019, original *P* value = 0.00032). Similarly, the association between *NBS1 E185Q QQ* genotype and early age (<50 years old) at diagnosis remained significant (FDR = 0.01724, original *P* value = 0.00287). The association between *ERCC2 D312N DN/NN* genotype and early age at diagnosis, however, was no longer significant (FDR = 0.114, original *P* value = 0.019).

Polygenic models of age, stage and TP53 mutations

As shown in Table V, there was significant trend in associating younger age at diagnosis (<50) with increasing numbers of risk genotypes for *ERCC2 312 DN/NN*, *MSH6 39 EE* and *NBS1 185 QQ* ($P_{trend} < 0.001$). After adjusted for stage and race, younger age at diagnosis was associated with combined one and two risk genotypes with OR of 2.24 (95% CI = 1.38, 3.62) and 4.32 (95% CI = 1.68, 11.13), respectively. There was a significant trend in associating advanced stage of tumor with *XRCC1 280 RH*, *ERCC4 415 QQ*, *MLH1 219 VV* and *XRCC3 241 MM* ($P_{trend} = 0.004$). After adjusted for age and race, advanced stage at diagnosis was associated with combined one and two risk genotypes with OR of 2.09 (95% CI = 1.28, 3.42) and 2.07 (95% CI = 0.57, 7.46), respectively. There was a significant trend in associating *TP53* mutations with increasing numbers of risk genotypes for *XRCC1 399 QQ*, *XPC 939 QQ*, *ERCC4 415 QQ* and *XPC 499 AA* ($P_{trend} < 0.001$). After adjusted for age, stage and race, *TP53* mutations were associated with combined two and three risk genotypes with OR of 5.75 (95% CI = 2.08, 15.87) and 27.51 (95% CI = 4.97, 152.16), respectively.

Discussion

Efficient mechanisms of DNA repair require multiple interacting proteins whose coordinated activities maintain genetic stability. Our data are consistent with studies, which have reported no association or minor associations between individual genetic variations in DNA repair genes and cancer risk. However, we found significant associations with increasing numbers of risk-associated alleles for age and stage at diagnosis. Our data provide evidence that multiple DNA repair SNPs may contribute to early age at diagnosis, advanced disease and *TP53* mutations in breast cancer. Our study results reflect the heterogeneity and complexity of breast cancer etiology.

We observed that cases <50 years of age had a greater frequency of later age at first live birth and nulliparity than women ≥50 years of age. Uninterrupted menstrual cycles and gestational hormonal exposure to mammary epithelium during pregnancy may enhance the carcinogenic process in susceptible subpopulations through

Table III. Association of NER genetic variants with age, breast cancer stage and *TP53* mutation status

