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# The genetic etiology of familial breast cancer: Assessing the role of rare genetic variation using next generation sequencing

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# Chapter 3

## Rare variants in *XRCC2* as breast cancer susceptibility alleles

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## Abstract

**Background** Recently, rare germline variants in *XRCC2* were detected in non-*BRCA1/2* familial breast cancer cases, and a significant association with breast cancer was reported. However, the breast cancer risk associated with these variants needs further evaluation.

**Methods** The coding regions and exon–intron boundaries of *XRCC2* were scanned for mutations in an international cohort of 3548 non-*BRCA1/2* familial breast cancer cases and 1435 healthy controls using various mutation scanning methods. Predictions on functional relevance of detected missense variants were obtained from three different prediction algorithms.

**Results** The only protein-truncating variant detected was found in a control. Rare non-protein-truncating variants were detected in 20 familial cases (0.6%) and nine healthy controls (0.6%). Although the number of variants predicted to be damaging or neutral differed between prediction algorithms, in all instances these categories were evenly represented among cases and controls.

**Conclusions** Our data do not confirm an association between *XRCC2* variants and breast cancer risk, although a relative risk smaller than two could not be excluded. Variants in *XRCC2* are unlikely to explain a substantial proportion of familial breast cancer.

Recently, Park and colleagues identified germline variants in x-ray repair cross complementing gene-2 (*XRCC2* (MIM 600375; NM_005431.1)) in a small number of breast cancer cases with a positive family history for the disease.[1] The overall difference in the prevalence of protein-truncating and potentially deleterious rare missense variants between cases and controls was reported to be statistically significant. XRCC2 is involved in the repair of double-strand breaks via homologous recombination. In addition, a homozygous protein-truncating variant in *XRCC2* has been detected in a Fanconi anaemia patient with consanguineous parents.[2] Since most known high and moderate-risk breast cancer genes have a function in DNA damage repair, and some are also Fanconi anaemia genes, it seems plausible that mutations in *XRCC2* represent breast cancer susceptibility alleles. In order to evaluate the association between *XRCC2* variants and breast cancer risk, we analysed the coding regions of *XRCC2* in a cohort of 3548 non-*BRCA1/2* familial breast cancer cases and 1435 healthy controls derived from various geographical locations. A more detailed description of the study population and mutations-scanning methods can be found in the online supplementary table S1.

We detected only one protein-truncating variant, a one-base-pair deletion, c.343T[8]>[7], present in a 41-year-old Italian control. Rare non-protein truncating variants in *XRCC2* were detected in 20 familial cases (0.6%) and nine healthy controls (0.6%). Polyphen2,[3] SIFT[4] and AlignGVGD[5] were used to predict the effect of detected missense variants on XRCC2 protein function. Although the number of variants predicted to be damaging or neutral differed between the prediction algorithms (table 1), in all instances these categories were evenly represented among cases and controls. The only common variant in the coding region of *XRCC2*, c.563G>A (rs3218536), was found to have equal minor allele frequencies in familial cases (0.085) and healthy controls (0.086).

Thus, our data do not confirm an association between *XRCC2* variants and breast cancer risk. It is possible that the study by Park et al represents a false-positive finding, or, alternatively, our data are a false-negative finding. The association reported by Park et al was based on six likely pathogenic variants in 1308 cases and zero in 1120 controls, which was significant in Fisher's exact test. However, it should be noted that of these six variants, four were considered possibly or probably damaging, based on in silico prediction. Moreover, the number of positives in cases and controls is extremely small, and the statistic is, therefore, likely to be very unstable. In the NHLBI ESP Exome Variant Server,[6] a publicly available database describing variants found in exomes of patients with heart, lung and blood disease and healthy controls, rare variants in the coding region of *XRCC2* are reported in 0.5% of all exomes, interestingly including two variants resulting in a gained stop codon. Whereas an allele frequency of 0.5% corresponds quite well with our findings, and with the frequency found by Park et al in their cases, it seems at odds with what they detect in controls. Even when including the innocuous missense change, Park et al found one rare *XRCC2* variant in 1120 controls (0.09%). This frequency might have been an underestimation given that the study employed High Resolution Melting Curve analyses for mutation detection rather than direct sequencing. Indeed, when specifically testing two truncating variants in another 1436 controls, they found one of them to be positive, indicating that the frequency of pathogenic variants among controls is greater than what is suggested by their finding of zero out 1120.

