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 RAD51 and 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.
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) (Syrrjäkoski 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 BigDye Terminator 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 χ² 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 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 Initiative 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
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 1 Identified germline variants in RAD51, XRCC3, and XRCC2 genes

| Gene   | Genomic location | 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  | 15:40987656      | 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.
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
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.

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 > A | 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.562-1651T > 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.122-5469G > T | 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.40-4608C > T | 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 + 5510T > 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.
As the only identified coding variant was a neutral mis-sense 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 mis-sense in each gene has been detected in breast cancer patients (Golmard et al. 2013; Kato et al. 2000). Furthermore, the RAD51 mis-sense 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 familial 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.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
HN and LMP designed the study and drafted the manuscript. LMP, JIK, SR, HN and LMP designed the study and drafted the manuscript. LMP, JIK, SR, Mietrik M, Attottmaki 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

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