EPIDEMIOLOGY

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

Maria A. Loizidou · Marios A. Cariolou · Susan L. Neuhausen · Robert F. Newbold · Evy Bashiardes · Yiola Marcou · Thalia Michael · Maria Daniel · Eleni Kakouri · Panayiotis Papadopoulos · Simon Malas · Andreas Hadjisavvas · Kyriacos Kyriacou

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 vs. TC/CC = 0.58; 95% CI, 0.37–0.92; $P = 0.019$ 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, 0.64–0.91; $P = 0.0022$). *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, 1.02–1.45; $P = 0.031$; OR AA vs. AG/GG = 1.53, 95% CI, 1.07–2.18; $P = 0.019$, 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.

Keywords Breast cancer · Case–control study · Cyprus · BRCA1/2 interacting genes · Genetic epidemiology · SNP

M. A. Loizidou · T. Michael · A. Hadjisavvas (✉) ·
K. Kyriacou
Department of Electron Microscope/Molecular Pathology,
The Cyprus Institute of Neurology and Genetics, 6. International
Airport Ave., Ayios Dometios, P.O. Box 23462, 1683 Nicosia,
Cyprus
e-mail: ahsavvas@cing.ac.cy

M. A. Cariolou · E. Bashiardes
Department of Cardiovascular Genetics and Laboratory
of Forensic Genetics, The Cyprus Institute of Neurology
and Genetics, Nicosia, Cyprus

S. L. Neuhausen
Department of Epidemiology, School of Medicine, University
of California Irvine, Irvine, CA 92697-7550, USA

R. F. Newbold
Brunel Institute of Cancer Genetics and Pharmacogenomics,
Faculty of Life Sciences, Brunel University, Uxbridge, UK

Y. Marcou · M. Daniel · E. Kakouri · P. Papadopoulos
Bank of Cyprus Oncology Center, Nicosia, Cyprus

S. Malas
Department of Oncology, Limassol General Hospital, Limassol,
Cyprus

Introduction

Breast cancer is the most common malignancy affecting women worldwide, and it is the leading cancer in females 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 that are involved in pathways critical to genomic integrity have been implicated in inherited predisposition to breast cancer, including *BRCA1*, *BRCA2*,

⚫ Springer

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(s) 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 two additional SNPs in the *PBOV1* (*UROC28*) and *DBC2* genes that 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 and 2006 from 1,109 female breast cancer patients diagnosed between 40 and 70 years old and 1,177 age-matched healthy controls. Participants were women who were previously diagnosed with breast cancer between January 1999 and December 2006. The majority of patients were ascertained from the Bank of Cyprus Oncology Centre which 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 four district mammography screening centers that operate in Cyprus. Eligible controls were women with no previous history of breast cancer and who had a negative mammography result. All study participants, both patients 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, epidemiologic and pathologic 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.

Gene and SNP selection

Sixty-two SNPs in the *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 that interact with either *BRCA1* 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.

Genotyping

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 U 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 U of shrimp alkaline phosphatase for 40 min at 37°C to dephosphorylate unincorporated dNTPs, followed by enzyme inactivation for 5 min at 85°C. 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 700–1,400 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 (3,200 g for 5 min), reaction products were spotted on a 384-format Spectro-Chip (Sequenom Inc., San Diego, CA, USA). Spectro-CHIPs were processed in a MassARRAY Compact Analyzer (Bruker Daltonics, Bremen, Germany) by Mass-ARRAY Workstation (version 3.3) software (Sequenom Inc., San Diego, CA, USA). Acquisition data were analyzed 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 χ^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 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, rs28908468) 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 vs. 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, 0.64–0.91; $P = 0.0022$). 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, 1.02–1.45; $P = 0.031$). 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, 1.07–2.18; $P = 0.019$).