SNP	Genotype	Age ≥50/<50	OR[a] (95% CI)	Stage I–II/III–IV	OR[b] (95% CI)	*TP53* Mutation No/Yes	OR[c] (95% CI)
ERCC2 D312N	*DD*	117/43	Referent	98/62	Referent	83/11	Referent
	DN	96/57	1.77 (1.08, 2.89)	99/54	0.89 (0.55, 1.42)	94/9	0.67 (0.26, 1.74)
	NN	28/16	1.72 (0.83, 3.55)	26/18	1.17 (0.58, 2.34)	24/1	0.32 (0.04, 2.62)
	DD	117/43	Referent	98/62	Referent	83/11	Referent
	DN/NN	124/73	1.76 (1.10, 2.81)	125/72	0.94 (0.60, 1.47)	118/10	0.60 (0.24, 1.52)
ERCC2 K751Q	*KK*	105/40	Referent	90/55	Referent	81/10	Referent
	KQ	113/59	1.40 (0.86, 2.28)	104/68	1.07 (0.67, 1.69)	93/13	1.14 (0.47, 2.78)
	QQ	33/20	1.71 (0.87, 3.35)	33/20	1.01 (0.52, 1.96)	31/0	N/A
	KK	105/40	Referent	90/55	Referent	81/10	Referent
	KQ/QQ	146/79	1.47 (0.92, 2.33)	137/88	1.06 (0.68, 1.63)	124/13	0.85 (0.35, 2.07)
ERCC4 R415Q	*RR*	227/104	Referent	198/133	Referent	180/19	Referent
	RQ	28/15	1.32 (0.67, 2.60)	33/10	0.47 (0.22, 0.98)	26/2	0.68 (0.15, 3.15)
	QQ	5/2	0.88 (0.17, 4.64)	3/4	2.21 (0.48, 10.21)	3/2	6.66 (0.88, 50.54)
	RR/RQ	255/119	Referent	231/143	Referent	206/21	Referent
	QQ	5/2	0.85 (0.16, 4.49)	3/4	2.41 (0.52, 11.10)	3/2	6.98 (0.93, 52.40)
ERCC5 D1104H	*DD*	145/63	Referent	129/79	Referent	114/13	Referent
	DH	93/55	1.25 (0.78, 1.99)	92/56	0.87 (0.55, 1.37)	84/11	0.97 (0.39, 2.38)
	HH	17/3	0.32 (0.09, 1.18)	11/9	1.24 (0.47, 3.25)	8/0	N/A
	DD	145/63	Referent	129/79	Referent	114/13	Referent
	DH/HH	110/58	1.12 (0.71, 1.77)	103/65	0.90 (0.58, 1.41)	92/11	0.91 (0.37, 2.23)
XPC A499V	*AA*	148/79	Referent	135/92	Referent	112/18	Referent
	AV	88/34	0.78 (0.48, 1.28)	78/44	0.91 (0.57, 1.45)	76/6	0.49 (0.18, 1.32)
	VV	17/7	0.85 (0.33, 2.15)	16/8	0.77 (0.31, 1.90)	18/0	N/A
	AV/VV	105/41	Referent	94/52	Referent	94/6	Referent
	AA	148/79	1.27 (0.79, 2.02)	135/92	1.13 (0.73, 1.77)	112/18	2.51 (0.94, 6.69)
XPC K939Q	*KK*	106/54	Referent	100/60	Referent	81/7	Referent
	KQ	115/52	0.90 (0.56, 1.44)	100/67	1.18 (0.75, 1.86)	100/7	0.81 (0.26, 2.51)
	QQ	39/12	0.62 (0.30, 1.29)	31/20	1.20 (0.62, 2.31)	26/10	5.18 (1.67, 16.0)
	KK/KQ	221/106	Referent	200/127	Referent	181/14	Referent
	QQ	39/12	0.66 (0.33, 1.31)	31/20	1.10 (0.59, 2.02)	26/10	5.80 (2.23, 15.09)

[a]Adjusted for stage (early/late) and race (Caucasian/African-American).
[b]Adjusted for race (Caucasian/African-American) and age (continuous).
[c]Adjusted for stage (early/late) and race (Caucasian/African-American) and age (continuous).

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Table IV. Association of MMR/DSBR genetic variants with age, breast cancer stage and *TP53* mutation status

