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Title

Genetic variation in genes interacting with *BRCA1/2* and risk of breast cancer in the Cypriot population.

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Abstract

Inability to correctly repair DNA damage is known to play a role in the development

of breast cancer. Single nucleotide polymorphisms (SNPs) of DNA repair genes have

been identified which modify the DNA repair capacity, which in turn may affect the

risk of developing breast cancer. To assess whether alterations in DNA repair genes

contribute to breast cancer, we genotyped 62 SNPs in 29 genes in 1,109 Cypriot

women with breast cancer and 1,177 age-matched healthy controls. Five SNPs were

associated with breast cancer. SNPs rs13312840 and rs769416 in the *NBS1* gene were

associated with a decrease in breast cancer risk (OR TT *v* TC/CC = 0.58; 95% CI,

0.37 to 0.92; p = 0.019 and OR GG vs GT/TT = 0.23, 95% CI 0.06–0.85, p = 0.017

respectively). The variant allele of *MRE11A* rs556477 was also associated with a

reduced risk of developing the disease (OR AA vs AG/GG = 0.76; 95% CI, 0.64 to

0.91; p = 0.0022). *MUS81* rs545500 and *PBOV1* rs6927706 SNPs were associated

with an increased risk of developing breast cancer (OR GG vs GC/CC = 1.21, 95%

CI, 1.02 to 1.45; p = 0.031; OR AA vs AG/GG = 1.53, 95% CI, 1.07 to 2.18; p =

0.019, respectively). Finally, haplotype-based tests identified significant associations

between specific haplotypes in *MRE11A* and *NBS1* genes and breast cancer risk.

Further large-scale studies are needed to confirm these results.

Introduction

Breast cancer is the most common malignancy affecting women worldwide and it is the leading female cancer in Cyprus, with approximately 400 new cases diagnosed annually [1]. *In vitro* studies have shown variability in inter-individual DNA repair capacity and have demonstrated that reduced ability to repair DNA is associated with an increased risk for breast cancer [2-4]. It has also been suggested that deficient DNA repair capacity predisposes to both familial and sporadic forms of breast cancer [5-7].

Ten different genes which are involved in pathways critical to genomic integrity have been implicated in inherited predisposition to breast cancer, including *BRCA1*, *BRCA2*, *p53*, *PTEN*, *CHEK2*, *ATM*, *NBS1*, *RAD50*, *BRIP1* and *PALB2*. The association of germline mutations in DNA repair genes with an increased susceptibility to breast cancer highlights the importance of these pathways in the development of breast cancer [8].

The DNA repair pathway is clearly involved in familial breast cancer. Thus, it was hypothesized that common single nucleotide polymorphisms (SNPs) of genes involved in the DNA repair pathway may influence breast cancer risk. Many studies have investigated the role of SNPs in DNA repair genes in relation to breast cancer and have reported associations with breast cancer risk [9-12].

Analysis of members of the DNA repair pathway appears to be a good rationale for identifying novel susceptibility loci. In particular, genes which have a direct interaction with the *BRCA1* and *BRCA2* genes are very good candidates. Recently, two more susceptibility genes, namely *BRIP1*/*FANCJ* and *PALB2*/*FANCN*, which interact with *BRCA1* and *BRCA2* genes respectively, have been identified [13, 14].

BRCA1 and *BRCA2* participate in the biological response to DNA damage that includes the activation of cell cycle checkpoints and the recruitment of the DNA damage repair machinery. Both *BRCA1* and *BRCA2* are implicated in DNA repair by homologous recombination and their proteins have distinct roles in double-strand break repair [15].

Despite the progress that has been made in improving our understanding of the functions of the *BRCA1* protein, a complete picture has not yet been attained. It has been hypothesized that *BRCA1* acts as a coordinator of the various functions of DNA damage, recognition, response and repair, and double-strand break repair. *BRCA1* interacts with many DNA repair proteins and protein complexes including the *RAD50-MRE11A-NBS1* (MRN) complex. The proteins associated with *BRCA1* are involved in response to and in the repair of DNA damage in several ways by acting as DNA damage sensors, signal transducers and repair effectors. Hence, these proteins are instrumental in the repair of DNA breakages and in the maintenance of genomic integrity [16-18]. The exact role(s) of the *BRCA2* protein also still remain elusive. It has been demonstrated that *BRCA2* plays an important role in homologous recombination, both in meiosis and in the repair of double-strand breaks. Fewer proteins are known to interact with *BRCA2* compared to *BRCA1* [19]. These include

RAD51, which mediates DNA repair via homologous recombination (HR) [15], and *PALB2*, which is required for *BRCA2* nuclear localization and stability as well as for some of its functions in HR and double-strand break repair [20]. Overall, *BRCA1* and *BRCA2* act in response to DNA damage and participate in multi-protein complexes that are involved in tumor suppression processes [17].

