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Polymorphisms in DNA repair genes and epithelial ovarian cancer risk

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DNA repair gene polymorphisms and mutations are known to influence cancer risk. We studied whether polymorphisms in DNA double strand break (DSB) repair genes are associated with epithelial ovarian cancer (EOC) risk. Up to 1,600 cases and 4,241 controls from 4 separate genetic association studies from 3 countries were genotyped for 13 single nucleotide polymorphisms (SNP) in 6 genes (*BRCA1*, *NBS1*, *RAD51*, *RAD52*, *XRCC2* and *XRCC3*) involved in homologous recombination of DNA double strand breaks. Genotype specific risks were estimated as odds ratios (OR) by unconditional logistic regression. No association was detected between EOC risk and *BRCA1* Q356R, *BRCA1* P871L, *RAD51* g135c, *RAD51* g172t, *RAD52* c2259t, *NBS1* L34L, *NBS1* E185Q, *NBS1* A399A, *NBS1* P672P, *XRCC2* g4324c, *XRCC2* c41657t and *XRCC3* T241M. The *XRCC2* R188H polymorphism was associated with a modest reduction in EOC risk: OR for heterozygotes was 0.8 (95% confidence interval [CI] = 0.7–1.0) and for rare homozygotes 0.3 (0.1–0.9). The *XRCC3* a4541g polymorphism, situated in the 5′UTR, and the intronic *XRCC3* a17893g polymorphism were not associated with EOC risk in general, but when the serous EOC subset only was analysed, the OR for heterozygotes for a4541g was 1.0 (0.9–1.2) and for the rare homozygotes 0.5 (0.3–0.9). For the *XRCC3* a17893g polymorphism, the OR for the heterozygotes and the rare homozygotes were 0.8 (0.7–0.9) and 0.9 (0.7–1.2), respectively. In our study, some polymorphisms in *XRCC2* and *XRCC3* genes were associated with EOC risk. Further research on the role of these genes on epithelial ovarian cancer is warranted.

Key words: ovarian cancer risk; polymorphisms; DNA repair

Without properly functioning DNA repair mechanisms, cells may accumulate various types of DNA damage. DNA double-strand breaks (DSB) are a common form of DNA damage induced during normal DNA replication and by environmental agents such as ionising radiation and genotoxic chemicals.[1] These lesions are particularly harmful, because if left unrepaired, they can cause chromosomal loss, translocations and deletions that may subsequently lead to the activation of proto-oncogenes, loss of function of tumour suppressor genes or global genomic instability.[2]

The repair of DSB in human cells is controlled by 2 different pathways: homologous recombination (HR) and non-homologous end-joining (NHEJ). In HR the broken strand is repaired using the homologous chromosome or sister chromatid as a template, whereas in NHEJ the broken strands are crudely joined together at a site of microhomology.[3,4] Homologous recombination is a high fidelity process, whereas NHEJ frequently results in small deletions at the site of fusion and is error-prone. Genes participating in HR include *RAD51*, *RAD52*, *BRCA1*, *BRCA2*, *XRCC2* and *XRCC3*. The protein products of *MRE11*, *RAD50* and *NBS1* form a multi-unit complex that binds to DNA DSB, signalling and recruiting components from the HR and NHEJ pathways.[5]

Mutations in *BRCA1* and *BRCA2* cause breast, ovarian and other cancers,[6,7] and an association has been suggested between a common polymorphism in *BRCA2* and breast and ovarian cancer risk.[8,9] Some recent studies suggest that polymorphisms in *XRCC2* and *XRCC3* influence susceptibility to breast cancer, skin cancer or acute myeloid leukemia.[10–12] Profound genetic instability has been noted in *XRCC2*-knockout mice and in *XRCC3*-mutant hamster cell lines,[13,14] which gives further evidence for the important role of these DNA repair genes in protecting cells from harmful mutations.

Epithelial ovarian cancer (EOC) accounts for 5% of all cancers among women.[15] The etiology of EOC is not fully understood, but both epidemiological and biological observations suggest that ovulation may play a role in ovarian cancer development. During a normal monthly ovulation, the surface epithelium of the ovary is degraded enzymatically to release the ovum and the resulting wound is subsequently repaired. This natural process is speculated to carry the potential for malignant transformation; repetitive ovulation increases the frequency of epithelial cell division, which subsequently increases DNA replication. Replication can induce DNA damage and the resulting lesions must be recognized and repaired to prevent the accumulation of potentially oncogenic mutations.[16,17] In support of the repetitive ovulation hypothesis, nulliparity has been found to be a risk factor for ovarian cancer and lack of ovulation, resulting from oral contraceptive pill use and pregnancy, is associated with a decreased risk of ovarian cancer.[18–21]

The known highly penetrant, rare cancer predisposition alleles, such as germline mutations in the *BRCA1* or *BRCA2* tumor suppressor genes, are estimated to account for <10% of all ovarian cancer cases and <30% of the excess familial risk of ovarian cancer.[22] It is likely that the unexplained heritable component of ovarian cancer susceptibility is due to multiple weakly penetrant

Abbreviations: CI, confidence interval; DSB, double strand break; EOC, epithelial ovarian cancer; EPIC, European prospective Investigation of Cancer; FROC, Family Registry for Ovarian Cancer; HR, homologous recombination; NHEJ, non-homologous end-joining; OR, odds ratio.