SNP	Genotype	Age ≥50/<50	OR[a] (95% CI)	Stage I–II/III–IV	OR[b] (95% CI)	*TP53* mutation no/yes	OR[c] (95% CI)
MLH1 I219V	*II*	143/59	Referent	126/76	Referent	103/15	Referent
	IV	90/52	1.51 (0.94, 2.42)	87/55	1.06 (0.67, 1.67)	84/8	0.62 (0.24, 1.57)
	VV	20/5	0.64 (0.23, 1.81)	13/12	1.72 (0.74, 4.02)	17/1	0.42 (0.05, 3.56)
	II/IV	143/59	Referent	213/131	Referent	187/23	Referent
	VV	110/57	0.53 (0.19, 1.46)	13/12	1.68 (0.73, 3.83)	17/1	0.52 (0.07, 4.24)
MSH3 R940Q	*RR*	184/85	Referent	165/104	Referent	148/17	Referent
	RQ	65/31	1.02 (0.62, 1.69)	59/37	1.00 (0.62, 1.63)	51/7	1.23 (0.47, 3.19)
	QQ	12/5	0.93 (0.32, 2.74)	11/6	0.89 (0.32, 2.49)	11/0	N/A
	RR	184/85	Referent	165/104	Referent	148/17	Referent
	RQ/QQ	77/36	1.01 (0.63, 1.62)	70/43	0.99 (0.62, 1.56)	62/7	1.00 (0.39, 2.58)
MSH3 T1036A	*TT*	124/55	Referent	108/71	Referent	96/12	Referent
	TA	100/43	0.97 (0.60, 1.57)	91/52	0.87 (0.55, 1.38)	84/10	0.96 (0.39, 2.38)
	AA	34/21	1.35 (0.71, 2.54)	31/24	1.13 (0.61, 2.09)	27/2	0.49 (0.10, 2.37)
	TT/TA	224/98	Referent	199/123	Referent	180/22	Referent
	AA	34/21	1.36 (0.75, 2.48)	31/24	1.19 (0.66, 2.15)	27/2	0.49 (0.11, 2.29)
MSH6 G39E	*GG*	213/104	Referent	191/126	Referent	176/19	Referent
	GE	38/11	0.58 (0.29, 1.19)	31/18	0.90 (0.48, 1.69)	29/5	2.07 (0.65, 6.57)
	EE	5/4	1.72 (0.45, 6.59)	7/2	0.42 (0.09, 2.08)	2/0	N/A
	GG/GE	251/115	Referent	222/144	Referent	201/24	Referent
	EE	5/4	1.84 (0.48, 7.04)	7/2	0.43 (0.09, 2.11)	2/0	N/A
NBS1 E185Q	*EE*	136/57	Referent	113/80	Referent	108/14	Referent
	EQ	106/45	1.02 (0.64, 1.64)	99/52	0.73 (0.47, 1.14)	81/6	0.52 (0.19, 1.44)
	QQ	14/18	3.12 (1.45, 6.75)	18/14	1.02 (0.48, 2.21)	18/4	1.40 (0.39, 5.04)
	EE/EQ	242/102	Referent	212/132	Referent	186/20	Referent
	QQ	14/18	3.09 (1.47, 6.49)	18/14	1.17 (0.56, 2.47)	17/4	1.81 (0.53, 6.19)
XRCC3 T241M	*TT*	113/46	Referent	102/57	Referent	86/8	Referent
	TM	100/56	1.43 (0.88, 2.32)	98/58	1.12 (0.70, 1.79)	85/11	1.36 (0.51, 3.60)
	MM	40/16	1.03 (0.51, 2.08)	30/26	1.79 (0.94, 3.39)	32/5	1.70 (0.50, 5.73)
	TT/TM	213/102	Referent	200/115	Referent	171/19	Referent
	MM	40/16	0.85 (0.45, 1.62)	30/26	1.69 (0.94, 3.04)	32/5	1.44 (0.49, 4.25)

[a]Adjusted for stage (early/late) and race (Caucasian/African-American).
[b]Adjusted for race (Caucasian/African-American) and age (continuous).
[c]Adjusted for stage (early/late) and race (Caucasian/African-American) and age (continuous).

