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RAD51, XRCC3, and XRCC2 mutation screening in Finnish breast cancer families

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Abstract

Majority of the known breast cancer susceptibility genes have a role in DNA repair and the most important high-risk genes BRCA1 and BRCA2 are specifically involved in the homologous recombination repair (HRR) of DNA double-strand breaks. A central player in HRR is RAD51 that binds DNA at the damage site. The RAD51 paralogs RAD51B, RAD51C, RAD51D, XRCC2, and XRCC3 facilitate the binding of RAD51 to DNA. While germline mutations in RAD51C and RAD51D are associated with high ovarian cancer risk and RAD51B polymorphisms with breast cancer, the contribution of RAD51, XRCC3, and XRCC2 is more unclear. To investigate the role of RAD51, XRCC3, and XRCC2 in breast cancer predisposition and to identify putative recurrent founder mutations in the Finnish population where such mutations have been observed in most of the currently known susceptibility genes, we screened 182 familial Finnish breast or ovarian cancer patients for germline variation in the RAD51and XRCC3 genes and 342 patients for variation in XRCC2, with a subset of the patients selected on the basis of decreased RAD51 protein expression on tumors. We also performed haplotype analyses for 1516 breast cancer cases and 1234 controls to assess the common variation in these genes. No pathogenic mutations were detected in any of the genes and the distribution of haplotypes was similar between cases and controls. Our results suggest that RAD51, XRCC3, and XRCC2 do not substantially contribute to breast cancer predisposition in the Finnish population.

Keywords: Breast cancer; RAD51; XRCC3; XRCC2

Introduction

Most of the known breast cancer susceptibility genes function in DNA damage repair. The most important predisposition genes BRCA1 and BRCA2, conferring high life-time risks of breast and ovarian cancer, are involved in the homologous recombination repair (HRR) of DNA double-strand breaks (DSB) (Mavaddat et al. 2010). The moderate-penetrance genes ATM, CHEK2, PALB2, and BRIP1 also have a role in DNA repair. A large proportion of the unexplained familial risk of breast cancer is likely explained by clustering of several common low-penetrance variants and so far, large number of low-risk loci have been identified (Michailidou et al. 2013). However, the currently known high, moderate, and low-penetrance alleles together only explain approximately 35% of the familial risk of breast cancer and thus, other susceptibility loci are likely to exist and

genes involved in the homologous recombination repair are attractive candidates.

A central player in the homologous recombination is the RAD51 recombinase that binds to single-stranded DNA at break sites (Suwaki et al. 2011). The binding of RAD51 to DNA is facilitated by several proteins including BRCA2 and the five RAD51 paralogs RAD51B, RAD51C, RAD51D, XRCC2, and XRCC3. Deleterious germline mutations in the RAD51C and RAD51D genes confer an increased risk of ovarian cancer (Loveday et al. 2011, 2012) whereas common polymorphisms in the RAD51B gene are associated with male and female breast cancer (Figueroa et al. 2011; Orr et al. 2012). The contribution of RAD51, XRCC3, and XRCC2 to breast cancer susceptibility remains unclear. Deleterious germline mutations in the XRCC2 gene have been identified in exome sequencing of familial breast cancer patients but the association was not confirmed in a larger case–control study (Park et al. 2012; Hilbers et al. 2012). Several association studies of XRCC3 have yielded controversial results yet a meta-analysis by He et al.

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suggests an association between common *XRCC3* polymorphisms and breast cancer risk (He et al. 2012). A likely deleterious missense mutation in the *XRCC3* gene has been identified in one breast and ovarian cancer family (Golmard et al. 2013). In the *RAD51* gene, one possibly disease-associated missense mutation has been identified in bilateral breast cancer patients whereas three studies report no deleterious *RAD51* mutations among breast cancer cases (Kato et al. 2000; Lose et al. 2006; Rapakko et al. 2006; Le Calvez-Kelm et al. 2012).

The presence of recurrent founder mutations in the Finnish population creates an advantage for the identification of new susceptibility genes. We have previously identified Finnish founder mutations in the ovarian cancer susceptibility genes *RAD51C* and *RAD51D* (Pelttari et al. 2011, 2012) and recently, we identified a recurrent nonsense mutation in the *FANCM* gene that associated especially with triple-negative breast cancer (Kiiski et al. 2014). In Finland, recurrent mutations explain most of the familial breast cancer risk caused by the currently known susceptibility genes, such as *BRCA1*, *BRCA2*, *PALB2*, and *CHEK2* (Sarantaus et al. 2000; Erkko et al. 2007; Vahteristo et al. 2002), whereas in other more diverse populations several rare mutations in each gene have been identified.

Inactivating mutations in tumor suppressor genes usually lead to decreased protein expression and further to tumor progression (Vogelstein and Kinzler 2004). Loss-of-function mutations have been identified in all the known breast cancer susceptibility genes involved in DNA damage response. We have previously shown that carriers of the truncating *CHEK2* c.1100delC mutation often have reduced or absent CHEK2 protein expression in breast tumors (Vahteristo et al. 2002). We have also previously identified two germline mutations in the *MRE11* gene among breast cancer patients whose tumors showed decreased expression of the MRN complex proteins MRE11, RAD50, and NBS1 that play an important role in the DNA damage response (Bartkova et al. 2008). The breast tumors were studied by immunohistochemical staining of MRE11, RAD50, and NBS1, and patients whose tumors had reduced expression of all three proteins were selected for further germline DNA analysis. These results indicate that loss or reduction of protein expression in the tumor may be a sign of underlying inactivating germline mutations.

To evaluate the contribution of *RAD51*, *XRCC3*, and *XRCC2* mutations to breast cancer predisposition, we screened 182 familial Finnish breast or ovarian cancer patients for germline variation in the *RAD51* and *XRCC3* genes and 342 patients for the *XRCC2* gene. To facilitate the mutation discovery, a subset of the patients was selected on the basis of decreased RAD51 protein expression on their breast tumors. We also studied the common variation in these genes with a haplotype analysis in 1516 breast cancer cases and 1234 controls.