The *NBS1* haplotype GGCGCAC (rs769416, rs769420, rs13312840, rs1805794, rs6413508, rs12677527, rs1805 787), 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 = 0.39–0.97; $P = 0.037$). We also found a reduced risk for breast cancer for a rare haplotype in *NBS1* (OR = 0.42; 95% CI = 0.26–0.66; $P = 2 \times 10^{-4}$). In addition, the *MRE11A* haplotype AGCG (rs556477, rs601341, rs1083

1234, rs1009456) is associated with a significantly increased risk for breast cancer (OR = 1.32; 95% CI = 1.13–1.54; $P = 0.0004$). 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 that 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. who found an increased risk for breast cancer in non-Hispanic Caucasian women aged 55 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

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

Gene	rs Number	Controls				Cases				MAF		HWE
		AA	Aa	aa	Total	AA	Aa	aa	Total	Controls	Cases	Controls
ATF1	rs2230674	1,071	86	2	1,159	1,021	81	1	1,103	0.04	0.04	0.69
ATM	rs1800057	1,087	85	2	1,174	1,015	85	0	1,100	0.04	0.04	0.68
	rs2234997	1,153	4	1	1,158	1,093	10	0	1,103	0	0	0.01
	rs2235000	1,160	1	0	1,161	1,102	2	0	1,104	0	0	1
	rs3218688	979	2	0	981	926	0	0	926	0	0	1
	rs3218695	1,177	0	0	1,177	1,109	0	0	1,109	0	0	Monomorphic
	rs3218708	1,177	0	0	1,177	1,109	0	0	1,109	0	0	Monomorphic
	rs4987945	1,169	1	0	1,170	1,100	1	0	1,101	0	0	1
ATR	rs2227928	401	520	229	1,150	344	517	218	1,079	0.43	0.44	0.011
	rs2229032	899	242	17	1,158	833	253	16	1,102	0.12	0.13	0.89
BARD1	rs2070094	466	551	156	1,173	461	485	159	1,105	0.37	0.36	0.75
	rs2229571	341	580	249	1,170	316	540	241	1,097	0.46	0.47	0.95
	rs3738888	1,150	7	0	1,157	1,099	4	0	1,103	0	0	1
BLM	rs11852361	1,123	46	2	1,171	1,046	52	2	1,100	0.02	0.03	0.094
	rs7167216	1,127	44	2	1,173	1,055	47	2	1,104	0.02	0.02	0.081
BRIP1	rs4986764	475	534	161	1,170	465	502	141	1,108	0.37	0.35	0.57
CHEK1	rs506504	1,064	105	5	1,174	986	116	2	1,104	0.05	0.05	0.19
CHEK2	rs17879961	1,158	0	0	1,158	1,101	1	0	1,102	0	0	1
DBC2	rs2241261	342	560	252	1,154	288	546	264	1,098	0.46	0.49	0.26
DDB2	rs830083	871	271	15	1,157	803	277	21	1,101	0.13	0.14	0.3
DMC1	rs2227914	1,146	3	0	1,149	1,099	1	1	1,101	0	0	1
EME1	rs12450550	918	221	24	1,163	838	239	23	1,100	0.12	0.13	0.021
FANCA	rs1800282	978	149	19	1,146	883	189	16	1,088	0.08	0.1	<0.0001
	rs7190823	573	476	113	1,162	541	462	98	1,101	0.3	0.3	0.33
	rs9282681	1,096	51	0	1,147	1,065	42	2	1,109	0.02	0.02	1
FANCC	rs1800364	1,159	1	0	1,160	1,104	1	0	1,105	0	0	1
FANCD2	rs2272125	787	346	38	1,171	710	346	43	1,099	0.18	0.2	1
FANCE	rs9462088	1,051	115	4	1,170	990	106	4	1,100	0.05	0.05	0.56
MLH1	rs1800149	1,156	0	1	1,157	1,101	0	2	1,103	0	0	0.00043
	rs2020872	1,177	0	0	1,177	1,109	0	0	1,109	0	0	Monomorphic
	rs2308317	1,177	0	0	1,177	1,109	0	0	1,109	0	0	Monomorphic
	rs1800734	494	496	137	1,127	446	505	136	1,087	0.34	0.36	0.47
MRE11A	rs1009456	1,040	105	0	1,145	992	89	5	1,086	0.05	0.05	0.17
	rs10831234	949	190	12	1151	899	193	6	1,098	0.09	0.09	0.48
	rs556477	444	550	167	1,161	494	473	130	1,097	0.38	0.33	0.9
MSH2	rs2303428	929	217	18	1,164	870	211	16	1,097	0.11	0.11	0.22
MSH6	rs1042821	653	451	64	1,168	646	388	70	1,104	0.25	0.24	0.24
	rs1800935	655	428	90	1,173	608	413	83	1,104	0.26	0.26	0.094
MUS81	rs545500	673	430	55	1,158	589	435	77	1,101	0.23	0.27	0.22
NBS1	rs1805787	548	483	104	1,135	549	447	103	1,099	0.3	0.3	0.94
	rs1805794	543	502	109	1,154	511	497	96	1,104	0.31	0.31	0.68
	rs6413508	1,167	6	1	1,174	1,097	4	0	1,101	0	0	0.012
	rs769416	1,141	10	0	1,151	1,098	3	0	1,101	0	0	1
	rs769420	1,177	0	0	1,177	1,109	0	0	1,109	0	0	Monomorphic
	rs12677527	546	505	115	1,166	512	497	96	1,105	0.32	0.31	0.95
	rs13312840	1,122	55	0	1,177	1,075	32	1	1,108	0.02	0.02	1