In this study we hypothesized that germline variations in genes encoding proteins that interact with *BRCA1/2*, are potential candidates for modifying breast cancer risk in the Cypriot population. Consequently disturbances in the interactions with *BRCA1* and *BRCA2* may prevent their tumor suppression function(s) and consequently modify inter-individual DNA repair capacity. As part of an ongoing study we assessed genetic variation in 60 SNPs in 29 genes, which interact with *BRCA1* or *BRCA2* genes and their association with breast cancer in a case-control study of Cypriot women. Furthermore, we investigated the role of 2 additional SNPs in the *PBOV1* (*UROC28*) and *DBC2* genes which are both upregulated in breast cancer [21, 22].

Materials and Methods

Study population

To investigate the associations between genetic factors and breast cancer risk in the Cypriot population, we conducted a population-based case-control study, with the acronym *MASTOS* (Greek word for breast). The population of this study are women participating in the *MASTOS* study. Blood samples were collected between 2004-2006 from 1109 female breast cancer patients diagnosed between 40-70 years old and 1177 aged-matched healthy controls. Cases participating in the study were women who were previously diagnosed with breast cancer between January 1999 and December

2006. The majority of cases were ascertained from the Bank of Cyprus Oncology Centre operates as a referral centre and offers treatment and follow-up for 80-90% of all breast cancer cases diagnosed in Cyprus. The rest of the patients, were recruited at the Oncology Departments of the Nicosia, Limassol, Larnaca and Paphos district hospitals. The control group consisted of healthy women who were participating in the National program for breast cancer screening with the use of mammography. Volunteers were enrolled in the study during the same calendar period as the cases, from the 4-district mammography screening centers that operate in Cyprus. Eligible controls were women with no previous history of breast cancer who had a negative mammography result. All study participants, both cases and controls, were of Greek Cypriot Caucasian origin, thus reducing any potential bias due to population stratification. In addition, the study population was representative of the whole island population and thus consisted of women, who resided in all five districts of the country, minimizing potential selection bias. The participation rate of cases and controls was very high covering around 98% of eligible cases and controls. In addition to blood samples, a risk factor questionnaire, which 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 reviewed and approved by the National Bioethics Committee of Cyprus. All participants provided written informed consent.

<u>Gene and SNP Selection</u>

Sixty-two SNPs in *ATF1*, *ATM*, *ATR*, *BARD1*, *BLM*, *BRIP1*, *CHEK1*, *CHEK2*, *DDB2*, *DMC1*, *EME1*, *FANCA*, *FANCC*, *FANCD2*, *FANCE*, *MLH1*, *MRE11A*, *MSH2*, *MSH6*, *MUS81*, *NBS1*, *PALB2*, *PCNA*, *RFC1*, *RAD50*, *RAD51C*, *RAD51L1*, *RAD52* and *XPC* genes were genotyped. The genetic variants were selected based on three main criteria: (1): all SNPs chosen belong to genes which interact with either *BRCA1* and/or *BRCA2*; (2) the SNPs chosen are either functional SNPs (based on potential protein changes, evolutionary conservation and location in putative functional regions [23-25] or (3) SNPs which were reported by other groups to modify cancer risk [14, 26-32]. For *MRE11A* and *RAD50*, we genotyped the tagging SNPs in Allen-Brady *et al.* [33] and for *NBS1*, we genotyped the tagging SNPs in Lu *et al.* [32]. SNPs in the *PBOV1* and *DBC2* genes were selected based on their minor allele frequency (MAF) >0.05.

<u>Genotyping</u>

DNA was isolated from blood samples using standard procedures (phenol-chloroform method). SNPs were genotyped by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) of allele-specific primer extension products (Mass Array, Sequenom Inc., San Diego, CA, USA). Assay design was based on published sequences retrieved from the National Center of Biotechnology Information (NCBI) databases. A 34-plex and a 28-plex multiplex assay were designed using the Sequenom MassARRAY Assay Design software (version 3.0). SNPs were genotyped using Sequenom iPLEX chemistry on a MALDI-TOF Compact Mass Spectrometer (Sequenom Inc., San Diego, CA, USA).