Materials and methods

Subjects

The patient samples originated from two unselected series of breast cancer cases and additional familial breast and ovarian cancer patients collected at Helsinki University Hospital Departments of Oncology and Clinical Genetics (Eerola et al. 2000; Fagerholm et al. 2008). The unselected breast cancer cases were ascertained at Helsinki University Hospital Department of Oncology in 1997–1998 and 2000 ($n = 884$) (Syrjäkoski et al. 2000; Kilpivaara et al. 2005) and Department of Surgery in 2001–2004 ($n = 986$) (Fagerholm et al. 2008) including 79% and 87%, respectively, of all consecutive, newly diagnosed breast cancer cases during the collection periods. *BRCA1* and *BRCA2* mutation carriers were excluded from the familial patient series as previously described (Vahteristo et al. 2001, 2002; Vehmanen et al. 1997). RAD51 protein expression was analyzed in 1240 paraffin-embedded invasive breast tumors from these patients as described (Fagerholm et al. 2013).

The *RAD51* and *XRCC3* genes were screened in 182 and the *XRCC2* gene in 342 *BRCA1/2*-negative familial breast or ovarian cancer patients. Out of these, 71 were selected on the basis of absent or decreased RAD51 expression on tumors. The *RAD51-XRCC3* screening included two ovarian cancer probands and four cases affected with both breast and ovarian cancer and the *XRCC2* screening included five cases with breast and ovarian cancer; the rest of the screened patients were breast cancer cases. The patients had a strong family background of breast cancer with at least three breast or ovarian cancers among first or second degree relatives, including the proband. A haplotype analysis was performed in 1516 breast cancer cases (including 592 familial *BRCA1/2*-negative patients) and 1234 population controls that had been genotyped on the iCOGS chip (Michailidou et al. 2013). The population controls were healthy female blood donors from the same geographic region.

This study was performed with written informed consents from the patients and with permission from the Ethical review board of Helsinki University Hospital.

Sequencing

The protein coding regions of the *RAD51, XRCC3,* and *XRCC2* genes were amplified by PCR in genomic DNA samples isolated from peripheral blood of the patients. The primers were designed with Primer3 software (http://bioinfo.ut.ee/primer3/). The PCR conditions are described in Additional file 1: Table S1. The PCR fragments were purified with ExoSAP-IT (Affymetrix) and

subsequently sequenced using ABI BigDyeTerminator 3.1 Cycle Sequencing kit (Life Technologies). The capillary sequencing was performed at the Institute for Molecular Medicine Finland (FIMM), University of Helsinki, using 3730xl DNA Analyzer (Life Technologies). The sequence chromatograms were analyzed with FinchTV (Geospiza) and Variant Reporter software (Life Technologies).

Bioinformatics and statistical methods

The pathogenicity of identified missense variants was evaluated with MutationTaster (Schwarz et al. 2010), SIFT, and PON-P (Olatubosun et al. 2012). The haplotype analysis was performed using PHASE v2.1.1 software (Stephens et al. 2001; Stephens and Scheet 2005) and the frequencies of haplotypes were compared between all breast cancer cases versus controls and familial breast cancer cases versus controls. The haplotypes were constructed using all single-nucleotide polymorphisms (SNPs) included in the iCOGS chip (Michailidou et al. 2013) that were located at the *RAD51* ($n = 14$), *XRCC3* ($n = 10$), and *XRCC2* ($n = 10$) gene loci and were not monomorphic in our study population. To test the association of the individual polymorphisms included in the haplotype analysis with breast cancer risk, two-sided p-values with odds ratios (OR) and 95% confidence intervals (CI) for each SNP were calculated using χ^2 test or Fisher's exact test when the count in any of the cells was five or less. Bonferroni's adjustment was used for multiple-testing correction. We also studied the association of the missense mutations with 10-year breast cancer-specific survival using univariate Cox's proportional regression models. The follow-up times were left-truncated at the date of ascertainment to account for the latency between diagnosis and study recruitment. The association analyses were performed using the R version 3.0.2 statistical software (http://www.r-project.org/).

Results

In the sequencing of the *RAD51* gene, only intronic and untranslated region (UTR) variants were identified. In *XRCC3* and *XRCC2*, one known missense variant was identified in each gene (Table 1). Both missenses were predicted to be polymorphisms, tolerated, and neutral by MutationTaster, SIFT, and PON-P, respectively, and both were detected at comparable frequencies (31.3% for rs861539 in *XRCC3* and 4.7% for rs3218536 in *XRCC2*) as in the Finnish population of the 1000Genomes (31.7% for rs861539 and 4.8% for rs3218536) and of the Exome Aggregation Consortium (ExAC) (31.8% for rs861539 and 3.5% for rs3218536) (Exome Aggregation Consortium (ExAC), Cambridge, MA; http://exac.broadinstitute.org [January 2015]), and in the Sequencing Intiative Suomi (SISu) (30.1% for rs861539 and 3.9% for rs3218536)

(http://sisu.fimm.fi/ [January 2015]) (Lim et al. 2014) dataset.

The association of *RAD51, XRCC3,* and *XRCC2* haplotypes with breast cancer risk was studied among 1516 breast cancer cases (including 592 familial cases) and 1234 population controls. The haplotypes were constructed with PHASE v2.1.1 software using 14 polymorphic sites for *RAD51* and ten for *XRCC3* and *XRCC2*. Eleven *RAD51*, twelve *XRCC3*, and eight *XRCC2* haplotypes were predicted among the samples (Table 2). The distribution of the haplotypes did not differ between all the breast cancer cases and controls ($p = 0.45$, $p = 0.49$ and $p = 0.55$ for *RAD51, XRCC3,* and *XRCC2*, respectively) nor between the familial cases and controls ($p = 0.66$, $p = 0.14$ and $p = 0.80$ for *RAD51, XRCC3,* and *XRCC2*, respectively). We also tested the association of individual SNPs included in the haplotype analysis with breast cancer but none of them showed significant association ($p = 0.060-0.951$) (Table 3). After Bonferroni's correction for multiple testing, p-value < 0.00167 was considered significant.