Table 1 continued

Gene	rs Number	Controls				Cases				MAF		HWE
		AA	Aa	aa	Total	AA	Aa	aa	Total	Controls	Cases	Controls
PALB2	rs45494092	1,170	1	0	1,171	1,097	4	0	1,101	0	0	1
	rs45532440	1,035	120	4	1,159	972	126	4	1,102	0.06	0.06	0.77
	rs45478192	1,177	0	0	1,177	1,109	0	0	1,109	0	0	Monomorphic
	rs45551636	1,076	92	2	1,170	1,010	92	2	1,104	0.04	0.04	1
PBOV1	rs6927706	1,083	63	1	1,147	1,017	83	2	1,102	0.03	0.04	0.61
PCNA	rs3626	827	242	36	1,105	838	196	34	1,068	0.14	0.12	0.0012
RAD50	rs2299015	742	370	45	1,157	743	323	37	1,103	0.2	0.18	1
	rs2522406	1,064	41	1	1,106	1,044	31	0	1,075	0.02	0.01	0.34
	rs2706377	1,052	66	21	1,139	1,046	37	14	1,097	0.05	0.03	<0.0001
	rs3187395	1,177	0	0	1,177	1,109	0	0	1,109	0	0	Monomorphic
RAD51C	rs28363317	1,164	6	0	1,170	1,095	6	0	1,101	0	0	1
RAD51L1	rs28908468	572	201	0	773	979	75	0	1,054	0.13	0.04	<0.0001
RAD52	rs7487683	1,135	35	3	1,173	1,072	33	0	1,105	0.02	0.01	0.0043
RFC1	rs2066791	1,177	0	0	1,177	1,109	0	0	1,109	0	0	Monomorphic
XPC	rs2228000	673	402	64	1,139	653	379	65	1,097	0.23	0.23	0.68
	rs2227999	1,040	128	4	1,172	963	138	4	1,105	0.06	0.07	1

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 patients than in controls (0.0147 vs. 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 that conferred a low risk (OR = 0.42) and had a combined frequency of 3.29% in controls and 1.37% in patients. 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 that 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 tumor 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. Rs6927706 is a non-synonymous SNP located in the coding region of PBOV1, a gene which is upregulated 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

Table 2 Genotypic specific risk (OR and 95% CI)

Gene	rs number	Dominant OR (95% CI); P value[a]	Recessive OR (95% CI); P value[a]	Log-additive OR (95% CI); 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

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 that 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 that 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 (all 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 multiethnic 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.