Briefly, PCR reactions were carried out in a final volume of 5 μl in standard 384-well plates. PCR was performed with 5 ng of genomic DNA, 1 unit of HotStarTaq DNA polymerase (Qiagen, Hilden, Germany), 500 μmol of each dNTP and 100 nmol of each PCR primer. PCR thermal cycling was carried out in an ABI-9700 instrument (Applied Biosystems, Foster City, CA, USA) for 15 min at 94°C, followed by 44 cycles of 20 s at 94°C, 30 s at 56°C and 60 s at 72°C. Next, PCR products were treated with 0.5 units of shrimp alkaline phosphatase for 40 minutes at 37°C to dephosphorylate unincorporated dNTPs, followed by enzyme inactivation for 5 min at 85oC. After adjusting the concentrations of the extension primers to equilibrate signal-to-noise ratios, the post-PCR primer extension reaction of the iPLEX gold assay was performed in a final 10 μl volume extension reaction containing 0.2 ul of termination mix, 0.0041 μl of iPLEX enzyme (Sequenom Inc., San Diego, CA, USA) and 700–1400 nM of extension primers. A two-step 200 short cycles program was used for the iPLEX reaction: initial denaturation was for 30 s at 94°C followed by five cycles of 5 s at 52°C and 5 s at 80°C. An additional 40 annealing and extension cycles were then looped back to 5 s at 94°C, 5 s at 52°C and 5 s at 80°C. Final extension was carried out at 72°C for 3 min. The iPLEX reaction products were desalted by diluting samples with 16 μl of water and adding 6 mg of clean resin. Following a quick centrifugation (3200 g for 5 minutes), reaction products were spotted on a 384-format SpectroChip (Sequenom Inc., San Diego, CA, USA). SpectroCHIPs were processed in a MassARRAY Compact Analyzer (Bruker Daltonics, Bremen, Germany) by MassARRAY Workstation (version 3.3) software (Sequenom Inc., San Diego, CA, USA). Acquisition data were analysed using MassARRAY TYPER 3.4 software (Sequenom Inc., San Diego, CA, USA).

For quality control, 48 random samples were genotyped in duplicate. Furthermore, ten samples were sequenced to confirm genotype calls from the MALDI-TOF platform. The genotype concordance rate between platforms was 99%. The order of the DNA samples on 384-well plates was randomized in order to ensure the same study conditions for samples from cases and controls. Genotyping call rates ranged from 95 to 99% and duplicate concordance rates were higher than 99%. The SNP that had 20% missing data was excluded from further analysis.

Data Analysis

Hardy Weinberg equilibrium (HWE) was assessed in the control samples by applying an exact test. The primary tests of association were the univariate analyses between each SNP and breast cancer. Genotype frequencies in cases and controls were compared using the X^2 test. 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 as well as under the log-additive model after adjusting for breast cancer risk factors including age (under or over 55 years), menopause status (pre- or post-menopausal), family history of breast cancer (first degree relative with breast cancer) and use of hormone replacement therapy. Statistical analysis was carried out using SNPStats, a web-based application designed for analysis of association studies [34].

Associations between breast cancer and common haplotypes of the *ATM*, *MRE11A* and *NBS1* genes were also investigated using SNPStats, which allows the estimation of maximum likelihood estimates of haplotype frequencies using the Expectation-Maximization (EM) algorithm. Logistic regression was performed to test the

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association between haplotypes and breast cancer risk. For assessing the contribution of the *MRE11A* haplotypes in breast cancer risk, a haplotype tagging SNP genotyped previously was also included in haplotype reconstruction [35]. Haplotypes with a frequency of less than 1% were not considered further for analysis since they are likely to be a result of rare recombination events.

Results

Table 1 shows the genotype frequency in cases and controls for the 62 SNPs, of which the 61 were successfully genotyped. Six SNPs (rs1800149, rs2706377, rs1800282, rs7487683, rs3626, rs728908468) deviated from HWE in controls (p<0.01) and were excluded from further analysis. Of the remaining 55 SNPs, 8 were monomorphic in both groups. Significant differences in genotype frequencies between breast cancer patients and controls were observed in 5 of the 55 SNPs analyzed.

The associations of SNPs and breast cancer risk in Cypriot women are shown in Table 2. Five of the 55 SNPs were associated at a p value of less than 0.05. Three SNPs were associated with a reduced risk for breast cancer while the three remaining were associated with an increased breast cancer risk. In detail, the variant allele of *NBS1* rs13312840 (924 T>C) was associated with a reduced risk of disease (OR TT *v* TC/CC = 0.58; 95% CI, 0.37 to 0.92; p = 0.019). Carriers of the *NBS1* rs769416 rare allele also had a reduced risk of breast cancer (OR GG vs GT/TT = 0.23, 95% CI 0.06–0.85, P = 0.017). Furthermore, the variant allele of *MRE11A* rs556477 was associated with a reduced risk of developing the disease (OR AA vs AG/GG = 0.76; 95% CI, 0.64 to 0.91; p = 0.0022). The variant allele of *MUS81* rs545500 was associated with an increased risk of developing breast cancer (OR GG vs GC/CC=

1.21, 95% CI, 1.02 to 1.45; p = 0.031). In addition, the rare allele of *PBOV1* rs6927706 was also associated with an increased risk of developing breast cancer (OR AA vs AG/GG = 1.53, 95% CI, 1.07 to 2.18; p = 0.019).

The *NBS1* haplotype GGCGCAC (rs769416, rs769420, rs13312840, rs1805794, rs6413508, rs12677527, rs1805787), which contains the *NBS1* rs13312840 C allele, to be associated with a reduced breast cancer risk compared with the most frequent haplotype GGTCCGC (OR=0.62; 95% CI= 0.39 to 0.97; p=0.037). We also found a reduced risk for breast cancer for a rare haplotype in *NBS1* (OR=0.42; 95% CI= 0.26 to 0.66; p=2 x 10-4). In addition, the *MRE11A* haplotype AGCG (rs556477, rs601341, rs10831234, rs1009456) is associated with a significantly increased risk for breast cancer (OR=1.32; 95% CI= 1.13 to 1.54; p=0.0004). None of the common *ATM* haplotypes were associated with breast cancer (Table 3).