Table V. Polygenic models of age stage and *TP53* mutation status

Risk genotypes	Total no. of risk genotypes	Age ≥50	%	Age < 50	%	OR[a] (95% CI)	*P*
ERCC2 312 DN/NN	0	127	48.8	37	30.6	Referent	
NBS1 185 QQ	1	123	47.3	73	60.3	**2.24 (1.38, 3.62)**	0.001
MSH6 39 EE	2	10	3.8	11	9.1	**4.32 (1.68, 11.13)**	0.002
						$P_{trend} < 0.001$	
		Stage I–II	%	Stage III–IV	%	OR[b] (95% CI)	
XRCC1 280 RH	0	179	76.5	95	64.6	Referent	
ERCC4 415 QQ	1	50	21.4	47	32.0	**2.09 (1.28, 3.42)**	0.003
MLH1 219 VV	2	5	2.1	5	3.4	2.07 (0.57, 7.46)	0.267
XRCC3 241 MM						$P_{trend} = 0.004$	
		TP53 mutation (no)	%	*TP53* mutation (yes)	%	OR[c] (95% CI)	
XRCC1 399 QQ	0–1	173	84.4	11	45.8	Referent	
ERCC4 415 QQ	2	29	14.1	9	37.5	**5.75 (2.08, 15.87)**	0.001
XPC 499 AA	3	3	1.5	4	16.7	**27.51 (4.97, 152.16)**	<0.001
XPC 939 QQ						$P_{trend} < 0.001$	

[a]Adjusted for stage (early/late) and race (Caucasian/African-American).
[b]Adjusted for race (Caucasian/African-American) and age (continuous).
[c]Adjusted for stage (early/late) and race (Caucasian/African-American) and age (continuous).

continuous estrogen-mediated proliferation. More women with late-onset breast cancer had a first live birth age ≤24 years in comparison with those with early-onset disease, suggesting longer breast cancer latency due to protection provided by pregnancy-induced differentiated mammary cells. A higher percentage of cases age ≥50 years with higher BMI as compared with cases age ≤50 years was observed and supports a potential role for estrogen production in adipose tissue as a risk factor for postmenopausal breast cancer.

XRCC1 is a scaffolding protein in BER, assisting in the coordinated activity of BER components DNA Ligase III, ADPRT, multiple DNA glycosylases and DNA polymerase β (35,36). Codon 194 of the *XRCC1* gene is located in a highly conserved linker region, known for its hydrophobicity and presumed DNA-binding secondary structure (37). *XRCC1 R194W* has been associated with markers of DNA damage and cancer risk, though inconsistently (38–41). We observed that Caucasian carriers of the *XRCC1 194RW* genotype were at increased risk for age <50 years at diagnosis (data not shown), which is consistent with

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a previous study shown that the *XRCC1 194RW* genotype was associated with breast cancer risk in women aged 45–55 (42).

NBS1 is a member of the MRE11-RAD50-NBS1 (MRN) complex which accumulates at sites of DSBs and functions in numerous damage response pathways, including cell cycle arrest, DSBR, DNA replication and initiation of apoptosis (43). MRN foci are observed at sites of ionizing radiation-induced DNA damage (44). In breast cancer patients, an increased number of MRN foci, representing DSBs, were present after DNA repair in irradiated peripheral blood lymphocytes as compared with controls (45). We observed that carriers of the *NBS1 185 QQ* genotype had a significantly increased risk of breast cancer development before age 50, supporting a previous study in women ≤55 years of age (46). Since codon 185 lies in the NBS1 BRCA1 C-terminal or BRCT protein–protein interaction domain shared by many DNA repair and cell cycle proteins, an amino acid substitution in this region may alter NBS1's ability to effectively complex with other DNA repair and regulatory proteins (47), potentially contributing to genetic instability and breast carcinogenesis at a younger age.

XRCC3 functions in the stabilization of RAD51 foci at DSBs (48). XRCC3 *241M*-expressing cells have a slightly decreased homology-directed repair activity and mitomycin C hypersensitivity (49). In lymphocytes from cancer-free *XRCC3 241M* allele carriers, higher levels of DNA adducts and X-ray-induced chromosomal deletions have been reported (50,51). In cell lines, the *XRCC3 241M* variant has been shown to result in greater numbers of binucleated cells and lower levels of cellular apoptosis (52). Our observation of increased advanced stage of breast cancer in *XRCC3 241 MM* carriers is in agreement with these observations.