Since the *XRCC2* p.(Arg188His) variant (rs3218536) has been previously associated with poor breast cancer survival (Lin et al. 2011), we performed 10-year breast cancer-specific survival analyses for the *XRCC2* p.(Arg188His) missense variant as well as the *XRCC3* p.(Thr241Met) (rs861539) variant that were both detected in the sequencing of the genes and also included in the haplotype analysis. Patients with available follow-up information from the sequencing dataset and from the haplotype analysis ($n = 1635$, events = 106 for *XRCC2*; $n = 1542$, events = 80 for *XRCC3*) were combined for the survival analysis, including 1183 or 1176 cases from the unselected series and 452 or 366 additional familial cases for the *XRCC2* and *XRCC3* analysis, respectively. Given that most of the familial patients were prevalent cases with more than six months between breast cancer diagnosis and recruitment to the study, the data was left-truncated at the date of ascertainment. Neither of the missenses associated with breast cancer survival (hazard ratio (HR) = 0.67, 95% CI = 0.32-1.40, $p = 0.288$ for rs3218536; HR = 0.92, 95% CI = 0.66-1.29, $p = 0.627$ for rs861539).

Discussion

We screened the *RAD51, XRCC3,* and *XRCC2* genes for germline variation in familial *BRCA1/2*-negative breast or ovarian cancer patients in order to evaluate the role of these genes in breast cancer predisposition in Finland and to identify putative recurrent founder mutations. To facilitate the variant discovery, we selected patients with strong family background of breast cancer from the homogeneous Finnish population where recurrent founder mutations in most of the breast cancer genes are present. In addition, a subset of the patients had decreased RAD51

Table 1 Identified germline variants in *RAD51*, *XRCC3*, and *XRCC2* genes

Gene	Genomic location[a]	HGVS[b]	Function	rs-number	AA[c]	Aa[d]	Aa[e]	MAF[f]	1000G-FIN MAF[g]
RAD51	15:40987528	c.-98G > C	5′UTR	rs1801320	154	27	1	0.080	0.113
RAD51	15:40987565	c.-61G > T	5′UTR	rs1801321	103	56	23	0.280	0.312
RAD51	15:40987568	c.-58C > G	5′UTR		181	1	0	0.003	
RAD51	15:40987725	c.-3 + 102C > T	intronic	rs3092981	151	22	9	0.110	0.183
RAD51	15:40991153	c.87 + 110A > G	intronic	rs2304579	153	28	1	0.082	0.113
RAD51	15:40998303	c.226-72delA	intronic	rs55943660	156	26	0	0.071	0.108
RAD51	15:40998342	c.226-33 T > G	intronic	rs45457497	136	43	3	0.135	0.129
RAD51	15:41001187	c.344-36 T > G	intronic	rs45455000	153	26	3	0.088	0.108
RAD51	15:41020898	c.531-12C > T	intronic		181	1	0	0.003	
XRCC3	14:104177282	c.55 + 88C > G	intronic		181	1	0	0.003	
XRCC3	14:104174944	c.108G > A	p.(=)		181	1	0	0.003	
XRCC3	14:104174824	c.193 + 34C > T	intronic	rs1799795	171	11	0	0.030	0.032
XRCC3	14:104173300	c.406 + 40C > T	intronic	rs374684710	177	5	0	0.014	
XRCC3	14:104169435	c.561 + 75G > A	intronic		181	1	0	0.003	
XRCC3	14:104165753	c.722C > T	p.(Thr241Met)	rs861539	91	68	23	0.313	0.317
XRCC3	14:104165647	c.774 + 54G > A	intronic	rs150986165	181	1	0	0.003	0.005
XRCC3	14:104165611	c.774 + 90G > T	intronic		181	1	0	0.003	
XRCC3	14:104165465	c.821 + 5G > A	intronic		181	1	0	0.003	
XRCC3	14:104165411	c.822-57C > T	intronic	rs17101777	181	1	0	0.003	
XRCC3	14:104165107	c.*28C > T	3'UTR		181	1	0	0.003	
XRCC3	14:104165100	c.*35A > G	3'UTR		181	1	0	0.003	
XRCC2	7:152373252	c.-88G > C	downstream	rs3218384	203	117	22	0.235	0.204
XRCC2	7:152373233	c.-69 T > G	5'UTR	rs3218385	324	16	2	0.029	0.032
XRCC2	7:152357877	c.40-10C > T	intronic	rs3218472	333	9	0	0.013	0.011
XRCC2	7:152346007	c.563G > A	p.(Arg188His)	rs3218536	310	32	0	0.047	0.048

[a]The genomic location is denoted according to NCBI37/Hg19 genome build and the variant coding refers to transcripts ENST00000267868 in *RAD51*, ENST00000352127 in *XRCC3*, and ENST00000359321 in *XRCC2*; [b]variant description according to HGVS nomenclature; number of [c]common homozygotes, [d]heterozygotes, and [e]rare homozygotes; [f]minor allele frequency (MAF) observed in this study; [g]MAF in 1000Genomes Finnish population.

protein expression on their breast tumors as we hypothesized that loss of protein expression might be a sign of underlying inactivating germline mutations. We also performed haplotype analyses in an extensive series of breast cancer cases and population controls to study the common variation in these genes.