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TABLE I – DETAILS OF SNPS GENOTYPED IN THIS STUDY

Gene	DbSNP reference[1]	SNP	Position	Amino acid change
BRCA1	Rs1799950	A/G at nt 1186	Exon 11	Gln356Arg
BRCA1	Rs799917	C/T at nt 2731	Exon 11	Pro871 Leu
NBS1	Rs1063045	G/A at nt 6582	Exon 2	Leu34Leu
NBS1	Rs1805794	C/G at nt 11122	Exon 5	Glu185GIn
NBS1	Rs709816	T/C at nt 33890	Exon 10	Asp399Asp
NBS1	Rs1061302	A/G at nt 43179	Exon 13	Pro672Pro
RAD51	Rs1801320	G/C at nt 135	5'UTR	No change
RAD51	Rs1801321	G/T at nt 172	5'UTR	No change
RAD52	Rs11226	C/T at nt 2259	3'UTR	No change
XRCC2	Rs3218536	G/A at nt 31479	Exon4	Arg188His
XRCC3	Rs1799794	A/G at nt 4541	5'UTR	No change
XRCC3	Rs1799796	A/G at nt 17893	Intron 5	No change
XRCC3	Rs861539	C/T at nt 18067	Exon 7	Thr241 Met

[1]http://www.ncbi.nlm.nih.gov/SNP.

alleles. Mutations in genes functioning in the HR pathway of DNA DSB repair cause inherited susceptibility to EOC and polymorphisms in these genes are suspected to influence the risk of breast and other cancers. The purpose of our study was to test the hypothesis that polymorphisms in these genes are associated with risk of invasive EOC. We aimed to identify common coding variants that may have functional effects as well as non-coding variants that may be in linkage disequilibrium with variants in unidentified regulatory regions.

Material and methods

Selection of candidate genes and SNP

DSB repair genes considered for our study were: *NBS1*, *RAD50*, *RAD51*, *RAD52*, *XRCC2*, *XRCC3* and *BRCA1*. Candidate SNP were initially identified through public SNP databases and literature searches. The URLs of these databases are listed in the section below. SNP were accepted for our study if their population frequency was >0.05. When suitable SNP could not be identified by electronic or literature searches, we carried out a variation search using either single-stranded conformation polymorphism (SSCP) or denaturing high pressure liquid chromatography (DHPLC) as described previously.[10] Possible SNP were verified by DNA sequencing using an ABI Prism 377 sequencer.[10] The association of a SNP in *BRCA2* with ovarian cancer incidence has been described elsewhere.[9] The SNP chosen for our study are presented in Table I.

SNP databases

The SNP databases used can be found at: CGAP-GAI, http://cgap.nci.nih.gov/; HGBase, http://hgvbase.cgb.ki.se/; Nijmegen Breakage Syndrome, http://vmresearch.org; NCBI SNP database, http://snp.cshl.org/; Sanger Centre, http://www.sanger.ac.uk; and SNP Consortium, http://snp.cshl.org / data.

Study subjects

United Kingdom SEARCH study. The SEARCH ovarian cancer study is an ongoing, population-based ovarian cancer case control study covering the regions served by the East Anglia and West Midlands cancer registries in the United Kingdom. Eligible women are those diagnosed since 1991 with invasive epithelial ovarian cancer under the age of 70 years. Participants are asked to provide written consent, to complete an epidemiological questionnaire and to provide a 20-ml whole blood sample. Currently, of 1,785 eligible patients, 1,019 women have agreed to take part. The first 864 cases (with 864 controls) were available for this analysis. Female controls have been selected randomly from the EPIC-Norfolk component of the European Prospective Investigation of Cancer (EPIC), a prospective study of diet and cancer being carried out in the same population from which the cases have been drawn. The EPIC Norfolk cohort comprises 25,000 individuals resident in Norfolk (East Anglia), aged 45–74 years at first interview in 1993. Blood for DNA extraction was collected during the second health check in 1998–2000. The ethnic background of cases and controls is similar, with over 98% being Caucasian Europeans. DNA was extracted from blood samples by Whatman International Ltd (Ely, UK) using a chloroform/phenol method. The study was approved by the Anglia and Oxford Multi-centre Research Ethics Committee.

Danish MALOVA study. The MALOVA study is a population-based, Danish case-control study of ovarian cancer. Eligible cases were women aged 35–79 years, who were diagnosed with an ovarian tumour from December 1994 to May 1999. The study included 18 different hospitals from the municipalities of Copenhagen and Frederiksberg as well as the counties of Copenhagen, Frederiksborg, Roskilde, Vestsjßlland, Storstrøm, Funen, Southern Jutland and Northern Jutland. In total 698 invasive epithelial ovarian cancers, 219 ovarian borderline tumors and 450 benign ovarian tumors were enrolled. Controls were drawn from the general female population within the study area (aged 35–79 years) selected at random using the computerized Central Population Register. After providing written consent, cases and controls had a personal interview and gave blood samples (pre-operatively for cases). Samples from 438 cases and 1,112 controls were available for our study. DNA was extracted from blood using the QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA).