Discussion

Breast cancer is a complex polygenic disease. Published data suggest that a proportion of breast cancer can be explained by common low-penetrance alleles that increase susceptibility [36]. High-penetrance mutations in genes which are involved in DNA repair pathways such as *BRCA1* and *BRCA2* predispose to familial breast cancer [37, 38]. Previously our group characterized novel mutations in these genes in Cypriot families [39, 40]. The importance of common inherited variants in DNA repair genes in relation to breast cancer risk is still being elucidated, but is currently receiving increased attention. Our group as part of an ongoing investigation has studied genetic variation in DNA repair genes in relation to breast cancer risk in the Cypriot population and has reported a number of SNPs that modify breast cancer risk [35, 41].

A number of large studies which focused on the contribution of common SNPs in DNA repair genes in breast cancer, using tagging-SNP approaches have also been completed [9, 42, 43]. In this case-control study we evaluated both functional as well as tagging SNPs in DNA repair genes in relation to breast cancer risk in Cypriot women.

We found that Cypriot women who carry *NBS1* rs13312840 C and rs769416 T alleles have a reduced risk of breast cancer. The *NBS1* protein is involved in non-homologous end-joining (NHEJ) pathway that repairs DNA double-strand breaks (DSBs). The first step of this pathway consists of the recognition of DSBs by the MRN complex whose core contains the *MRE11*, *RAD50* and *NBS1* proteins. *NBS1* is the key regulator of this protein complex [44, 45]. The *NBS1* rs13312840 T>C SNP is located on the 5' UTR (-1120) of the gene that is the transcription factor *GATA-1* binding site. The activation domains of *GATA-1* are capable of activating transcription in mammalian cells through *GATA* motifs [46]. Our results are in contrast to those of a recent study by Lu *et al.* which found an increased risk for breast cancer in non-Hispanic Caucasian women aged 55 years or younger who were carriers of the C allele [32]. Conflicting evidence for association may be due to population-specific and/or age-specific differences. The protective effect of the *NBS1* rs13312440 SNP observed in our study could be attributed to the SNP itself or to linkage disequilibrium with another variant.

To the best of our knowledge this is the first study investigating the role of *NBS1* rs769416 SNP and breast cancer risk. The rs769416 SNP causes an amino acid change (Gly to Lys) at codon 216 of the *NBS1* gene. This SNP is not located within

one of the three functional regions of the *NBS1* protein but it may have an alternative splicing regulatory effect, based on the Functional Single Nucleotide Polymorphism (F-SNP) database [47]. Our result on the association of rs769416 SNP and breast cancer needs to be interpreted with caution, since this is a rare SNP in our population and the most likely explanation for this association is chance.

Haplotype analysis with the combination of the seven *NBS1* SNPs showed that the frequency of the GGCGCAC haplotype (rs769416, rs769420, rs13312840, rs1805794, rs6413508, rs12677527, rs1805787) was lower in cases than in controls (0.0147 versus 0.0225; p=0.035) suggesting a protective effect. There was also evidence for a protective effect of the rare pooled *NBS1* haplotypes. This protective effect is driven by the difference in frequencies of the pooled rare haplotypes which conferred a low risk (OR=0.42) and had a combined frequency of 3.29% in controls and 1.37% in cases. It is possible that these pooled haplotypes are a marker for a single, rare, protective mutation in the Cypriot population. There may be value in sequencing this region, in order to help identify the protective variant(s). Both these findings need to be replicated in independent studies in order to confirm or refute this effect.

Our data support the notion that *MUS81* rs545500 C allele carriers are at an increased risk for breast cancer. Rs545500 is a non-synonymous SNP located in the coding region of *MUS81*, a structure-specific DNA nuclease which plays an important role in DNA repair by homologous recombination [48]. This polymorphism results in an amino acid change from a positively charged hydrophilic arginine to an uncharged hydrophobic proline residue, which may have an effect on the 3D structure or a protein-protein binding interface of the *MUS81* protein [25]. The role of the *MUS81*

gene in breast cancer has not been investigated. However, it was demonstrated that *MUS81* homozygote and heterozygote knockout mice have a predisposition to develop cancer. Proper bialellic expression of *MUS81* is critical for the maintenance of genomic integrity and tumour suppression [49]. Therefore, the rs545500 SNP could predispose individuals to breast cancer, but functional studies need to be performed in order to identify the actual role of this variant in carcinogenesis.