No truncating mutations were identified in any of the genes. In *RAD51*, only intronic and UTR variants were identified which is in line with the previous studies where no cancer-predisposing mutations were identified among early-onset breast cancer patients (Lose et al. 2006; Rapakko et al. 2006; Le Calvez-Kelm et al. 2012). However, one of the detected 5'UTR polymorphisms, rs1801320, has been found to affect the splicing of *RAD51* within the 5'UTR and to modify breast cancer risk among *BRCA2* mutation carriers (Levy-Lahad et al. 2001; Antoniou et al. 2007). In *XRCC3* and *XRCC2*, known missense variants p.(Thr241Met) and p.(Arg188His),

respectively, were detected. Both of these variants were detected at comparable frequencies as in the Finnish population of the 1000Genomes, ExAC, and SISu datasets and neither was predicted to be pathogenic *in silico*. A large association study by Breast Cancer Association Consortium (BCAC) found no association with breast cancer risk for neither of the variants (Breast Cancer Association Consortium 2006). However, a meta-analysis by He *et al.*, including also the BCAC study, suggests the *XRCC3* p.(Thr241Met) variant is associated with a mild increase in breast cancer risk (OR = 1.10, 95% CI = 1.03-1.16) (He et al. 2012). In our data set, the p.(Thr241Met) and p.(Arg188His) variants did not associate with an increased breast cancer risk nor did they form risk-associated haplotypes. Furthermore, the overall distribution of *RAD51*, *XRCC3*, or *XRCC2* haplotypes did not differ between all breast cancer cases and controls or between familial cases and controls. Previously, *XRCC2* p.(Arg188His) has been

Table 2 Detected haplotypes with frequency estimates for population controls and breast cancer cases

RAD51 haplotype	Haplotype count	Frequency controls	Frequency all cases	Haplotype count	Frequency controls	Frequency fam cases
GCGCACTTAGAGAC	1510	26.66%	28.11%	999	26.66%	28.80%
GCGCATTTGGAGAC	1510	27.22%	27.63%	996	27.22%	27.36%
GCGTACTTAGGGAC	956	18.07%	16.81%	656	18.07%	17.74%
GTGCACTTAGAGAC	913	16.25%	16.89%	579	16.25%	15.03%
CCGCGCCGAAAGGG	431	8.51%	7.29%	301	8.51%	7.69%
GCTCATTTGGAGAC	138	2.76%	2.32%	97	2.76%	2.45%
GTGCACTTAGATAC	37	0.45%	0.86%	21	0.45%	0.84%
GCGCACTTAGGGAC	2	0.04%	0.03%	2	0.04%	0.08%
CCGCGCTTAGAGAC	1	0.04%	0%	1	0.04%	0%
GCGCACTTGGAGAC	1	0.001%	0.02%	0	0%	0%
CCGCGCCGAAAGAC	1	0%	0.03%	0	0%	0%
	All BC cases vs controls: *p* = 0.45			Familial BC cases vs controls: *p* = 0.66		
XRCC3 haplotype	Haplotype count	Frequency controls	Frequency all cases	Haplotype count	Frequency controls	Frequency fam cases
CATGCGCGGG	1599	28.57%	29.58%	1071	28.56%	31.07%
TACGCGCTGG	1586	28.32%	29.25%	1046	28.32%	29.30%
CGTACGCGGG	1159	22.33%	20.05%	778	22.33%	19.17%
CATGCGCGGA	602	10.82%	10.98%	378	10.83%	9.26%
CGTGCGTGGG	227	4.13%	4.03%	151	4.14%	3.98%
TACGCGCTAG	200	3.28%	3.92%	139	3.28%	4.90%
CGTGCGCGGG	75	1.49%	1.25%	55	1.49%	1.52%
CGTACACGGG	29	0.49%	0.56%	18	0.49%	0.51%
CGTGTGCGGG	17	0.41%	0.23%	12	0.41%	0.17%
CGTGCGTGGA	4	0.12%	0.11%	2	0.11%	0.03%
TGCGCGCTGG	1	0.05%	0.005%	1	0.05%	0.003%
CATGCGCTGG	1	0%	0.02%	1	0%	0.04%
	All BC cases vs controls: *p* = 0.49			Familial BC cases vs controls: *p* = 0.14		
XRCC2 haplotype	Haplotype count	Frequency controls	Frequency all cases	Haplotype count	Frequency controls	Frequency fam cases
GGGCGCACCT	3633	66.79%	65.48%	2438	66.80%	66.73%
GGGCGCACCG	1253	22.53%	22.97%	814	22.53%	21.79%
GGACGCACCT	247	4.13%	4.78%	159	4.13%	4.81%
GGGCCCATGT	123	2.15%	2.31%	84	2.15%	2.62%
AGGTGGACCT	123	2.25%	2.21%	83	2.25%	2.28%
GAGCGCGCGT	119	2.11%	2.21%	73	2.11%	1.77%
GGGCGCACGT	1	0.02%	0%	1	0.02%	0%
GAGCGCACCT	1	0%	0.02%	0	0%	0%
	All BC cases vs controls: *p* = 0.55			Familial BC cases vs controls: *p* = 0.80		

BC = breast cancer; Fam = familial.
Separate analyses were performed for all breast cancer cases versus controls and familial breast cancer cases versus controls. The SNPs included in the analysis are described in Table 3.

associated with poor breast cancer survival (Lin et al. 2011) but in our study, no survival effect was found for this variant or for the *XRCC3* p.(Thr241Met) variant.