XPC is involved in the NER pathway. The *XPC 939Q* allele is associated with decreased repair of DNA damage induced by benzo(a)pyrene diolepoxide and gamma-radiation in healthy control lymphocytes (53). In a study of XPC 939Q-carrier bladder cancer patients, smokers had an increased risk for *TP53* transversion mutations (54). In the current study, the *XPC 939 QQ* genotype was significantly associated with *TP53* mutation status, which was more prevalent in ever-smokers (data not shown). Our results suggest that the *XPC 939Q* allele may modify smoking-related *TP53* mutations in breast cancer.

Only 11% of breast cancer cases were confirmed mutation carriers in *TP53* exons 5–9. Although lower than other reported breast cancer studies, *TP53* mutation frequencies may differ in various populations, reflecting variations in environmental exposure, including smoking and air pollution, ethnicity and geographic location. Higher numbers of DCIS/stage 0 and lower frequency of stages II–IV may contribute to frequency variation. However, *TP53* transition and non-transition frequencies were similar to a previous North Carolina study population (55). Mutations in *TP53* can arise through multiple mechanisms, initiated by exogenous and endogenous agents (26). Higher frequencies of *TP53* mutations were identified in cases aged <50 years (Table I; 34). In addition, our data showed differences in subgroup *TP53* mutation types; cases aged <50 and stages II–IV were more likely to have non-transitions and transitions, respectively (data not shown). While transitional mutations may result from oxidative and spontaneous deamination of methylated cytosine and transversions via carcinogen exposure, including tobacco byproducts (26), deletions and complex mutations may arise from errors in DNA replication and through DNA damage induced by ionizing radiation. Accordingly, analysis of *TP53* mutation subtypes may help to elucidate carcinogen exposure leading to breast cancer development.

Previous studies using a subset of this patient population examined the association of *TP53* mutations with polymorphisms in drug metabolic enzymes responsible for the activation and detoxification of endogenous and exogenous chemical carcinogens (26). This study demonstrated that the combined *GSTP1 105 VV*, *CYP1B1 432 LV/VV* and *GSTM1*-positive genotypes were associated with mutations in *TP53* relative to women with breast cancer that had other genotype patterns. Although the sample sizes were too small to include a comprehensive analysis of both DNA repair and drug metabolic pathways, the combined results of this and the current study suggest that genetically determined individual responses to environmental chemical exposures can affect relative susceptibility of the individual to cancer initiation.

These initial data represent early genotyping efforts in an ongoing study. Future studies will incorporate a more comprehensive evaluation of case demographics, tumor phenotype and clinical follow-up information. Low *TP53* mutation frequencies were detected with many DNA repair genotype associations. However, the association between younger age at diagnosis with *ERCC2 312 DN/NN* genotypes and *NBS1 185 QQ* genotype as well as the association between the *XPC 939 QQ* genotype and *TP53* mutations warrant further investigation. Genetic background and *TP53* mutation status may influence tumor sensitivity to chemotherapy and radiotherapy, clinical outcome and survival (37,56–59). Women diagnosed with breast cancer and having specific DNA repair genotypes may benefit from individually tailored breast cancer management aimed to improve quality of life issues and survivorship.

Our pilot study provides evidence that multiple DNA repair SNPs are associated with early age at diagnosis, advanced disease and *TP53* mutations in breast cancer. Notably, the association was stronger in carriers with multiple risk genotypes. Although these preliminary data require validation in larger independent populations, our results suggest that DNA repair SNPs not only impact breast cancer risk but may also influence age at diagnosis and aggressiveness of tumor phenotype. Screening of DNA repair SNPs may increase the accuracy of prediction of breast cancer risk and aid in improving clinical outcomes through the identification of cancer cases suitable for more aggressive treatment.

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