United States FROC study. The United States subjects were ascertained from the Family Registry for Ovarian Cancer (FROC) of 6 counties in Northern California. Included were ovarian cancer cases (n = 327) from Caucasians (20–64 years old) diagnosed between March 1, 1997 and July 31, 2001 and age/ethnic matched self-reported healthy female controls without cancer (n = 427), obtained from the same 6 counties by random-digit dialing. Buccal rinses were obtained from 83 cancer cases and 55 controls of FROC and bloods from all other subjects. DNA was purified from peripheral blood leucocytes using the Puregene Kit (Gentra Systems, Minneapolis, MN). DNA was isolated from exfoliated cells in buccal mouthwash rinses as described previously.[23] The study protocol was approved by the Institutional Review Boards at all participating study sites.

United Kingdom Royal Marsden Hospital and young ovarian cancer study (UK RMH/YOV). The fourth case control study comprised cases drawn from 2 different sources: 244 were women with invasive EOC who were <70 years of age at the time of diagnosis and were seen at the Royal Marsden Hospital, London between July 1993 and September 1995[24] and 118 were women who had taken part in a United Kingdom national study of early onset ovarian cancer.[25] Women diagnosed in the United Kingdom between 1984–93 with EOC diagnosed under 30 years of age who were still alive in 1998 were eligible for our study. Control samples (n up to 1,878) for these cases were a set of EPIC samples (separate from the samples selected as controls for SEARCH) that had been previously genotyped for a breast cancer case-control

association study[10] (see above for description of EPIC). Genomic DNA was extracted by standard methods using an Extrragen automated extractor. Both these studies were approved by the relevant local research ethics committees.

Genotyping

Genotyping was carried out centrally in Strangeways Research Laboratory using the 5′-nuclease assay (TaqMan, Applied Biosystems, Warrington, UK) and the ABI PRISM 7700/7900 sequence detection system (Applied Biosystems). Primers and probes were designed with the Primer Express Oligo Design Software v1.0 (Applied Biosystems). The primer and probe sequences and amplification conditions are listed in Table II.

For the United Kingdom RMH/YOV study, 15-ul assays (25-ul for *BRCA1* P871L) were carried out on 20 ng of genomic DNA according to the manufacturer's instructions. Amplification was carried out using MJ Tetrad thermal cyclers (GRI), and the plates were read "post-PCR" on an ABI PRISM 7700 Sequence detector using the Allelic Discrimination sequence detection software (Applied Biosystems). We included 8 non-template controls (H_2O) and 8 positive controls (allele-specific) in each 96-well plate. Positive controls were artificial DNA templates made by annealing together long oligonucleotides that span each SNP.

For the other 3 studies, each assay was carried out using 10 ng DNA in a 5 μl reaction with primers at 900 nM and probes at 200 nM concentrations. All reactions were carried out using 384-well arrays with 12 duplicate samples in each plate for quality control. There were no discordant genotypes in duplicates. Genotypes were called using the Allelic Discrimination sequence detection software (Applied Biosystems,). DNA samples that did not give a clear genotype result at the first attempt were not repeated because this is a high-throughput process. There are variations in the number of samples successfully genotyped for each polymorphism. We were unable to obtain satisfactory genotype calling for the *BRCA1* Q356R assay in the Danish samples.

Statistical analysis

We present age-unadjusted odds ratios (OR) as there was no association of genotype with age in controls, and so age is not a confounding variable. Odds ratios were not adjusted for other known risk factors for ovarian cancer such as reproductive factors because it is unlikely that any such factors will act as true confounders.

Genotype frequencies in the controls were tested for deviation from Hardy-Weinberg Equilibrium using a standard χ^2 test. (1 *df*). The primary test of association used was a comparison of genotype frequencies in cases and controls. This was done for each study separately using χ^2 tests (2 *df*). The data were then pooled and genotype frequencies were compared in cases and controls using unconditional logistic regression models with terms for genotype and study and an appropriate likelihood ratio test. Genotype specific risks, with the common homozygote as the baseline comparator, were estimated as OR by unconditional logistic regression.

For genes with more than one polymorphism we investigated possible haplotype effects, using the logistic regression procedure suggested by Cordell and Clayton.[26] This method includes a main effect term for each polymorphism in the logistic regression model, rather than modelling the full haplotype effect. A likelihood ratio test was used to test this model for significance. All statistical tests and *p*-values are 2-tailed.