References

1. Parkin DM, Bray F, Ferlay J et al (2005) Global cancer statistics, 2002. CA Cancer J Clin 55(2):74–108
2. Bau DT, Mau YC, Ding SL et al (2007) DNA double-strand break repair capacity and risk of breast cancer. Carcinogenesis 28(8):1726–1730
3. Kennedy DO, Agrawal M, Shen J et al (2005) DNA repair capacity of lymphoblastoid cell lines from sisters discordant for breast cancer. J Natl Cancer Inst 97(2):127–132
4. Machella N, Terry MB, Zipprich J et al (2008) Double-strand breaks repair in lymphoblastoid cell lines from sisters discordant for breast cancer from the New York site of the BCFR. Carcinogenesis 29(7):1367–1372
5. Balmain A, Gray J, Ponder B (2003) The genetics and genomics of cancer. Nat Genet 33:238–244
6. Helzlsouer KJ, Harris EL, Parshad R et al (1996) DNA repair proficiency: potential susceptibility factor for breast cancer. J Natl Cancer Inst 88(11):754–755
7. Lunn RM, Helzlsouer KJ, Parshad R et al (2000) XPD polymorphisms: effects on DNA repair proficiency. Carcinogenesis 21(4):551–555
8. Walsh T, King MC (2007) Ten genes for inherited breast cancer. Cancer Cell 11(2):103–105
9. Haiman CA, Hsu C, de Bakker PI et al (2008) Comprehensive association testing of common genetic variation in DNA repair pathway genes in relationship with breast cancer risk in multiple populations. Hum Mol Genet 17(6):825–834
10. Kuschel B, Auranen A, McBride S (2002) Variants in DNA double-strand break repair genes and breast cancer susceptibility. Hum Mol Genet 11(12):1399–1407
11. Goode EL, Ulrich CM, Potter JD (2002) Polymorphisms in DNA repair genes and associations with cancer risk. Cancer Epidemiol Biomarkers Prev 11(12):1513–1530
12. Garcia-Closas M, Egan KM, Newcomb PA et al (2006) Polymorphisms in DNA double-strand break repair genes and risk of breast cancer: two population-based studies in USA and Poland, and meta-analyses. Hum Genet 119(4):376–388
13. Seal S, Thompson D, Renwick A et al (2006) Truncating mutations in the Fanconi anemia J gene BRIP1 are low-penetrance breast cancer susceptibility alleles. Nat Genet 38(11):1239–1241
14. Rahman N, Seal S, Thompson D (2007) PALB2, which encodes a BRCA2-interacting protein, is a breast cancer susceptibility gene. Nat Genet 39(2):165–167
15. Narod SA, Foulkes WD (2004) BRCA1 and BRCA2: 1994 and beyond. Nat Rev Cancer 4(9):665–676
16. Wang Y, Cortez D, Yazdi P et al (2000) BASC, a super complex of BRCA1-associated proteins involved in the recognition and repair of aberrant DNA structures. Genes Dev 14:927–939
17. Greenberg RA, Sobhian B, Pathania S et al (2006) Multifactorial contributions to an acute DNA damage response by BRCA1/BARD1-containing complexes. Genes Dev 20:34–46
18. Scully R, Xie A, Nagaraju G (2004) Molecular functions of BRCA1 in the DNA damage response. Cancer Biol Ther 3:521–527
19. Liu Y, West SC (2002) Distinct functions of BRCA1 and BRCA2 in double-strand break repair. Breast Cancer Res 4:9–13
20. Xia B, Sheng Q, Nakanishi K et al (2006) Control of BRCA2 cellular and clinical functions by a nuclear partner, PALB2. Mol Cell 22(6):719–729
21. An G, Ng AY, Meka CS et al (2000) Cloning and characterization of UROC28, a novel gene overexpressed in prostate, breast, and bladder cancers. Cancer Res 60(24):7014–7020

22. Hamaguchi M, Meth JL, von Klitzing C et al (2002) DBC2, a candidate for a tumor suppressor gene involved in breast cancer. Proc Natl Acad Sci USA 99(21):13647–13652

23. Rudd MF, Williams RD, Webb EL et al (2005) The predicted impact of coding single nucleotide polymorphisms database. Cancer Epidemiol Biomarkers Prev 14(11 Pt 1):2598–2604

24. Savas S, Kim DY, Ahmad MF et al (2004) Identifying functional genetic variants in DNA repair pathway using protein conservation analysis. Cancer Epidemiol Biomarkers Prev 13(5):801–807

25. Nakken S, Alseth I, Rognes T (2007) Computational prediction of the effects of nonsynonymous single nucleotide polymorphisms in human DNA repair genes. Neuroscience 145(4):1273–1279

26. Beiner ME, Rosen B, Fyles A et al (2006) Endometrial cancer risk is associated with variants of the mismatch repair genes MLH1 and MSH2. Cancer Epidemiol Biomarkers Prev 15(9):1636–1640

27. Sigurdson AJ, Hauptmann M, Chatterjee N et al (2004) Kincohort estimates for familial breast cancer risk in relation to variants in DNA base excision repair, BRCA1 interacting and growth factor genes. BMC Cancer 4:9

28. Kilpivaara O, Vahteristo P, Falck J et al (2004) CHEK2 variant I157T may be associated with increased breast cancer risk. Int J Cancer 111(4):543–547

29. Barroso E, Milne RL, Fernandez LP et al (2006) FANCD2 associated with sporadic breast cancer risk. Carcinogenesis 27(9):1930–1937

30. Landi S, Gemignani F, Canzian F et al (2006) DNA repair and cell cycle control genes and the risk of young-onset lung cancer. Cancer Res 66(22):11062–11069