Our findings also suggest that the *PBOV1* rs6927706 polymorphism may be a risk factor for breast cancer. Rs56927706 is a non-synonymous SNP located in the coding region of *PBOV1*, a gene which is up-regulated in prostate, breast, and bladder cancers [21]. The polymorphism results in an amino acid change at codon 73 from a hydrophobic isoleucine to a hydrophilic threonine residue. Bioinformatics analysis indicates that this SNP could be involved in splicing regulation [47]. However further work is warranted since the exact roles of the *PBOV1* protein as well as its functional domains are not well known at present.

Our current data suggest that the *MRE11A* rs556477 G allele may be associated with a reduced breast cancer risk. 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 [50]. The rs556477 common variant is located in intron 15 of the *MRE11A* gene. The rs556477 MAF is 40% in Caucasians as reported in NCBI's dbSNP database; the same as that observed in our population. The functionality of this SNP is not clear. Using the TFSEARCH webtool (http://www.cbrc.jp/research/db/TFSEARCH.html), we searched for potential

transcription factors binding sites at this position. The rs556477 SNP is located in a region that is a potential transcription factor binding site of activator protein 1 (*AP-1*), which plays a critical role in signal transduction pathways in many cells. A recent study has shown that inhibition of *AP-1* transcription factors suppresses breast cancer growth. Inhibitors which are capable of blocking *AP-1* activation may be promising agents for the treatment and prevention of breast cancer [51]. The reduced risk of breast cancer for carriers of rs556477 SNP found in our study is in contrast with the above finding since it is expected that the creation of an *AP-1* binding site will result in an increased breast cancer risk. However it must be taken into account that the prediction that rs556477 A to G substitution results in a gain of an *AP-1* binding site is based on *in silico* analysis and this remains to be proven by *in vitro* data. Furthermore, the *MRE11A* rs556477 polymorphism may not be causal, but could be in linkage disequilibrium with a true protective variant.

In the current study, we present evidence for an increased breast cancer risk for women carrying the *MRE11A* AGCG (rs556477, rs601341, rs10831234, rs1009456) haplotype. It is noteworthy that in a previous study conducted by our group there was evidence for an increased breast cancer risk for women homozygous for the MRE11A rs601341 A allele [35]. The rs601341 A to G substitution results in potential binding of ubiquitous transcription factor Ying Yang 1 (*YY1*) that has a fundamental role in normal biologic processes such as differentiation, replication and cell proliferation. *YY1* overexpression and/or activation results in uncontrolled cellular proliferation, resistance to apoptotic stimuli and tumorigenesis [52]. Given the intronic position of the two associated SNPs, it is unlikely that these SNPs in and by themselves, are

disease associated. Rather, in all likelihood they are in linkage disequilibrium with other variants which cause the associations observed.

Our study has several strengths, including a high participation rate of eligible cases (98%) and a population sample from a homogeneous ethnic background (100% of study participants are Greek Cypriots) thus reducing any potential bias due to population stratification. In addition, our study population (both cases and controls) was from all over the country minimizing potential selection bias.

However, there were limitations in our study, one of which is the possibility of survivor bias. This is one of the known disadvantages of all retrospective case-control studies. In our study, samples from breast cancer cases were collected between 2004 and 2006 for cases diagnosed between 1999 and 2006. Our study may therefore have excluded a number of women with the most aggressive form of breast cancer, diagnosed between 1999 and 2003. It is possible that this could lead to "survivor bias" if genotypes differ between those who succumb quickly compared with longer-term breast cancer survivors.

The SNP selection for this study was based solely on functionality and their position in genes interacting with BRCA1/2 rather than allele frequency. As a result of this, a number of monomorphic / low-polymorphic SNPs were included in the study. It is noted that this is the first time that these SNPs were studied in the Cypriot population and their allele frequencies were a priori unknown. Rare SNPs can also contribute to disease risk [53]. However, our study did not have sufficient power to detect such associations and the possibility that some of the low-polymorphic SNPs studied,

contribute to breast cancer risk cannot be ruled out.

Another limitation of our study is that we did not consider the possibility of gene-gene interactions or gene-environment 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 the small numbers available. Furthermore, we did not account for multiple testing. When multiple comparisons are being made, statistically significant associations may be identified by chance alone. Replication in independent, well-powered studies is the gold standard of bona fide true associations from chance findings. A Cypriot replication set is not available to attempt to replicate the variants identified, and replication will need to be performed in other populations.

In conclusion, this study provides support for the hypothesis that genetic variants in DNA repair genes influence breast cancer risk and provides further evidence for the polygenic model of breast cancer. However, large-scale genetic epidemiologic studies are warranted to further examine and corroborate the associations observed between polymorphisms and breast cancer in multi-ethnic groups. In addition, elucidation of the functional impact of the breast cancer associated SNPs is needed in order to provide further insights into their mechanistic effects on risk.