Our study had 80% power to detect a relative risk of at least 2.1 ($p<0.05$) for a variant with an allele frequency of 0.5% (see online supplementary figure S1). Our results could, therefore, represent a false-negative finding if the relative risk associated with *XRCC2* variants would be lower than two. We note that variants in a number of other DNA damage repair genes, such as *CHEK2* and *BRIP1*, have been associated with breast cancer with such low

risks.[7,8] Park et al did not provide a quantitative estimate of the risk, but in the two families with an *XRCC2* variant for which other family members were also available for DNA analysis, cosegregation of the variant with breast cancer was incomplete. This suggests that if an association between *XRCC2* and breast cancer exists at all, it may not be very strong. A much larger sample than that studied by us here would need to be analysed to address this.

Another potential source of controversy is the selection criteria used for constituting the case- and control-series. Park and colleagues analysed 1308 breast cancer cases diagnosed before age 45 years and 1120 healthy controls recruited through population-based sampling by the Australian Breast Cancer Family Registry. In addition, they scanned 689 index cases from multicase breast cancer families and 150 male breast cancer cases. The current study included mostly clinic-based cohorts of cases that were forwarded for *BRCA*-mutation analysis because the prior probability of detecting a *BRCA1* or *BRCA2* mutation exceeded 10%.[9] Hence, both studies attempted to enrich for 'genetic' familial breast cancer cases, but in slightly different ways, perhaps leading to different representations of certain case subgroups. Ethnic backgrounds of the cohorts may also differ proportionally between the studies, although European ancestry was represented in both, and variants were detected in comparable frequencies among individuals of Italian, Spanish, Dutch and US origin.

Neither group studied the effect of missense variants on protein function other than by in silico prediction algorithms. Whereas truncating variants are likely to cause reduction in activity of XRCC2 in homologous recombination, the effect of missense variants (if any) may be subtler. If and how loss of XRCC2 function translates into breast cancer risk will be difficult to assess, but the existence of an effect cannot be excluded at this moment. In mice, complete loss of Xrcc2 is embryonic lethal, and leads to increased genetic instability at the cellular level,[10,11] a hallmark of many breast cancers.

In summary, our data do not confirm an association between *XRCC2* variants and breast cancer risk, although a relative risk smaller than two could not be excluded. Our inability to reproduce the previously reported association might point at a more general obstacle in applying exome sequencing in order to find new genes involved in common complex diseases. Exome sequencing, typically, yields many rare candidate variants. Accordingly, the prior odds that any of these variants are truly associated with disease are small, even when such a variant has been detected in two independent exomes. In any case, our data suggest that variants in *XRCC2* are unlikely to explain a substantial proportion of familial breast cancer.

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**Table 1** Rare variants detected in the coding region of *XRCC2*

| Variant* | Protein alteration† | rs-number | Polyphen-2 | Align GVGD‡ | SIFT§ | Cases (%) n=3548 | Controls (%) n=1435 |
|---|---|---|---|---|---|---|---|
| c.140A>G | p.H47R | - | Poss. damaging | C0 | 0.01 | 1 (0.03) | 0 (0) |
| c.189A>G | p.A63A | - | Silent | - | 0.37 | 2 (0.06) | 0 (0) |
| c.223G>C | p.E75Q | - | Benign | C0 | 0.14 | 1 (0.03) | 0 (0) |
| c.283A>G | p.I95V | rs140214637 | Benign | C0 | 0.38 | 6 (0.17) | 2 (0.14) |
| c.343T[8]>[7] | p.L117fs | - | Truncating | - | - | 0 (0) | 1 (0.07) |
| c.353T>C | p.V118A | rs185815454 | Benign | C0 | 1.00 | 1 (0.03) | 0 (0) |
| c.359G>A | p.C120Y | - | Prob. Damaging | C65 | 0.00 | 0 (0) | 1 (0.07) |
| c.398T>C | p.L133P | - | Prob. Damaging | C65 | 0.07 | 0 (0) | 1 (0.07) |
| c.490G>C | p.E164Q | - | Prob. Damaging | C0 | 0.04 | 1 (0.03) | 0 (0) |
| c.509A>C | p.E170A | - | Poss. damaging | C65 | 0.00 | 1 (0.03) | 2 (0.14) |
| c.562C>T | p.R188C | rs139219364 | Prob. Damaging | C15 | 0.01 | 1 (0.03) | 0 (0) |
| c.581C>T | p.T194M | - | Prob. Damaging | C15 | 0.00 | 1 (0.03) | 0 (0) |
| c.595A>C | p.M199L | - | Benign | C0 | 0.16 | 0 (0) | 1 (0.07) |
| c.620A>G | p.E207G | rs61762969 | Benign | C0 | 0.39 | 1 (0.03) | 1 (0.07) |
| c.659A>T | p.D220V | - | Benign | C0 | 0.45 | 1 (0.03) | 0 (0) |
| c.714G>C | p.R238S | - | Prob. Damaging | C65 | 0.05 | 1 (0.03) | 0 (0) |
| c.742C>G | p.Q248E | rs190900560 | Benign | C0 | 0.46 | 1 (0.03) | 0 (0) |
| c.772C>T | p.R258C | - | Benign | C0 | 0.27 | 0 (0) | 1 (0.07) |
| c.808T>G | p.F270V | rs145085742 | Prob. Damaging | C45 | 0.00 | 1 (0.03) | 0 (0) |