Like most of the known breast cancer susceptibility genes, *RAD51, XRCC3,* and *XRCC2* also have a role in DNA double-strand break repair by homologous

Table 3 SNPs from the haplotype analysis with ORs and *p*-values for breast cancer association

Gene	rs-number	HGVS	MAF$_{controls}$	MAF$_{cases}$	OR	95% CI	*p*-value
RAD51	rs1801320	c.-98G > C	0.09	0.07	0.82	0.66-1.01	0.184
RAD51	rs3092981	c.-3 + 102C > T	0.17	0.18	1.07	0.91-1.27	0.614
RAD51	rs5030791	c.-3 + 203G > T	0.03	0.02	0.83	0.59-1.18	0.583
RAD51	rs2619681	c.-3 + 1398 T > C	0.18	0.17	0.88	0.75-1.05	0.352
RAD51	rs2304579	c.87 + 110A > G	0.09	0.07	0.82	0.67-1.01	0.184
RAD51	rs4924496	c.225 + 1936 T > C	0.29	0.29	1.06	0.90-1.24	0.549
RAD51	rs45503494	c.343 + 494 T > C	0.09	0.07	0.82	0.67-1.02	0.205
RAD51	rs45455000	c.344-36 T > G	0.09	0.07	0.82	0.67-1.02	0.202
RAD51	rs12592524	c.435 + 2149G > A	0.30	0.30	1.07	0.91-1.25	0.518
RAD51	rs4144242	c.436-4016G > A	0.09	0.07	0.82	0.67-1.02	0.202
RAD51	rs4924500	c.530 + 4654A > G	0.18	0.17	0.88	0.74-1.04	0.314
RAD51	rs45532539	c.531-3201G > T	0.004	0.009	1.92	0.97-4.10	0.062
RAD51	rs45507396	c.*929A > G	0.09	0.07	0.82	0.66-1.01	0.187
RAD51	rs45585734	c.*1113C > G	0.09	0.07	0.82	0.66-1.01	0.187
XRCC3	rs861539	c.722C > T	0.32	0.33	1.06	0.90-1.24	0.489
XRCC3	rs861537	c.562-1162G > A	0.29	0.26	0.89	0.76-1.04	0.060
XRCC3	rs861536	c.562-1651 T > C	0.32	0.33	1.06	0.90-1.24	0.475
XRCC3	rs12432907	c.561 + 1132G > A	0.23	0.21	0.92	0.78-1.08	0.092
XRCC3	rs3212092	c.561 + 866C > T	0.004	0.002	0.57	0.20-1.51	0.246
XRCC3	rs3212081	c.407-478G > A	0.005	0.006	1.15	0.55-2.49	0.704
XRCC3	rs3212079	c.407-801C > T	0.04	0.04	0.97	0.73-1.28	0.951
XRCC3	rs861531	c.406 + 533G > T	0.32	0.33	1.06	0.90-1.24	0.459
XRCC3	rs3212042	c.56-652G > A	0.03	0.04	1.17	0.86-1.58	0.456
XRCC3	rs3212028	c.-261 + 1368G > A	0.11	0.11	1.07	0.88-1.29	0.427
XRCC2	rs3218552	c.*1874G > A	0.02	0.02	0.95	0.66-1.37	0.879
XRCC2	rs3218550	c.*1772G > A	0.02	0.02	1.07	0.74-1.55	0.729
XRCC2	rs3218536	c.563G > A	0.04	0.05	1.08	0.82-1.43	0.256
XRCC2	rs3218504	c.122-4868C > T	0.02	0.02	0.94	0.65-1.36	0.878
XRCC2	rs6964582	c.122-5014G > C	0.02	0.02	1.02	0.70-1.47	0.676
XRCC2	rs3218501	c.122-5469C > G	0.02	0.02	0.93	0.64-1.34	0.839
XRCC2	rs3218491	c.121 + 4038A > G	0.02	0.02	1.04	0.72-1.52	0.817
XRCC2	rs3111465	c.40-4608 T > C	0.02	0.02	1.02	0.70-1.48	0.676
XRCC2	rs3094406	c.40-4998G > C	0.04	0.05	0.99	0.76-1.30	0.252
XRCC2	rs3218408	c.39 + 5510 T > G	0.23	0.23	1.09	0.93-1.28	0.447

The SNPs are presented in the same order as in the haplotypes in Table 2.

recombination. XRCC2 and XRCC3, two of the five human RAD51 paralogs, help to load RAD51 on the site of DNA damage (Suwaki et al. 2011). *XRCC2* gene has been recently linked to breast cancer since rare germline mutations in the gene were identified in breast cancer families (Park et al. 2012). However, no association with breast cancer risk was detected in a subsequent large case–control study (Hilbers et al. 2012) and another study by Golmard *et al.* (Golmard et al. 2013) reports no pathogenic *XRCC2* mutations among early-onset or familial breast cancer patients (Golmard et al. 2013). Interestingly, one Fanconi anemia patient has been found to carry a homozygous truncating *XRCC2* mutation (Shamseldin et al. 2012) while biallelic mutations in four breast and ovarian cancer susceptibility genes, *BRCA2*, *BRIP1*, *PALB2*, and *RAD51C*, are associated with Fanconi anemia (Kee and D'Andrea 2012). Given the unclear role of *XRCC2* in breast cancer susceptibility, we sequenced the gene in an extensive series of 342 patients with a strong family history breast cancer.

As the only identified coding variant was a neutral missense mutation, our results indicate that *XRCC2* is not a major breast cancer susceptibility gene, in line with the studies by Hilbers *et al*. and Golmard *et al*. In contrast to *XRCC2*, no truncating mutations in *XRCC3* or *RAD51* genes have been reported and only one possibly disease-associated missense in each gene has been detected in breast cancer patients (Golmard et al. 2013; Kato et al. 2000). Furthermore, the *RAD51* missense mutation was later also detected once among 1330 breasts cancer cases as well as once among 1123 controls (Le Calvez-Kelm et al. 2012). The absence of mutations in our study as well as the results of the previous studies indicates that *XRCC3* and *RAD51* are not major breast cancer susceptibility genes.

Conclusions

In conclusion, the absence of mutations among breast cancer families and similar distribution of haplotypes between breast cancer cases and controls suggests that *RAD51*, *XRCC3*, and *XRCC2* do not substantially contribute to familial breast cancer predisposition in the Finnish population. Taken together, it is unlikely that *RAD51*, *XRCC3*, and *XRCC2* have a significant contribution to breast cancer susceptibility. However, we cannot exclude possible unique or very rare risk variants.

Additional file

Additional file 1: Table S1. Primers and PCR conditions for the sequencing of the *RAD51*, *XRCC3*, and *XRCC2* genes.