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Table 1. Genotype frequencies in cases and controls for the 62 SNPs studied

Gene		Controls				Cases				MAF		HWE
	rs number	AA	Aa	aa	Total	AA	Aa	aa	Total	Controls	Cases	Controls
ATF1	rs2230674	1071	86	2	1159	1021	81	1	1103	0.04	0.04	0.69
ATM	rs1800057	1087	85	2	1174	1015	85	0	1100	0.04	0.04	0.68
	rs2234997	1153	4	1	1158	1093	10	0	1103	0	0	0.01
	rs2235000	1160	1	0	1161	1102	2	0	1104	0	0	1
	rs3218688	979	2	0	981	926	0	0	926	0	0	1
	rs3218695	1177	0	0	1177	1109	0	0	1109	0	0	Monomorphic
	rs3218708	1177	0	0	1177	1109	0	0	1109	0	0	Monomorphic
	rs4987945	1169	1	0	1170	1100	1	0	1101	0	0	1
ATR	rs2227928	401	520	229	1150	344	517	218	1079	0.43	0.44	0.011
	rs2229032	899	242	17	1158	833	253	16	1102	0.12	0.13	0.89
BARD1	rs2070094	466	551	156	1173	461	485	159	1105	0.37	0.36	0.75
	rs2229571	341	580	249	1170	316	540	241	1097	0.46	0.47	0.95
	rs3738888	1150	7	0	1157	1099	4	0	1103	0	0	1
BLM	rs11852361	1123	46	2	1171	1046	52	2	1100	0.02	0.03	0.094
	rs7167216	1127	44	2	1173	1055	47	2	1104	0.02	0.02	0.081
BRIP1	rs4986764	475	534	161	1170	465	502	141	1108	0.37	0.35	0.57
CHEK1	rs506504	1064	105	5	1174	986	116	2	1104	0.05	0.05	0.19
CHEK2	rs17879961	1158	0	0	1158	1101	1	0	1102	0	0	1
DBC2	rs2241261	342	560	252	1154	288	546	264	1098	0.46	0.49	0.26
DDB2	rs830083	871	271	15	1157	803	277	21	1101	0.13	0.14	0.3
DMC1	rs2227914	1146	3	0	1149	1099	1	1	1101	0	0	1
EME1	rs12450550	918	221	24	1163	838	239	23	1100	0.12	0.13	0.021

		AA	Aa	aa		AA	Aa	aa				
FANCA	rs1800282	978	149	19	1146	883	189	16	1088	0.08	0.1	<0.0001
	rs7190823	573	476	113	1162	541	462	98	1101	0.3	0.3	0.33
	rs9282681	1096	51	0	1147	1065	42	2	1109	0.02	0.02	1
FANCC	rs1800364	1159	1	0	1160	1104	1	0	1105	0	0	1
FANCD2	rs2272125	787	346	38	1171	710	346	43	1099	0.18	0.2	1
FANCE	rs9462088	1051	115	4	1170	990	106	4	1100	0.05	0.05	0.56
MLH1	rs1800149	1156	0	1	1157	1101	0	2	1103	0	0	0.00043
	rs2020872	1177	0	0	1177	1109	0	0	1109	0	0	Monomorphic
	rs2308317	1177	0	0	1177	1109	0	0	1109	0	0	Monomorphic
	rs1800734	494	496	137	1127	446	505	136	1087	0.34	0.36	0.47
MRE11A	rs1009456	1040	105	0	1145	992	89	5	1086	0.05	0.05	0.17
	rs10831234	949	190	12	1151	899	193	6	1098	0.09	0.09	0.48
	rs556477	444	550	167	1161	494	473	130	1097	0.38	0.33	0.9
MSH2	rs2303428	929	217	18	1164	870	211	16	1097	0.11	0.11	0.22
MSH6	rs1042821	653	451	64	1168	646	388	70	1104	0.25	0.24	0.24
	rs1800935	655	428	90	1173	608	413	83	1104	0.26	0.26	0.094
MUS81	rs545500	673	430	55	1158	589	435	77	1101	0.23	0.27	0.22
NBS1	rs1805787	548	483	104	1135	549	447	103	1099	0.3	0.3	0.94
	rs1805794	543	502	109	1154	511	497	96	1104	0.31	0.31	0.68
	rs6413508	1167	6	1	1174	1097	4	0	1101	0	0	0.012
	rs769416	1141	10	0	1151	1098	3	0	1101	0	0	1
	rs769420	1177	0	0	1177	1109	0	0	1109	0	0	Monomorphic
	rs12677527	546	505	115	1166	512	497	96	1105	0.32	0.31	0.95
	rs13312840	1122	55	0	1177	1075	32	1	1108	0.02	0.02	1
PALB2	rs45494092	1170	1	0	1171	1097	4	0	1101	0	0	1
	rs45532440	1035	120	4	1159	972	126	4	1102	0.06	0.06	0.77
	rs45478192	1177	0	0	1177	1109	0	0	1109	0	0	Monomorphic
	rs45551636	1076	92	2	1170	1010	92	2	1104	0.04	0.04	1
PBOV1	rs6927706	1083	63	1	1147	1017	83	2	1102	0.03	0.04	0.61
PCNA	rs3626	827	242	36	1105	838	196	34	1068	0.14	0.12	0.0012
RAD50	rs2299015	742	370	45	1157	743	323	37	1103	0.2	0.18	1
	rs2522406	1064	41	1	1106	1044	31	0	1075	0.02	0.01	0.34
	rs2706377	1052	66	21	1139	1046	37	14	1097	0.05	0.03	<0.0001
	rs3187395	1177	0	0	1177	1109	0	0	1109	0	0	Monomorphic
RAD51C	rs28363317	1164	6	0	1170	1095	6	0	1101	0	0	1
RAD51L1	rs28908468	572	201	0	773	979	75	0	1054	0.13	0.04	<0.0001
RAD52	rs7487683	1135	35	3	1173	1072	33	0	1105	0.02	0.01	0.0043
RFC1	rs2066791	1177	0	0	1177	1109	0	0	1109	0	0	Monomorphic
XPC	rs2228000	673	402	64	1139	653	379	65	1097	0.23	0.23	0.68
	rs2227999	1040	128	4	1172	963	138	4	1105	0.06	0.07	1

