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EPIDEMIOLOGY

DNA-repair genetic polymorphisms and risk of breast cancer in Cyprus

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Abstract The DNA repair pathway is known to play a role in the etiology of breast cancer. A number of studies have demonstrated that common germline variants in genes involved in the DNA repair pathway influence breast cancer risk. To assess whether alterations in DNA repair genes contribute to breast cancer, we genotyped 12 single nucleotide polymorphisms (SNPs) in 1,109 Cypriot women with breast cancer and 1,177 age-matched healthy controls. We found significant associations with breast cancer for SNPs in the *BRCA2* and *MRE11A* genes. Carriers of the *BRCA2* rs1799944 variant (991 Asp) were found to have an increased risk of breast cancer (OR = 1.41, 95% CI 1.08–1.83, $P = 0.01$) with $P_{trend} = 0.0076$. Homozygous carriers of the *MRE11A* rs601341 A allele had an increased risk of breast cancer (OR = 1.36, 95% CI 1.08–1.71, $P = 0.009$) with $P_{trend} = 0.0087$. This study suggests that genetic variants in *BRCA2* and *MRE11A* are associated with breast cancer risk.

Keywords Breast cancer · Case-control study · Cyprus · DNA repair genes · Genetic epidemiology · SNP

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Introduction

Breast cancer is the most common malignancy affecting women worldwide and it is the leading female cancer in Cyprus, with approximately 350–400 new cases diagnosed annually [1].

The DNA repair pathway is essential for maintaining genomic stability of mammalian cells. Deficiencies in DNA repair mechanisms lead to high penetrance genetic syndromes such as Fanconi anemia and Bloom syndrome, which have cancer as a predominant phenotype [2]. Ten different genes, involved in pathways critical to genomic integrity, have been implicated in inherited predisposition to breast cancer. Germline mutations in these genes significantly increase breast cancer risk and thus support a major role of the DNA repair pathway in breast carcinogenesis. The most important of these genes are *BRCA1* and *BRCA2* [3]. There is also evidence from in vitro studies that reduced DNA repair capacity is associated with increased breast cancer risk [4, 5].

The known breast cancer susceptibility genes have been estimated to explain only 5% of breast cancer cases, thus it is likely that other breast cancer susceptibility genes exist [6]. Based on the fact that the DNA repair pathway is involved in familial breast cancer it was suggested that single nucleotide polymorphisms (SNPs) in genes involved

in DNA repair may influence DNA repair capacity and, in turn, confer an altered susceptibility to develop breast cancer. Several studies have investigated the role of SNPs in DNA repair genes in relation to breast cancer and have reported associations with breast cancer risk [7–10].

In this study, we hypothesized that genetic variation in the DNA repair pathway may modify susceptibility to breast cancer in Cypriot women. As part of an ongoing study we assessed genetic variation in 12 SNPs in 11 DNA repair-related pathway genes, specifically *BARD1*, *BRCA2*, *ERCC2*, *FANCA*, *MLH1*, *MRE11A*, *MSH2*, *OGG1*, *RAD51*, *RAD52*, and *TP53* and their association with breast cancer in a case-control study of Cypriot women.

Materials and methods

Subjects

The study population was women participating in the *MASTOS* study, a population-based case-control study of breast cancer in Cyprus [11]. Blood samples were collected between 2004 and 2006 from 1,109 female breast cancer patients diagnosed between 40 and 70 years old and 1,177 aged-matched healthy controls. Cases participating in the study were women previously diagnosed with breast cancer between January 1999 and December 2006. In addition to blood samples, a risk factor questionnaire that included extensive demographic, epidemiological, and pathological data was obtained from each participant through a standardized interview. Breast cancer cases were verified by reviewing histological reports. The study was approved by the National Bioethics Committee of Cyprus, and all participants provided signed informed consent.