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References

Antoniou AC, Sinilnikova OM, Simard J, Léoné M, Dumont M, Neuhausen SL, Struewing JP, Stoppa-Lyonnet D, Barjhoux L, Hughes DJ, Coupier I, Belotti M, Lasset C, Bonadona V, Bignon YJ, Rebbeck TR, Wagner T, Lynch HT, Domchek SM, Nathanson KL, Garber JE, Weitzel J, Narod SA, Tomlinson G, Olopade OI, Godwin A, Isaacs C, Jakubowska A, Lubinski J, Gronwald J et al (2007) RAD51 135G → C modifies breast cancer risk among BRCA2 mutation carriers: results from a combined analysis of 19 studies. Am J Hum Genet 81:1186–1200, doi:S0002-9297(07)63768-9

Bartkova J, Tommiska J, Oplustilova L, Aaltonen K, Tamminen A, Heikkinen T, Mistrik M, Aittomäki K, Blomqvist C, Heikkilä P, Lukas J, Nevanlinna H, Bartek J (2008) Aberrations of the MRE11-RAD50-NBS1 DNA damage sensor complex in human breast cancer: MRE11 as a candidate familial cancer-predisposing gene. Mol Oncol 2:296–316, doi:10.1016/j.molonc.2008.09.007

Breast Cancer Association Consortium (2006) Commonly studied single-nucleotide polymorphisms and breast cancer: results from the Breast Cancer Association Consortium. J Natl Cancer Inst 98:1382–1396, doi:98/19/1382

Eerola H, Blomqvist C, Pukkala E, Pyrhönen S, Nevanlinna H (2000) Familial breast cancer in southern Finland: how prevalent are breast cancer families and can we trust the family history reported by patients? Eur J Cancer 36:1143–1148

Erkko H, Xia B, Nikkilä J, Schleutker J, Syrjäkoski K, Mannermaa A, Kallioniemi A, Pylkäs K, Karppinen SM, Rapakko K, Miron A, Sheng Q, Li G, Mattila H, Bell DW, Haber DA, Grip M, Reiman M, Jukkola-Vuorinen A, Mustonen A, Kere J, Aaltonen LA, Kosma VM, Kataja V, Soini Y, Drapkin RI, Livingston DM, Winqvist R (2007) A recurrent mutation in PALB in Finnish cancer families. Nature 446:316–319, doi:10.1038/nature05609

Fagerholm R, Hofstetter B, Tommiska J, Aaltonen K, Vrtel R, Syrjäkoski K, Kallioniemi A, Kilpivaara O, Mannermaa A, Kosma VM, Uusitupa M, Eskelinen M, Kataja V, Aittomäki K, von Smitten K, Heikkilä P, Lukas J, Holli K, Bartkova J, Blomqvist C, Bartek J, Nevanlinna H (2008) NAD(P)H:quinone oxidoreductase 1 NQO1*2 genotype (P187S) is a strong prognostic and predictive factor in breast cancer. Nat Genet 40:844–853

Fagerholm R, Sprott K, Heikkinen T, Bartkova J, Heikkilä P, Aittomäki K, Bartek J, Weaver D, Blomqvist C, Nevanlinna H (2013) Overabundant FANCD2, alone and combined with NQO1, is a sensitive marker of adverse prognosis in breast cancer. Ann Oncol 24:2780–2785, doi:10.1093/annonc/mdt290

Figueroa JD, Garcia-Closas M, Humphreys M, Platte R, Hopper JL, Southey MC, Apicella C, Hammet F, Schmidt MK, Broeks A, Tollenaar RA, Van't Veer LJ, Fasching PA, Beckmann MW, Ekici AB, Strick R, Peto J, Dos Santos Silva I, Fletcher O, Johnson N, Sawyer E, Tomlinson I, Kerin M, Burwinkel B, Marme F, Schneeweiss A, Sohn C, Bojesen S, Flyger H, Nordestgaard BG et al (2011) Associations of common variants at 1p11.2 and 14q24.1 (RAD51L1) with breast cancer risk and heterogeneity by tumor subtype: findings from the Breast Cancer Association Consortium. Hum Mol Genet 20:4693–4706, doi:10.1093/hmg/ddr368

Golmard L, Caux-Moncoutier V, Davy G, Al Ageeli E, Poirot B, Tirapo C, Michaux D, Barbaroux C, D'Enghien CD, Nicolas A, Castéra L, Sastre-Garau X, Stern MH, Houdayer C, Stoppa-Lyonnet D (2013) Germline mutation in the RAD51B gene confers predisposition to breast cancer. BMC Cancer 13:484-2407-13-484, doi:10.1186/1471-2407-13-484

He XF, Wei W, Su J, Yang ZX, Liu Y, Zhang Y, Ding DP, Wang W (2012) Association between the XRCC3 polymorphisms and breast cancer risk: meta-analysis based on case–control studies. Mol Biol Rep 39:5125–5134, doi:10.1007/s11033-011-1308-y

Hilbers FS, Wijnen JT, Hoogerbrugge N, Oosterwijk JC, Collee MJ, Peterlongo P, Radice P, Manoukian S, Feroce I, Capra F, Couch FJ, Wang X, Guidugli L, Offit K, Shah S, Campbell IG, Thompson ER, James PA, Trainer AH, Gracia J, Benitez J, van Asperen CJ, Devilee P (2012) Rare variants in XRCC2 as breast cancer susceptibility alleles. J Med Genet 49:618–620, doi:10.1136/jmedgenet-2012-101191

Kato M, Yano K, Matsuo F, Saito H, Katagiri T, Kurumizaka H, Yoshimoto M, Kasumi F, Akiyama F, Sakamoto G, Nagawa H, Nakamura Y, Miki Y (2000) Identification of Rad51 alteration in patients with bilateral breast cancer. J Hum Genet 45:133–137, doi:10.1007/s100380050199