AA, common homozygote; Aa, heterozygote; aa, rare homozygote.

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Table 2. Genotypic specific risk (OR and 95% CI)[a]

Gene	Rs number	Dominant OR (95%CI)[a] ; p value[a]	Recessive OR (95%CI)[a] ; p value[a]	Log-additive OR (95%CI)[a] ; p value[a]
ATF1	rs2230674	1.02 (0.74-1.42); 0.89	0.60 (0.05-7.34); 0.68	1.01 (0.73-1.40); 0.94
ATM	rs1800057	1.10 (0.79-1.52); 0.57	-	1.07 (0.78-1.47); 0.69
	rs2234997	2.31 (0.75-7.12); 0.13	-	1.83 (0.68-4.91); 0.23
	rs4987945	1.27 (0.07-21.89); 0.87	-	-
ATR	rs2227928	1.14 (0.95-1.38); 0.15	1.04 (0.84-1.30); 0.71	1.07 (0.95-1.21); 0.25
	rs2229032	1.09 (0.89-1.34); 0.42	1.01 (0.48-2.15); 0.97	1.07 (0.89-1.30); 0.45
BARD1	rs2070094	0.98 (0.82-1.17); 0.8	1.07 (0.83-1.37); 0.6	1.01 (0.89-1.14); 0.93
	rs2229571	0.99 (0.82-1.20); 0.93	0.96 (0.78-1.18);0.69	0.98 (0.87-1.11); 0.77
	rs3738888	0.46 (0.13-1.66); 0.23	-	-
BLM	rs11852361	1.28 (0.85-1.95); 0.24	0.95 (0.13-7.12); 0.96	1.25 (0.84-1.85); 0.27
	rs7167216	1.26 (0.82-1.94); 0.3	0.95 (0.13-7.14); 0.96	1.22 (0.81-1.84); 0.34
BRIP1	rs4986764	0.94 (0.79-1.12);0.49	0.96(0.75-1.25);0.78	0.96(0.85-1.09); 0.53
CHEK1	rs506504	1.19 (0.89-1.59); 0.24	0.48 (0.09-2.59); 0.37	1.15 (0.87-1.51); 0.34
DBC2	rs2241261	1.18 (0.97-1.43); 0.095	1.16 (0.94-1.43); 0.17	1.12 (0.99-1.27); 0.061
DDB2	rs830083	1.14(0.93-1.39);0.2	1.51(0.74-3.07);0.25	1.14 (0.95-1.37); 0.15
DMC1	rs2227914	0.63 (0.09-4.39);0.64	-	1.10 (0.25-4.73); 0.9
EME1	rs12450550	1.15 (0.94-1.42); 0.18	0.94 (0.52-1.72); 0.85	1.11 (0.92-1.33); 0.27
FANCA	rs7190823	1.02 (0.86-1.22); 0.8	0.91 (0.68-1.23); 0.56	1.00 (0.87-1.14); 0.95
	rs9282681	0.84 (0.54-1.30);0.44	-	0.89 (0.58-1.35); 0.57
FANCC	rs1800364	1.25 (0.07-21.55); 0.88	-	-
FANCD2	rs2272125	1.12 (0.93-1.34); 0.24	1.08 (0.68-1.72); 0.74	1.09 (0.93-1.28); 0.27
FANCE	rs9462088	0.95 (0.71-1.26); 0.71	0.73 (0.15-3.53); 0.7	0.94 (0.71-1.24); 0.67
MLH1	rs1800734	1.09 (0.91-1.31); 0.33	1.01 (0.77-1.31); 0.96	1.05 (0.92-1.20); 0.46
MRE11A	rs1009456	0.93 (0.68-1.26);0.63	-	0.98 (0.73-1.32); 0.9
	rs10831234	1.04 (0.83-1.31);0.72	0.69 (0.25-1.93);0.47	1.02 (0.82-1.26); 0.85
	rs556477	**0.76 (0.64-0.91); 0.0022**	0.81 (0.62-1.05); 0.11	**0.82 (0.72-0.93); 0.0027**
MSH2	rs2303428	1.02 (0.82-1.26); 0.89	0.93 (0.45-1.89); 0.83	1.01 (0.83-1.22); 0.94
MSH6	rs1042821	0.85 (0.71-1.01); 0.066	1.23 (0.84-1.79); 0.29	0.92 (0.80-1.07); 0.27
	rs1800935	1.09 (0.91-1.30); 0.34	1.07 (0.77-1.48); 0.69	1.07 (0.93-1.22); 0.36
MUS81	rs545500	**1.21 (1.02-1.45); 0.031**	1.43(0.98-2.08);0.06	**1.21(1.04-1.39); 0.012**
NBS1	rs1805787	0.92 (0.77-1.10); 0.36	0.97 (0.72-1.31); 0.84	0.95 (0.83-1.08); 0.43
	rs1805794	1.08 (0.91-1.28); 0.4	0.93 (0.69-1.26);0.65	1.03 (0.90-1.18); 0.65
	rs6413508	0.46 (0.13-1.66); 0.23	-	0.46 (0.14-1.50); 0.18
	rs769416	**0.23 (0.06-0.85); 0.017**	-	-
	rs12677527	1.04 (0.88-1.24); 0.64	0.88 (0.65-1.19); 0.4	1.00 (0.87-1.14); 0.98
	rs13312840	**0.58 (0.37-0.92); 0.019**	-	**0.61 (0.39-0.95); 0.028**
PALB2	rs45494092	3.75 (0.40-35.04); 0.2	-	-
	rs45532440	1.06 (0.81-1.40); 0.66	0.73 (0.18-3.04);0.67	1.05 (0.80-1.36); 0.74
	rs45551636	1.04 (0.76-1.43); 0.8	0.67 (0.09-4.89); 0.69	1.03 (0.76-1.4); 0.85
PBOV1	rs6927706	**1.53 (1.07-2.18); 0.019**	1.63(0.12-21.60);0.71	**1.51(1.06-2.13); 0.02**
RAD50	rs2299015	0.89 (0.74-1.07); 0.21	0.90 (0.56-1.43); 0.65	0.91 (0.77-1.06); 0.22
	rs2522406	0.80 (0.49-1.31); 0.37	-	0.78 (0.48-1.26); 0.31
	rs3187395	0.32 (0.06-1.85); 0.19	-	-
RAD51C	rs28363317	0.93 (0.28-3.10); 0.91	-	-
XPC	rs2228000	1.01 (0.84-1.21); 0.91	0.96 (0.66-1.40); 0.85	1.00 (0.87-1.16); 0.99
	rs2227999	1.12 (0.86-1.46); 0.42	1.10 (0.26-4.64); 0.42	1.11 (0.86-1.43); 0.43