Genotyping

DNA was isolated from blood samples using standard procedures (phenol-chloroform method). The 12 single nucleotide polymorphisms (SNPs): *BARD1* rs1048108 (Pro24Ser), *BRCA2* rs1799944 (Asn991Asp), *ERCC2* rs13181 (Lys751Gln), *FANCA* rs2239359 (Gly501Ser), *MLH1* rs1799977 (Ile219Val), *MRE11A* rs601341, *MSH2* rs2059520, *OGG1* rs1052134 (Ser326Cys), *RAD51* rs1801320 and rs1801321 (135G > C-5′UTR and 172 G > T-5′UTR), *RAD52* rs11226 (2259 C > T-3′UTR) and *TP53* rs1042522 (Pro72Arg) were genotyped in all subjects participating in the study. All SNPs studied had a minor allele frequency of over 0.05. Genotyping was performed using the Taqman SNP genotyping assays from Applied Biosystems Inc. (ABI). For genotyping SNP rs1801320 the primers and probes described previously by Kuschel et al. were used [8]. Each assay was carried out using 10 ng genomic DNA in a 5 μl reaction using Taqman Universal PCR Master Mix (ABI), forward and reverse primers, and FAM and VIC-labelled probes purchased from Applied Biosystems (ABI Pre-Designed assays). All assays were carried out in 384-well plates. The fluorescence profile was read on an ABI PRISM 7900HT instrument and the results analyzed with Sequence Detection Software (ABI). For quality control, random samples were genotyped in duplicate and had identical genotyping assignments. Genotype call rates ranged from 99% to 100% and duplicate concordance rates were higher than 99%.

Data analysis

We performed a chi square test (χ^2) to assess Hardy–Weinberg equilibrium (HWE) in the control samples. Genotype frequencies were compared across groups using the χ^2 test and the Mantel–Haenzel test for linear trend. The association between breast cancer and each SNP was examined using logistic regression with the SNP genotype tested under models of complete dominance and recessive inheritance. Statistical analysis was carried out using the SPSS v 13 software (SPSS Inc., Chicago, Illinois) and GraphPad InStat v 3.06 (GraphPad Software, San Diego California).

Results

Genotype distributions of controls at each locus were consistent with HWE. However, the *RAD51* genotype frequencies in the controls were not in HWE ($P < 0.05$), but were similar to the frequencies reported by the HapMap Project (www.hapmap.org) [12]. This may be due to hidden population structures that specifically affect RAD51. Neither of the *RAD51* SNPs were associated with breast cancer. Table 1 summarizes the distribution of genotypes among cases and controls, as well as the allele frequencies of the 12 SNPs under study.

The median ages of both the breast cancer cases and the controls were 56 years. The mean age at diagnosis for the breast cancer cases was 51.6 years (standard deviation (SD) ± 9.2) and mean age at ascertainment for the controls was 56.4 years ((SD) ± 9.2).

The associations of the SNPs and breast cancer risk in Cypriot women are shown in Table 2. We found significant associations of the *BRCA2* rs1799944 (Asn991Asp) and *MRE11A* rs601341 variants and breast cancer risk. Carriers of *BRCA2* 991 Asp were found to have an increased risk of breast cancer (OR = 1.41, 95% CI 1.08–1.83, $P = 0.01$) with $P_{trend} = 0.0076$. Homozygous carriers of the *MRE11A* rs601341 A allele had an increased risk of breast cancer (OR = 1.36, 95% CI 1.08–1.71, $P = 0.009$) with $P_{trend} = 0.0087$. A marginal association ($P = 0.05$) was