Results

The observed genotype and allele frequencies for each of the 13 polymorphisms are presented in Table III. The genotype frequencies in the controls did not differ significantly from those expected under Hardy-Weinberg equilibrium (HWE) apart from *RAD52* c2259t that was marginally significant in the United Kingdom

TABLE II – PRIMERS AND PROBES USED FOR TAQMAN™ ASSAYS

SNP	Forward primer (5′-3′)	Reverse primer (5′-3′)	Vic-probe[1]	Fam-probe[1]
BRCA1 Q356R	CAAGGAACATCTTCAGTATC	AATGCTGATCCCCTGTGTGAG	AGCATGGCAGTTTCCGCTTATCCATTCT	AGCATGGCAGTTTCTGCTTATTCC
BRCA1 P871L	TCTAGGATT TCAAGGTTTCAAAGCGCCA	AATGTTGCACATTCCTCTTCTGC	ATTTGCTCCGTTTTCAAATCCAGGAA	ATTCT TCATTTGCTCTGTTTTCAAATCCAGGAA
NBS1 g6582a	GCGTTGAGTACGTTGTTGGAAG	ACAGCATGATTTCGGCTGATC	AAAAACTGTGCCATTCTGATTGAAA	AAAAACTGTGCCATTCTAATTAAAA
NBS1 E185Q	ACATAATATACCTTTCAATT TCTGGAGG	GACGTCCAATTGTAAAGCCAGAAT	ATGATC TGCTTCTTGGACTCAACTGCTTTCAGG	TGATC TGCTTCTTGGACTGAACTGCTTTCAGG
NBS1 t33890c	AGAAATCAAAGTCTCCAAAA TGGAA	GAGCTTGTTTTGCAGGACTCCT	AATGCTTTCACAAGATGCACCCACT	AATGCTTTCACAAGACGCACCCACT
NBS1 a43179g	CTGTTATTGACTGAATTTAGA TCACTGGT	TGATACAGTTGAAATACCTACC TTTTTGA	ACTTCCAGAAATCCATCTGGCTAAATG	ACTTCCAGAAATCCGTCTGGCATAAATG
RAD51 g135c	GCAGCGCTCCTCTCTCCAGC	GCTGGGAACTGCAACTCATCT	CAACGCCCGTGGCTTACGCT	CCCCAACGCCCCTGGCTTAC
RAD51 g172t	GTCCGCAGCCTCCTCTCTC	CAGCTGGGAACTGCAACTCA	CCCCGCGGGAGTGGCA	CCGCGGGCGTGGCAC
RAD52 c2259t	GTGGTGCAAACACAGCTCTCTC	AGTAATCCCTGTATTTTG TAAGGCAGA	ACAACCTCTTGGGCCCAAGTGATACT	CACTAACCTCTTGGGCTCAAGTGATACTC
XRCC2 R188H	TCTGCATTATAGTTTGTGTCG TTGC	CGCGTCAATGGAGGAGAAA	AAAAGAACCAGGCGATAGTCATTTA	CAAAAAGAACCAGGTGATAGTCATTTA CAAGC
XRCC3 a4541g	TCCGGCTCCTGAGGCTC	GGTCGTCGCTAAACAGACTTTGA	CTCTGTGCACACATCCTGCTGAGAACTTG	CTCTGTGCACACCCTGCTGAGAACTT
XRCC3 a17893g	GGTCGAGTGACAGTCCAAACG	GGACCTTCTTATTCACACACTCCAA	AGCATAGACAATGACAGCTGTCCCCAC	CAGCATAGACATGACGGCTGTCCC
XRCC3 T241M	GGCTAAAAATACGAGCTCAGGG	CCAGGCATCTGCAGTCCC	CACGCAGCATGGCCCCC	ACGCAGCGTGGCCCCC

[1]Variable nucleotide underlined.