Data in bold highlight the statically significant results
[a] Adjusted for age, menopause status, family history of breast cancer and use of hormone replacement therapy (HRT)

Table 3. Estimated haplotype frequencies in cases and controls and haplotypic specific risks

Gene	Haplotype	Control freq	Case freq	OR [95%CI]	χ^2 p-value	Global test p-value
ATM[a]	CCCCCTGC	0.92	0.9225	1.00		0.53
	GCCCCTGC	0.0367	0.0349	0.96 (0.69-1.32)	0.8	
	CCCCGTGC	0.0376	0.0329	0.89 (0.34-1.23)	0.48	
	Rare	0.0057	0.0097	1.6 (0.72-3.57)	0.25	
MRE11A[b]	GACG	0.3702	0.326	1.00		0.021
	AGCG	**0.2384**	**0.2752**	**1.32 (1.13-1.54)**	**4×10^{-4}**	
	AACG	0.2478	0.255	1.17 (1.00-1.36)	0.044	
	AGTG	0.0864	0.0891	1.16 (0.93-1.45)	0.19	
	AGCT	0.0444	0.0438	1.12 (0.84-1.51)	0.44	
	Rare	0.0128	0.0109	0.98 (0.52-1.85)	0.95	
NBS1[c]	GGTCCGC	0.3705	0.3886	1.00		0.00019
	GGTCCGG	0.2955	0.2925	0.94 (0.82-1.09)	0.44	
	GGTGCAC	0.2786	0.2905	0.99 (0.86-1.15)	0.92	
	GGCGCAC	**0.0225**	**0.0147**	**0.62 (0.39-0.97)**	**0.037**	
	Rare	**0.0329**	**0.0137**	**0.42 (0.26-0.66)**	**2×10^{-4}**	

Data in bold highlight the statically significant results

[a] ATM haplotypes are arranged in the order of rs1800057, rs3218688, rs3218695, rs4987945, rs2230674, rs2234997, rs2235000, rs3218708

[b] *MRE11A* haplotypes are arranged in the order of rs556477, rs601341, rs10831234, rs1009456

[c] *NBS1* haplotypes are arranged in the order of rs769416, rs769420, rs13312840, rs1805794, rs6413508, rs12677527, rs1805787