Kee Y, D'Andrea AD (2012) Molecular pathogenesis and clinical management of Fanconi anemia. J Clin Invest 122:3799–3806, doi:10.1172/JCI58321

Kiiski JI, Pelttari LM, Khan S, Freysteinsdottir ES, Reynisdottir I, Hart SN, Shimelis H, Vilske S, Kallioniemi A, Schleutker J, Leminen A, Bützow R, Blomqvist C, Barkardottir RB, Couch FJ, Aittomäki K, Nevanlinna H (2014) Exome sequencing identifies FANCM as a susceptibility gene for triple-negative breast cancer. Proc Natl Acad Sci U S A 111:15172–15177, doi:10.1073/pnas.1407909111

Kilpivaara O, Bartkova J, Eerola H, Syrjäkoski K, Vahteristo P, Lukas J, Blomqvist C, Holli K, Heikkilä P, Sauter G, Kallioniemi OP, Bartek J, Nevanlinna H (2005) Correlation of CHEK2 protein expression and c.1100delC mutation status with tumor characteristics among unselected breast cancer patients. Int J Cancer 113:575–580, doi:10.1002/ijc.20638

Le Calvez-Kelm F, Oliver J, Damiola F, Forey N, Robinot N, Durand G, Voegele C, Vallée MP, Byrnes G, Registry BC, Hopper JL, Southey MC, Andrulis IL, John EM, Tavtigian SV, Lesueur F (2012) RAD51 and breast cancer susceptibility: no evidence for rare variant association in the Breast Cancer Family Registry study. PLoS One 7:e52374, doi:10.1371/journal.pone.0052374

Levy-Lahad E, Lahad A, Eisenberg S, Dagan E, Paperna T, Kasinetz L, Catane R, Kaufman B, Beller U, Renbaum P, Gershoni-Baruch R (2001) A single nucleotide polymorphism in the RAD51 gene modifies cancer risk in BRCA2 but not BRCA1 carriers. Proc Natl Acad Sci U S A 98:3232–3236, doi:10.1073/pnas.051624098

Lim ET, Würtz P, Havulinna AS, Palta P, Tukiainen T, Rehnström K, Esko T, Mägi R, Inouye M, Lappalainen T, Chan Y, Salem RM, Lek M, Flannick J, Sim X, Manning A, Ladenvall C, Bumpstead S, Hämäläinen E, Aalto K, Maksimow M, Salmi M, Blankenberg S, Ardissino D, Shah S, Horne B, McPherson R, Hovingh GK, Reilly MP, Watkins H et al (2014) Distribution and medical impact of loss-of-function variants in the Finnish founder population. PLoS Genet 10:e1004494, doi:10.1371/journal.pgen.1004494

Lin WY, Camp NJ, Cannon-Albright LA, Allen-Brady K, Balasubramanian S, Reed MW, Hopper JL, Apicella C, Giles GG, Southey MC, Milne RL, Arias-Pérez JI, Menéndez-Rodríguez P, Benítez J, Grundmann M, Dubrowinskaja N, Park-Simon TW, Dörk T, Garcia-Closas M, Figueroa J, Sherman M, Lissowska J, Easton DF, Dunning AM, Rajaraman P, Sigurdson AJ, Doody MM, Linet MS, Pharoah PD, Schmidt MK et al (2011) A role for XRCC2 gene polymorphisms in breast cancer risk and survival. J Med Genet 48:477–484, doi:10.1136/jmedgenet-2011-100018

Lose F, Lovelock P, Chenevix-Trench G, Mann GJ, Pupo GM, Spurdle AB, Kathleen Cuningham Foundation Consortium for Research into Familial Breast Cancer (2006) Variation in the RAD51 gene and familial breast cancer. Breast Cancer Res 8:R26, doi:bcr1415

Loveday C, Turnbull C, Ramsay E, Hughes D, Ruark E, Frankum JR, Bowden G, Kalmyrzaev B, Warren-Perry M, Snape K, Adlard JW, Barwell J, Berg J, Brady AF, Brewer C, Brice G, Chapman C, Cook J, Davidson R, Donaldson A, Douglas F, Greenhalgh L, Henderson A, Izatt L, Kumar A, Lalloo F, Miedzybrodzka Z, Morrison PJ, Paterson J, Porteous M et al (2011) Germline mutations in RAD51D confer susceptibility to ovarian cancer. Nat Genet 43:879–882, doi:10.1038/ng.893

Loveday C, Turnbull C, Ruark E, Xicola RM, Ramsay E, Hughes D, Warren-Perry M, Snape K, Breast Cancer Susceptibility Collaboration (UK), Eccles D, Evans DG, Gore M, Renwick A, Seal S, Antoniou AC, Rahman N (2012) Germline RAD51C mutations confer susceptibility to ovarian cancer. Nat Genet 44:475–476, doi:10.1038/ng.2224

Mavaddat N, Antoniou AC, Easton DF, Garcia-Closas M (2010) Genetic susceptibility to breast cancer. Mol Oncol 4:174–191, doi:10.1016/j.molonc.2010.04.011

Michailidou K, Hall P, Gonzalez-Neira A, Ghoussaini M, Dennis J, Milne RL, Schmidt MK, Chang-Claude J, Bojesen SE, Bolla MK, Wang Q, Dicks E, Lee A, Turnbull C, Rahman N, Breast and Ovarian Cancer Susceptibility Collaboration, Fletcher O, Peto J, Gibson L, Dos Santos Silva I, Nevanlinna H, Muranen TA, Aittomäki K, Blomqvist C, Czene K, Irwanto A, Liu J, Waisfisz Q, Meijers-Heijboer H, Adank M et al (2013) Large-scale genotyping identifies 41 new loci associated with breast cancer risk. Nat Genet 45:353–361, doi:10.1038/ng.2563

Olatubosun A, Väliaho J, Härkönen J, Thusberg J, Vihinen M (2012) PON-P: integrated predictor for pathogenicity of missense variants. Hum Mutat 33:1166–1174, doi:10.1002/humu.22102