TABLE III – DISTRIBUTIONS OF GENOTYPES BY STUDY

Study					Total	Rare allele frequency	HWE *p*-value	Case-control comparison of genotype frequencies	
								χ^2 (2 *df*)	*p*-value
BRCA1 Q356R		QQ	QR	RR					
UK SEARCH	Controls	745	100	4	849	0.06	0.74	3.45	0.18
	Cases	644	87	0	731	0.06			
US FROC	Controls	371	49	0	420	0.06	0.20	1.51	0.47
	Cases	280	41	1	322	0.06			
UK RMH / YOV	Controls	663	89	0	752	0.06	0.08	2.91	0.23
	Cases	264	31	1	296	0.06			
Combined	Controls	1,779	238	4	1,868	0.06	0.17	0.23	0.89
	Cases	1,188	159	2	1,349	0.06			
BRCA1 P871L		PP	PL	LL	Total				
UK SEARCH	Controls	388	350	92	830	0.32	0.33	2.44	0.29
	Cases	322	331	69	722	0.33			
US FROC	Controls	177	174	44	395	0.33	0.90	2.98	0.22
	Cases	119	154	37	310	0.37			
Danish MALOVA	Controls	359	339	83	781	0.32	0.82	0.70	0.70
	Cases	129	137	33	299	0.34			
UK RMH / YOV	Controls	380	423	102	905	0.35	0.33	0.30	0.86
	Cases	130	135	32	297	0.34			
Combined	Controls	1,304	1,286	321	2,911	0.33	0.88	2.80	0.25
	Cases	700	757	171	1,628	0.34			
NBS1 g6582a		gg	ga	aa					
UK SEARCH	Controls	700	704	182	1,586	0.34	0.66	0.92	0.63
	Cases	309	352	82	743	0.35			
US FROC	Controls	168	196	54	418	0.36	0.79	1.68	0.43
	Cases	146	140	39	325	0.34			
Danish MALOVA	Controls	378	368	86	832	0.33	0.80	0.36	0.83
	Cases	142	134	28	304	0.31			
UK RMH / YOV	Controls	335	331	80	746	0.33	0.80	0.30	0.86
	Cases	135	125	29	289	0.32			
Combined	Controls	1,246	1,268	322	2,836	0.34	0.98	0.69	0.71
	Cases	732	751	178	1,661	0.33			
NBS1 E185Q		EE	EQ	QQ					
UK SEARCH	Controls	369	372	107	848	0.35	0.38	1.57	0.46
	Cases	307	335	79	721	0.34			
US FROC	Controls	162	169	52	383	0.36	0.46	1.62	0.44
	Cases	141	134	33	308	0.33			
Danish MALOVA	Controls	383	359	85	827	0.32	0.75	0.41	0.81
	Cases	142	130	27	299	0.31			
UK RMH / YOV	Controls	339	318	77	734	0.32	0.85	0.31	0.86
	Cases	124	109	25	258	0.31			
Combined	Controls	1,253	1,218	214	2,685	0.34	0.09	2.56	0.28
	Cases	714	708	164	1,586	0.33			
NBS1 t33890c		tt	tc	cc					
UK SEARCH	Controls	336	388	125	849	0.38	0.45	0.95	0.62
	Cases	283	350	98	731	0.37			
US FROC	Controls	142	176	70	387	0.41	0.24	0.96	0.62
	Cases	120	138	55	309	0.40			
Danish MALOVA	Controls	339	380	107	826	0.36	0.97	0.76	0.68
	Cases	124	146	34	304	0.35			
UK RMH / YOV	Controls	756	827	253	1,836	0.36	0.26	1.53	0.47
	Cases	116	107	38	261	0.35			
Combined	Controls	1,573	1,771	555	3,899	0.37	0.06	0.57	0.75
	Cases	643	741	225	1,609	0.37			
NBS1 a43179g		aa	ag	gg					
UK SEARCH	Controls	367	364	99	830	0.34	0.55	1.82	0.40
	Cases	306	333	73	712	0.34			
US FROC	Controls	158	174	50	382	0.36	0.85	1.21	0.54
	Cases	138	134	34	306	0.33			
Danish MALOVA	Controls	329	306	74	709	0.32	0.82	0.35	0.84
	Cases	124	111	24	259	0.31			
UK RMH / YOV	Controls	841	782	199	1,822	0.32	0.40	0.00	1.00
	Cases	131	122	31	284	0.32			
Combined	Controls	1,695	1,626	422	3,743	0.33	0.28	1.51	0.47
	Cases	699	700	162	1,561	0.33			
RAD51 g135c		gg	gc	cc					
UK SEARCH	Controls	745	100	2	847	0.06	0.48	0.41	0.82
	Cases	642	84	3	729	0.06			
US FROC	Controls	357	61	1	419	0.08	0.34	3.03	0.22
	Cases	270	52	4	326	0.09			
Danish MALOVA	Controls	616	78	5	699	0.06	0.15	1.00	0.61
	Cases	241	36	1	278	0.07			

(Continued)

TABLE III – DISTRIBUTIONS OF GENOTYPES BY STUDY (CONTINUED)