31. Poplawski T, Zadrozny M, Kolacinska A et al (2005) Polymorphisms of the DNA mismatch repair gene HMSH2 in breast cancer occurrence and progression. Breast Cancer Res Treat 94(3):199–204

32. Lu J, Wei Q, Bondy ML et al (2006) Polymorphisms and haplotypes of the NBS1 gene are associated with risk of sporadic breast cancer in non-Hispanic white women < or =55 years. Carcinogenesis 27(11):2209–2216

33. Allen-Brady K, Camp NJ (2005) Characterization of the linkage disequilibrium structure and identification of tagging-SNPs in five DNA repair genes. BMC Cancer 5:99

34. Sole X, Guino E, Valls J et al (2006) SNPStats: a web tool for the analysis of association studies. Bioinformatics 22(15):1928–1929

35. Loizidou MA, Michael T, Neuhausen SL et al (2009) DNA-repair genetic polymorphisms and risk of breast cancer in Cyprus. Breast Cancer Res Treat 115(3):623–627

36. Pharoah PD, Antoniou A, Bobrow M et al (2002) Polygenic susceptibility to breast cancer and implications for prevention. Nat Genet 31(1):33–36

37. Miki Y, Swensen J, Shattuck-Eidens D et al (1994) A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. Science 266(5182):66–71

38. Wooster R, Bignell G, Lancaster J et al (1995) Identification of the breast cancer susceptibility gene BRCA2. Nature 378(6559):789–792

39. Hadjisavvas A, Charalambous E, Adamou A et al (2004) Hereditary breast and ovarian cancer in Cyprus: identification of a founder BRCA2 mutation. Cancer Genet Cytogenet 151(2):152–156

40. Loizidou M, Marcou Y, Anastasiadou V et al (2007) Contribution of BRCA1 and BRCA2 germline mutations to the incidence of early onset breast cancer in Cyprus. Clin Genet 71(2):165–170

41. Loizidou MA, Michael T, Neuhausen SL et al (2008) Genetic polymorphisms in the DNA repair genes XRCC1, XRCC2 and XRCC3 and risk of breast cancer in Cyprus. Breast Cancer Res Treat 112(3):575–579

42. Pooley KA, Baynes C, Driver KE et al (2008) Common single-nucleotide polymorphisms in DNA double-strand break repair genes and breast cancer risk. Cancer Epidemiol Biomarkers Prev 17(12):3482–3489

43. Han J, Haiman C, Niu T et al (2009) Genetic variation in DNA repair pathway genes and premenopausal breast cancer risk. Breast Cancer Res Treat 115(3):613–622

44. Kobayashi J, Antoccia A, Tauchi H et al (2004) NBS1 and its functional role in the DNA damage response. DNA Repair (Amst) 3(8–9):855–861

45. Jazayeri A, Jackson SP (2002) Screening the yeast genome for new DNA-repair genes. Genome Biol 3(4):reviews1009.1–reviews1009.5

46. Merika M, Orkin SH (1993) DNA-binding specificity of GATA family transcription factors. Mol Cell Biol 13:3999–4010

47. Lee PH, Shatkay H (2008) F-SNP: computationally predicted functional SNPs for disease association studies. Nucleic Acids Res 36(Database issue):D820–D824

48. Osman F, Whitby MC (2007) Exploring the roles of Mus81-Eme1/Mms4 at perturbed replication forks. DNA Repair (Amst) 6(7):1004–1017

49. McPherson JP, Lemmers B, Chahwan R et al (2004) Involvement of mammalian Mus81 in genome integrity and tumor suppression. Science 304(5678):1822–1826

50. van den Bosch M, Bree RT, Lowndes NF (2003) The MRN complex: coordinating and mediating the response to broken chromosomes. EMBO Rep 4(9):844–849

51. Liu Y, Ludes-Meyers J, Zhang Y et al (2002) Inhibition of AP-1 transcription factor causes blockade of multiple signal transduction pathways and inhibits breast cancer growth. Oncogene 21(50):7680–7689

52. Gordon S, Akopyan G, Garban H et al (2006) Transcription factor YY1: structure, function, and therapeutic implications in cancer biology. Oncogene 25(8):1125–1142

53. Gorlov IP, Gorlova OY, Sunyaev SR et al (2008) Shifting paradigm of association studies: value of rare single-nucleotide polymorphisms. Am J Hum Genet 82(1):100–112