Table 1 Genotypes and allele frequencies for the 12 SNPs under study

Gene/SNP	Cases	Controls	*P*-value[a]	Gene/SNP	Cases	Controls	*P*-value[a]
MSH2 (rs2059520)				*RAD51* 135G > C (rs1801320)			
A/A	512	562	0.8	G/G	915	952	0.5
A/G	471	489		G/C	193	216	
G/G	108	119		C/C	0	0	
MAF[b]	0.31	0.31		MAF[b]	0.09	0.09	
Hardy–Weinberg[c]		0.41		Hardy–Weinberg[c]		0.0005	
MLH1 Ile219Val (rs1799977)				*RAD51* 172 G > T (rs1801321)			
Ile/Ile	543	568	0.78	G/G	340	400	0.24
Ile/Val	449	497		G/T	522	530	
Val/Val	98	110		T/T	236	236	
MAF[b]	0.3	0.31		MAF[b]	0.45	0.43	
Hardy–Weinberg[c]		0.93		Hardy–Weinberg[c]		0.01	
MRE11A (rs601341)				*BRCA2* Asn991Asp (rs1799944)			
G/G	385	452	0.02	Asn/Asn	945	1058	0.03
G/A	530	566		Asn/Asp	133	108	
A/A	190	156		Asp/Asp	8	4	
MAF[b]	0.41	0.37		MAF[b]	0.07	0.05	
Hardy–Weinberg[c]		0.31		Hardy–Weinberg[c]		0.49	
BARD1 Pro24Ser (rs1048108)				*OGG1* Ser326Cys (rs1052134)			
Pro/Pro	515	514	0.18	Ser/Ser	615	647	0.93
Pro/Ser	445	520		Ser/Cys	422	455	
Ser/Ser	138	138		Cys/Cys	71	72	
MAF[b]	0.33	0.34		MAF[b]	0.25	0.26	
Hardy–Weinberg[c]		0.71		Hardy–Weinberg[c]		0.5	
FANCA Gly501Ser (rs2239359)				*TP53* Pro72Arg (rs1042522)			
Gly/Gly	387	433	0.52	Pro/Pro	555	638	0.08
Gly/Ser	524	543		Pro/Arg	463	438	
Ser/Ser	190	186		Arg/Arg	85	97	
MAF[b]	0.41	0.39		MAF[b]	0.29	0.27	
Hardy–Weinberg[c]		0.47		Hardy–Weinberg[c]		0.08	
ERCC2 Lys751Gln (rs13181)				*RAD52* 2259C > T (rs11226)			
Lys/Lys	331	383	0.06	C/C	561	568	0.4
Lys/Gln	603	585		C/T	448	494	
Gln/Gln	171	208		T/T	92	108	
MAF[b]	0.43	0.43		MAF[b]	0.29	0.3	
Hardy–Weinberg[c]		0.55		Hardy–Weinberg[c]		0.97	

[a] Genotype frequency *P*-value

[b] MAF = minor allele frequency

[c] *P*-value from Chi-square test

observed between *TP53* rs1042522 (Pro72Arg) and risk of breast cancer. No significant associations with breast cancer were observed for the other nine SNPs studied.

Discussion

Breast cancer is a common, polygenic, and heterogeneous disease. Genetic epidemiology data suggest that part of breast cancer etiology can be explained by common, low-penetrance alleles that increase susceptibility to breast cancer risk [13]. DNA repair is essential for maintaining genomic integrity. Deficiencies in the DNA repair pathway lead to genetic instability which in turn may lead to cancer development. Genetic polymorphisms in DNA repair genes may contribute to differential DNA repair capability between individuals [14]. In an attempt to identify low-penentrance breast cancer susceptibility alleles, we

Table 2 Genotype frequencies and risk estimates calculated using the recessive and dominant inheritance models

Gene	SNP	Model	OR	95% CI	*P*-value
MSH2	rs2059520	Dominant	1.05	0.89–1.23	0.6
		Recessive	0.97	0.74–1.28	0.83
					0.76[a]
MLH1	rs1799977	Dominant	0.94	0.8–1.11	0.48
		Recessive	0.96	0.72–1.27	0.76
					0.5[a]
MRE11A	rs601341	Dominant	1.17	0.99–1.39	0.07
		Recessive	1.36	1.08–1.71	0.009
					0.0087[a]
BARD1	rs1048108	Dominant	0.88	0.75–1.04	0.15
		Recessive	1.08	0.84–1.39	0.56
					0.43[a]
FANCA	rs2239359	Dominant	1.1	0.92–1.3	0.3
		Recessive	1.09	0.88–1.37	0.42
					0.25[a]
ERCC2	rs13181	Dominant	1.13	0.95–1.35	0.18
		Recessive	0.85	0.68–1.06	0.16
					0.89[a]
RAD51	rs1801320	Dominant	0.93	0.75–1.15	0.5
		Recessive			
					0.5[a]
RAD51	rs1801321	Dominant	1.16	0.98–1.39	0.09
		Recessive	1.08	0.88–1.32	0.46
					0.13[a]
BRCA2	rs1799944	Dominant	1.41	1.08–1.83	0.01
		Recessive	2.16	0.65–7.2	0.2
					0.0076[a]
OGG1	rs1052134	Dominant	0.98	0.83–1.16	0.85
		Recessive	1.05	0.75–1.47	0.79
					0.96[a]
TP53	rs1042522	Dominant	1.18	1–1.39	0.05
		Recessive	0.93	0.68–1.25	0.62
					0.19[a]
RAD52	rs11226	Dominant	0.89	0.76–1.06	0.19
		Recessive	0.9	0.68–1.21	0.5
					0.19[a]