Orr N, Lemnrau A, Cooke R, Fletcher O, Tomczyk K, Jones M, Johnson N, Lord CJ, Mitsopoulos C, Zvelebil M, McDade SS, Buck G, Blancher C, KConFab C, Trainer AH, James PA, Bojesen SE, Bokmand S, Nevanlinna H, Mattson J, Friedman E, Laitman Y, Palli D, Masala G, Zanna I, Ottini L, Giannini G, Hollestelle A, Ouweland AM, Novaković S et al (2012) Genome-wide association study identifies a common variant in RAD51B associated with male breast cancer risk. Nat Genet 44:1182–1184, doi:10.1038/ng.2417

Park DJ, Lesueur F, Nguyen-Dumont T, Pertesi M, Odefrey F, Hammet F, Neuhausen SL, John EM, Andrulis IL, Terry MB, Daly M, Buys S, Le Calvez-Kelm F, Lonie A, Pope BJ, Tsimiklis H, Voegele C, Hilbers FM, Hoogerbrugge N, Barroso A, Osorio A, Breast Cancer Family R, Kathleen Cuningham Foundation Consortium for Research into Familial Breast Cancer, Giles GG, Devilee P, Benitez J, Hopper JL, Tavtigian SV, Goldgar DE, Southey

MC (2012) Rare mutations in XRCC2 increase the risk of breast cancer. Am J Hum Genet 90:734–739, doi:10.1016/j.ajhg.2012.02.027

Pelttari LM, Heikkinen T, Thompson D, Kallioniemi A, Schleutker J, Holli K, Blomqvist C, Aittomäki K, Bützow R, Nevanlinna H (2011) RAD51C is a susceptibility gene for ovarian cancer. Hum Mol Genet 20:3278–3288, doi:10.1093/hmg/ddr229

Pelttari LM, Kiiski J, Nurminen R, Kallioniemi A, Schleutker J, Gylfe A, Aaltonen LA, Leminen A, Heikkilä P, Blomqvist C, Bützow R, Aittomäki K, Nevanlinna H (2012) A Finnish founder mutation in RAD51D: analysis in breast, ovarian, prostate, and colorectal cancer. J Med Genet 49:429–432, doi:10.1136/jmedgenet-2012-100852

Rapakko K, Heikkinen K, Karppinen SM, Winqvist R (2006) Screening for RAD51 and BRCA2 BRC repeat mutations in breast and ovarian cancer families. Cancer Lett 236:142–147, doi:S0304-3835(05)00507-0

Sarantaus L, Huusko P, Eerola H, Launonen V, Vehmanen P, Rapakko K, Gillanders E, Syrjäkoski K, Kainu T, Vahteristo P, Krahe R, Pääkkönen K, Hartikainen J, Blomqvist C, Löppönen T, Holli K, Ryynänen M, Bützow R, Borg A, Wasteson Arver B, Holmberg E, Mannermaa A, Kere J, Kallioniemi OP, Winqvist R, Nevanlinna H (2000) Multiple founder effects and geographical clustering of BRCA1 and BRCA2 families in Finland. Eur J Hum Genet 8:757–763, doi:10.1038/sj.ejhg.5200529

Schwarz JM, Rödelsperger C, Schuelke M, Seelow D (2010) MutationTaster evaluates disease-causing potential of sequence alterations. Nat Methods 7:575–576, doi:10.1038/nmeth0810-575

Shamseldin HE, Elfaki M, Alkuraya FS (2012) Exome sequencing reveals a novel Fanconi group defined by XRCC2 mutation. J Med Genet 49:184–186, doi:10.1136/jmedgenet-2011-100585

Stephens M, Scheet P (2005) Accounting for decay of linkage disequilibrium in haplotype inference and missing-data imputation. Am J Hum Genet 76:449–462, doi:10.1086/428594

Stephens M, Smith NJ, Donnelly P (2001) A new statistical method for haplotype reconstruction from population data. Am J Hum Genet 68:978–989, doi:10.1086/319501

Suwaki N, Klare K, Tarsounas M (2011) RAD51 paralogs: roles in DNA damage signalling, recombinational repair and tumorigenesis. Semin Cell Dev Biol 22:898–905, doi:10.1016/j.semcdb.2011.07.019

Syrjäkoski K, Vahteristo P, Eerola H, Tamminen A, Kivinummi K, Sarantaus L, Holli K, Blomqvist C, Kallioniemi OP, Kainu T, Nevanlinna H (2000) Population-based study of BRCA1 and BRCA2 mutations in 1035 unselected Finnish breast cancer patients. J Natl Cancer Inst 92:1529–1531

Vahteristo P, Eerola H, Tamminen A, Blomqvist C, Nevanlinna H (2001) A probability model for predicting BRCA1 and BRCA2 mutations in breast and breast-ovarian cancer families. Br J Cancer 84:704–708, doi:10.1054/bjoc.2000.1626

Vahteristo P, Bartkova J, Eerola H, Syrjäkoski K, Ojala S, Kilpivaara O, Tamminen A, Kononen J, Aittomäki K, Heikkilä P, Holli K, Blomqvist C, Bartek J, Kallioniemi OP, Nevanlinna H (2002) A CHEK2 genetic variant contributing to a substantial fraction of familial breast cancer. Am J Hum Genet 71:432–438, doi:10.1086/341943

Vehmanen P, Friedman LS, Eerola H, McClure M, Ward B, Sarantaus L, Kainu T, Syrjäkoski K, Pyrhönen S, Kallioniemi OP, Muhonen T, Luce M, Frank TS, Nevanlinna H (1997) Low proportion of BRCA1 and BRCA2 mutations in Finnish breast cancer families: evidence for additional susceptibility genes. Hum Mol Genet 6:2309–2315

Vogelstein B, Kinzler KW (2004) Cancer genes and the pathways they control. Nat Med 10:789–799, doi:10.1038/nm1087