Study					Total	Rare allele frequency	HWE *p*-value	Case-control comparison of genotype frequencies	
								χ^2 (2 *df*)	*p*-value
UK RMH/YOV	Controls	722	116	2	840	0.07	0.23	3.23	0.20
	Cases	266	29	1	296	0.05			
Combined	Controls	2,440	355	10	2,805	0.07	0.44	1.04	0.60
	Cases	1,419	201	9	1,629	0.07			
RAD51 g172t		gg	gt	tt					
UK SEARCH	Controls	273	433	141	847	0.42	0.16	1.91	0.38
	Cases	226	363	141	730	0.44			
US FROC	Controls	149	189	74	412	0.41	0.30	0.06	0.97
	Cases	119	145	57	321	0.40			
Danish MALOVA	Controls	235	277	95	607	0.39	0.37	0.46	0.80
	Cases	112	130	51	293	0.40			
UK RMH/YOV	Controls	226	371	139	736	0.44	0.54	0.95	0.62
	Cases	94	157	49	300	0.43			
Combined	Controls	883	1,270	449	2,602	0.42	0.83	1.91	0.39
	Cases	551	795	298	1,644	0.42			
RAD52 c2259t		cc	ct	tt					
UK SEARCH	Controls	270	387	190	847	0.45	0.02	6.31	0.04
	Cases	218	368	138	724	0.45			
US FROC	Controls	120	208	87	415	0.46	0.86	3.12	0.21
	Cases	75	172	76	323	0.50			
Danish MALOVA	Controls	257	390	180	827	0.45	0.16	2.09	0.35
	Cases	81	153	70	304	0.48			
UK RMH/YOV	Controls	544	890	383	1,817	0.46	0.59	2.68	0.26
	Cases	101	144	53	298	0.42			
Combined	Controls	1,191	1,875	840	3,906	0.46	0.02	4.91	0.09
	Cases	475	837	337	1,649	0.46			
XRCC2 R188H		RR	RH	HH					
UK SEARCH	Controls	704	129	9	842	0.09	0.26	4.80	0.09
	Cases	629	98	2	729	0.07			
US FROC	Controls	331	68	5	404	0.10	0.48	1.81	0.40
	Cases	260	54	1	315	0.09			
Danish MALOVA	Controls	484	75	2	561	0.07	0.61	1.54	0.46
	Cases	238	31	0	269	0.06			
UK RMH/YOV	Controls	1,538	267	6	1,811	0.08	0.12	8.12	0.02
	Cases	251	23	1	275	0.05			
Combined	Controls	3,057	539	22	3,618	0.08	0.74	11.5	0.003
	Cases	1,378	206	4	1,588	0.07			
XRCC3 a4541g		aa	ag	gg					
UK SEARCH	Controls	552	261	29	842	0.19	0.79	1.78	0.41
	Cases	463	246	20	729	0.20			
US FROC	Controls	267	133	17	417	0.20	0.93	1.30	0.52
	Cases	204	112	9	325	0.20			
Danish MALOVA	Controls	536	259	38	833	0.20	0.35	2.96	0.23
	Cases	199	97	7	303	0.18			
UK RMH/YOV	Controls	1,196	535	77	1,808	0.19	0.08	0.49	0.78
	Cases	194	95	12	301	0.20			
Combined	Controls	2,551	1,188	161	3,900	0.19	0.13	4.90	0.09
	Cases	1,060	550	48	1,658	0.20			
XRCC3 a17893g		aa	ag	gg					
UK SEARCH	Controls	386	381	85	852	0.32	0.52	0.57	0.75
	Cases	329	319	81	729	0.33			
US FROC	Controls	191	183	41	415	0.32	0.77	0.97	0.61
	Cases	157	132	36	325	0.31			
Danish MALOVA	Controls	357	361	103	821	0.35	0.43	2.48	0.29
	Cases	140	120	46	306	0.35			
UK RMH/YOV	Controls	823	851	204	1,878	0.34	0.47	3.63	0.16
	Cases	143	121	40	304	0.33			
Combined	Controls	1,757	1,776	433	3,966	0.33	0.62	6.03	0.049
	Cases	769	692	203	1,664	0.33			
XRCC3 T241M		TT	TM	MM					
UK SEARCH	Controls	318	404	108	830	0.37	0.25	0.93	0.63
	Cases	297	347	105	749	0.37			
US FROC	Controls	130	174	40	344	0.37	0.11	4.89	0.09
	Cases	125	114	31	270	0.33			
Danish MALOVA	Controls	358	394	139	891	0.38	0.08	1.02	0.60
	Cases	144	168	49	361	0.37			
UK RMH/YOV	Controls	728	827	229	1,784	0.36	0.81	2.22	0.33
	Cases	130	121	39	290	0.34			
Combined	Controls	1,712	1,946	583	4,241	0.37	0.42	2.83	0.24
	Cases	676	762	227	1,665	0.37			