*Based on XRCC2 transcript NM_005431.1
†Based on peptide sequence NP_005422.1
‡Depth: until Branchiostoma floridae (lancelet). C65 is the most likely deleterious category, while C0 is most likely neutral.
§Normalised probabilities smaller than 0.05 are predicted to be deleterious.

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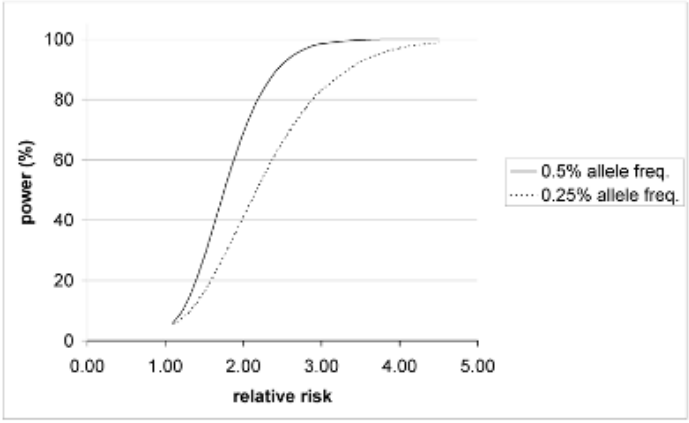
## Supplementary data




**Supplementary fig. 1 Power to detect a specific relative risk.**
For a study with 3548 cases and 1435 controls and α is 0.05.

**Supplementary table 1.** Description of the study populations and mutation scanning methods

| | Centre | Country | n | Mutation scanning method | Description |
|---|---|---|---|---|---|
| Cases | LUMC | The Netherlands | 847 | Sanger sequencing | Probands from the clinical genetics centre of Leiden, Rotterdam, Nijmegen and Groningen, who tested negative for BRCA1/2 mutations, either unselected but with an a priori change of a BRCA1 or BRCA2 mutation of ≥ 10% (n=472) or at least 2 cases with age of onset <50 (n=375) |
| | MSKCC | USA | 739 | Sanger sequencing | Probands from families with 3 or more breast cancer cases, no age restrictions, negative for mutations in BRCA1/2 |
| | Peter MacCallum Cancer Centre | Australia | 590 | HRMCA[1,3] | Affected probands from the familial cancer clinic, who tested negative for BRCA1/2 mutations from multigenerational affected families with an a priori chance of a BRCA1 or BRCA2 mutation of ≥ 10%. |
| | CNIO | Spain | 468 | DHPLC[2,3] | Patients from families with at least 2 breast cancer cases (at least one of the <50 years) and tested negative for BRCA1/2 mutations |
| | MBCSG | Italy | 463 | Sanger sequencing | Probands ascertaind at the Istituto Nazionale Tumori (INT) and Istituto Europeo di Oncologia (IEO), Milan, who tested negative for BRCA1/2 mutations and with early onset of disease (<36 years) or from families with at least 2 breast cancer cases diagnosed before age 50 or 1 breast cancer case and 1 ovrian cancer case, . |
| | Mayo Clinic | USA | 441 | Sanger sequencing | Cases from BRCA1/2 negative multi-case breast cancer families, from some families more than 1 case was screened for XRCC2 variants |
| Controls | MBCSG | Italy | 444 | Sanger sequencing | Age-matched female blood donors recruited through the Immunohematology and Transfusion Medicine Service of INT and Associazione Volontari Italiani Sangue (AVIS) of Milan |
| | CNIO | Spain | 392 | DHPLC[2,3] | Healthy controls from the Spanish College of Lawyers |
| | LUMC | The Netherlands | 367 | Sanger sequencing | Healthy controls from the Dutch blood bank |
| | Peter MacCallum Cancer Centre | Australia | 232 | HRMCA[1,3] | Healthy Caucasian female volunteers from southern England |

All primer sequences are available upon request.
[1] HRMCA = High resolution melting curve analysis
[2] DHPLC = Denaturating high performance liquid chromatography
[3] Detected variants were validated by Sanger sequencing