[a] P_{trend}

investigated the hypothesis that common variation in 11 DNA repair-related pathway genes modifies risk for breast cancer. We genotyped 12 SNPs in a cohort of 2,286 Cypriot women (1,109 breast cancer patients and 1,177 healthy controls). We found that SNPs in *BRCA2* and *MRE11A* may be associated with breast cancer risk.

For the *BRCA2* 991Asp allele, the additive model showed a significant trend ($P = 0.0076$) towards increased risk of breast cancer with the number of copies of the Asp allele among Cypriot women. It is located in the conserved BRC repeat region of the BRCA2 gene in exon 11 [15]. This variant has been found in many individuals with a family history of breast cancer and has been classified as a variant of no clinical significance in the Breast Cancer Information Core Database (BIC) [16]. On the other hand, in silico prediction methods suggest that this is a non-tolerated amino acid substitution within the limits of confidence in the alignments [17]. Therefore, until functional data become available, the pathogenicity of this variant cannot be excluded, and it may be a variant that increases risk moderately, but is indeed, not highly penetrant. There was no association between the presence of the *BRCA2* 991Asp allele and family history of breast cancer. A moderately strong association of this *BRCA2* polymorphism with malignant melanoma has been reported. The presence of this common *BRCA2* variant was associated with malignant melanoma risk ($P = 0.002$ after Bonferroni correction), in over 9% of the cases studied. The authors suggested that this variant is not a neutral missense mutation and that follow-up studies should be undertaken in melanoma and breast cancer populations to precisely define its pathogenicity [18]. The role of this SNP in breast cancer risk has been investigated in the Multiethnic Cohort study and no association was found [19]. Previous studies that we performed in our population revealed a different spectrum of mutations in the *BRCA1* and *BRCA2* genes compared to other populations [20, 21]. The over-representation of the *BRCA2* Asn991Asp polymorphism in the breast cancer group supports that this variant is associated with an increased breast cancer risk among Cypriot women and it is possible that this association is characteristic only for the Cypriot population.

In the current study, there was also evidence for an increased breast cancer risk for women homozygous for the *MRE11A* rs601341 A allele. The *MRE11A* gene forms a complex with *RAD50* and *NBS1* genes which is involved in the cellular response to DNA double strand breaks. Defects in the members of this tri-complex are linked to increased chromosomal instability which leads to cancer [22]. To our knowledge, the role of rs601341 in breast cancer has not been investigated but a protective effect of this SNP against follicular lymphoma has been reported [23]. rs601341 may be in LD with another variant in the region. Functional studies will need to be performed in the future to identify the actual causal variant.

There are contradictory reports regarding the role of the *TP53* Pro72Arg polymorphism and breast cancer. Our results suggest a marginal increased risk for breast cancer ($P = 0.05$) for carriers of the Pro allele. A meta-analysis conducted by the Breast Cancer Association Consortium concluded that this variant is not associated with breast cancer [24].

Our study has several strengths, including a high participation rate of eligible cases (98%) and a sample from a homogeneous ethnic background (100% of study participants are Greek Cypriots) thus reducing the bias due to population stratification. In addition, our study population (both cases and controls) was from all over the country minimizing potential selection bias. Limitations of this study are that our analysis did not consider the possibility of gene-gene interactions. It is possible that the risks observed are the result of interactions but we have not attempted to assess such effects since the estimate of an interaction effect will be unreliable because of small numbers. We also did not adjust for possible differences in lifestyle factors.

In conclusion our results suggest that genetic variation in the DNA repair pathway is associated with breast cancer risk in Cypriot women. The associations with SNPs rs1799944 and rs601341 should be considered for replication efforts in other larger studies to increase confidence in reported association and to clarify whether the association is only specific for the Cypriot population.

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