TABLE IV – GENOTYPE SPECIFIC RISKS (OR AND 95% CI) FOR EACH POLYMORPHISM BY STUDY

Study	Heterozygote risk		Rare homozygote risk	
	OR	95% CI	OR	95% CI
BRCA1 Q356R[1]				
UK SEARCH	1.0	0.7–1.4		
US FROC	1.1	0.7–1.7		
UK RMH / YOV	0.9	0.6–1.3		
Combined	1.0	0.8–1.2	0.7	0.2–3.2
BRCA1 P871L				
UK SEARCH	1.1	0.9–1.4	0.9	0.6–1.3
US FROC	1.3	1.0–1.8	1.3	0.8–2.0
Danish MALOVA	1.1	0.8–1.5	1.1	0.7–1.7
UK RMH / YOV	0.9	0.7–1.2	0.9	0.6–1.4
Combined	1.1	1.0–1.3	1.0	0.8–1.2
NBS1 g6582a				
UK SEARCH	1.1	0.9–1.4	0.9	0.7–1.3
US FROC	0.8	0.6–1.1	0.8	0.5–1.3
Danish MALOVA	1.0	0.7–1.3	0.9	0.5–1.4
UK RMH / YOV	0.9	0.7–1.2	0.9	0.6–1.4
Combined	1.0	0.9–1.1	0.9	0.7–1.1
NBS1 E185Q				
UK SEARCH	1.1	0.9–1.3	0.9	0.6–1.2
US FROC	0.9	0.7–1.2	0.7	0.4–1.2
Danish MALOVA	1.0	0.7–1.3	0.8	0.5–1.4
UK RMH / YOV	0.9	0.7–1.3	0.9	0.5–1.5
Combined	1.0	0.9–1.1	0.8	0.7–1.1
NBS1 t33890c				
UK SEARCH	1.1	0.9–1.3	0.9	0.7–1.3
US FROC	0.9	0.7–1.3	1.0	0.6–1.5
Danish MALOVA	1.1	0.8–1.4	0.9	0.6–1.3
UK RMH / YOV	0.8	0.6–1.1	1.0	0.7–1.5
Combined	1.0	0.9–1.1	0.9	0.8–1.1
NBS1 a43179g				
UK SEARCH	1.1	0.9–1.4	0.9	0.6–1.2
US FROC	0.9	0.6–1.2	0.8	0.5–1.3
Danish MALOVA	1.0	0.7–1.3	0.9	0.5–1.4
UK RMH / YOV	1.0	0.8–1.3	1.0	0.7–1.5
Combined	1.0	0.9–1.1	0.9	0.7–1.1
RAD51 g135c				
UK SEARCH	1.0	0.7–1.3	1.7	0.3–10.5
US FROC	1.1	0.8–1.7	5.3	0.6–48.0
Danish MALOVA	1.2	0.8–1.8	0.5	0.1–1.4
UK RMH / YOV	0.7	0.4–1.0	1.4	0.1–15.0
Combined	1.0	0.8–1.2	1.6	0.6–4.3
RAD51 g172t				
UK SEARCH	1.0	0.8–1.3	1.2	0.9–1.6
US FROC	1.0	0.7–1.3	1.0	0.6–1.5
Danish MALOVA	1.0	0.7–1.3	1.1	0.7–1.7
UK RMH / YOV	1.0	0.8–1.4	0.8	0.6–1.3
Combined	1.0	0.9–1.1	1.1	0.9–1.3
RAD52 c2259t				
UK SEARCH	1.2	1.0–1.5	0.9	0.6–1.1
US FROC	1.3	0.9–1.9	1.4	0.9–2.1
Danish MALOVA	1.2	0.9–1.7	1.2	0.8–1.8
UK RMH / YOV	0.9	0.7–1.1	0.7	0.5–1.1
Combined	1.1	1.0–1.3	1.0	0.8–1.2
XRCC2 R188H				
UK SEARCH	0.8	0.6–1.1	0.2	0.1–1.2
US FROC	1.0	0.7–1.5	0.3	0.1–2.2
Danish MALOVA	0.8	0.5–1.3	0.0	–
UK RMH / YOV	0.5	0.3–0.8	1.0	0.1–8.5
Combined	0.8	0.7–1.0	0.3	0.1–0.9
XRCC3 a4541g				
UK SEARCH	1.1	0.9–1.4	0.8	0.5–1.5
US FROC	1.1	0.8–1.5	0.7	0.3–1.6
Danish MALOVA	1.0	0.8–1.3	0.5	0.2–1.1
UK RMH / YOV	1.1	0.8–1.4	1.0	0.5–1.8
Combined	1.1	0.9–1.2	0.8	0.5–1.1
XRCC3 a17893g				
UK SEARCH	1.0	0.8–1.2	1.1	0.8–1.6
US FROC	0.9	0.6–1.2	1.1	0.7–1.8
Danish MALOVA	0.8	0.6–1.1	1.1	0.8–1.7
UK RMH / YOV	0.8	0.6–1.1	1.1	0.8–1.7
Combined	0.9	0.8–1.0	1.1	0.9–1.4

(Continued)

TABLE IV – GENOTYPE SPECIFIC RISKS (OR AND 95% CI) FOR EACH POLYMORPHISM BY STUDY (CONTINUED)

Study	Heterozygote risk		Rare homozygote risk	
	OR	95% CI	OR	95% CI
XRCC3 T241M				
UK SEARCH	0.9	0.7–1.1	1.0	0.7–1.4
US FROC	0.7	0.5–1.0	0.8	0.5–1.4
Danish MALOVA	1.1	0.8–1.5	1.0	0.6–1.5
UK RMH/YOV	0.8	0.6–1.1	1.0	0.6–1.4
Combined	0.9	0.8–1.0	1.0	0.8–1.2

[1]Number of rare homozygotes zero for some studies, thus rare homozygote risks not estimated for individual studies.

SEARCH controls only ($p = 0.02$). This is likely to be a chance finding, as the discrimination of genotypes for this assay was good. There were no significant differences in genotype frequencies in cases and control for any of the polymorphisms in *BRCA1*, *NBS1*, *RAD51* and *RAD52* when each study was analysed separately or when the data were combined (Table III). Genotype specific risks for the SNP in these genes were not significantly different from unity (Table IV).

There was a moderately strong association of the *XRCC2* R188H polymorphism with EOC (combined data genotype frequency heterogeneity test $p = 0.003$). The rare allele was associated with a reduced risk of disease in a dose-dependent manner: women having one rare (histidine) allele had a 20% reduction in risk (combined data OR = 0.8 [0.66–0.96]), whereas women who were homozygote for the histidine allele had their EOC risk reduced to less than half of the common homozygote risk (combined data OR = 0.31 [0.11–0.88]). There was no evidence for heterogeneity of risk between the studies ($p = 0.62$). The results were similar when the analysis was restricted to the 737 cases with serous disease (data not shown).

There was some evidence for an association of the *XRCC3* SNP a4541g and a17893g with invasive EOC (combined data $p = 0.087$ and 0.049 respectively). The genotype specific risks are shown in Table IV. These suggest that the rare allele is associated with a weak protective effect for each SNP. These associations were somewhat stronger when analysis was restricted to the 737 cases with serous type histopathology ($p = 0.024$ and 0.027). In the serous cases, the g-allele of a4541g in the 5′UTR was found to be associated with a recessive protective effect (OR ag *vs.* aa = 1.0 [0.87–1.2]; OR gg *vs.* aa = 0.50 [0.28–0.89]). The g-allele of a17893g in the 5′UTR was also found to be associated with a protective effect (OR ag *vs.* aa = 0.79 [0.0.66–0.94]; OR gg *vs.* aa = 0.95 [0.72–1.2]). There was no association with the missense T241M variant in *XRCC3*. Multiple SNP logistic regression models provided no evidence for haplotype specific effects for *BRCA1*, *NBS1*, *RAD51*, *XRCC2* and *XRCC3* ($p = 0.86$, 0.84, 0.84, 0.23 and 0.80 respectively).

Discussion

Common variants in genes involved in double strand break DNA repair are good candidates for low penetrance susceptibility to epithelial ovarian cancer. We have found some evidence that the missense variant R188H in *XRCC2* is associated with risk of invasive epithelial ovarian cancer, the rare allele having a protective effect under a co-dominant genetic model. This result needs to be interpreted with some caution. Even though the null hypothesis would be rejected at the 0.005 level for the primary test of association, the possibility of a Type I statistical error (false positive) must be considered. It has been suggested that stringent criteria should be applied to statistical tests for genetic association, *e.g.*, $p < 0.0001$, because of the large number of candidate polymorphisms across the human genome. A total of 7,600 cases with controls would be needed to detect a co-dominant allele with risk of 0.8 with 80% power at this level of significance.

Hidden population stratification is an alternative explanation for a spurious association. This occurs when allele frequencies differ between population sub-groups and cases and controls are drawn differentially from those sub-groups. It seems unlikely that population stratification is relevant in this investigation because the cases and controls in the four studies reported here were drawn from the same ethnic groups and minor allele frequencies were similar for all SNP in the four studies. Furthermore, if stratification were present, it is unlikely that the same degree of stratification would be seen in all four studies. It is also worth noting that the existence of significant population stratification that has resulted in a false genetic association has never been demonstrated empirically.[27]

Other direct evidence to support or refute our findings is lacking as there have been no studies published previously of *XRCC2* R188H in ovarian cancer. Two studies, however, have found marginal evidence for an increased risk of breast cancer associated with the rare allele, either by itself [28] or only when modulated by plasma folate levels.[29] Another study of this polymorphism in breast cancer found no association.[10] Furthermore, there is evidence for a functional effect of R188H that might predict the rare allele to be associated with a decreased risk of cancer.[28] Cell lines, in which the positively charged arginine at position 188 has been changed to the neutral alanine or deleted altogether, show significantly decreased survival compared to normal cell lines when treated with mitomycin C. Cell lines with the polymorphic histidine also showed decreased survival, but this effect was subtle and not significant statistically.

We also found weaker evidence that 2 SNP in *XRCC3* are associated with EOC. The association for a4541g was not significant at the 5% level ($p = 0.087$) and that for a17893g was only marginally significant ($p = 0.049$). As with R188H, these observations may have occurred by chance. Some support for the findings comes from a report that both these SNP were associated with breast cancer risk.[10]

We found no evidence for an association of 9 polymorphisms in *BRCA1*, *NBS1*, *RAD51* and *RAD52* with EOC. Our study included a minimum of 1,561 cases and 2,602 controls providing us with at least 93% power at the 5% level of significance to detect a co-dominant allele with frequency 0.3 that confers a relative risk of 1.2 or 86% power to detect a dominant allele with frequency 0.1 that confers a relative risk of 1.3. We cannot, however, exclude the possibility that the alleles investigated are associated with smaller risks, or that there are other susceptibility variants in these genes that are not correlated strongly with the polymorphisms examined.

Several other studies investigating putative associations between ovarian cancer and various SNP have been published,[30–39] but the number of patients in the studies has rarely exceeded 500. Apart from the *BRCA1* polymorphisms, none of the polymorphisms in our study have been examined previously. The small but significant differences in risk associated with *XRCC2* R188H variant and the 2 non-coding *XRCC3* variants, with what is known of the importance of these genes in homologous recombination pathway of DNA DSB, might indicate a true association, but confirmation of our results in other data sets is needed before drawing definitive conclusions.

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