DNA Glycosylases Involved in Base Excision Repair May Be Associated with Cancer Risk in BRCA1 and BRCA2 Mutation Carriers

Ana Osorio1,2, Roger L. Milne3, Karoline Kuchenbuecker4, Tereza Vaclová1, Guillermo Pita5, Rosario Alonso6, Paolo Peterlongo6, Ignacio Blanco7, Miguel de la Hoya8, Mercedes Duran9, Orland Diez10, Teresa Ramón y Cajal11, Irene Konstantopoulou12, Cristina Martínez-Bouzas13, Raquel Andrés Conejero14, Penny Soucy15, Lesley McGuffog6, Daniel Barrowdale4, Andrew Lee4, SWEBRCA16, Brita Arve17, Johanna Rantala18, Niklas Loman19, Hans Ehrencreun20, Olufunmilayo I. Olopade21, Mary S. Beattie22, Susan M. Domchek23, Katherine Nathanson23, Timothy R. Rebbeck24, Banu K. Arun25, Beth Y. Karlan26, Christine Walsh26, Jenny Lester26, Esther M. John27, Alice S. Whittemore28, Mary B. Daly29, Melissa Southey30, John Hopper31, Mary B. Terry32, Saundra S. Buys33, Ramunas Janavicius34, Cecilia M. Dorfling35, Elizabeth J. van Rensburg36, Linda Steele36, Susan L. Neuhausen36, Yuan Chun Ding36, Thomas v. O. Hansen37, Lars Jønson37, Bent Ejlertsen38, Anne-Marie Gerdes39, Mar Infante9, Belén Herráez2, Leticia Thais Moreno5, Jeffrey N. Weitzel40, Josef Herzog40, Kisa Weeman40, Siranoush Manoukian41, Bernard Peissel41, Daniella Zaffaroni41, Giulietta Scuvera41, Bernardo Bonanni42, Frédérique Mariette43, Sara Volorio43, Alessandra Viel44, Liliana Varesco45, Laura Papi46, Laura Ottini47, Maria Grazia Tibiletti48, Paolo Radice49, Drakoulis Yannakakos12, Judy Garber50, Steve Ellis4, Debra Frost4, Radka Platte4, Elena Fineberg4, Gareth Evans61, Fiona Laloo51, Louise Izatt52, Ros Eeles53, Julian Adlard54, Rosemarie Davidson55, Trevor Cole56, Diana Eccles57, Jackie Cook58, Shirley Hodgson59, Carole Brewer60, Marc Tischkowitz61, Fiona Douglas62, Mary Porteous63, Lucy Side64, Lisa Walker65, Patrick Morrison66, Alan Donaldson67, John Kennedy68, Claire Foo69, Andrew K. Godwin70, Rita Katharina Schmutzler71, Barbara Wappenschmidt71, Kerstin Rhiem71, Christoph Engel72, Alfons Meindl73, Nina Ditsch73, Norbert Arnold74, Hans Jörg Plendl75, Dieter Niederacher76, Christian Sutter77, Shan Wang-Gohrke78, Doris Steinemann79, Sabine Preisler-Adams80, Karin Kast81, Raymonda Varon-Mateeva82, Andrea Gehrig83, Dominique Stoppa-Lyonnet84,85, Olga M. Sinilnikova87,88, Sylvie Mazoyer88, Francesca Damiola88, Bruce Poppe89, Kathleen Claes89, Marian Piedmonte90, Kathy Tucker91, Floor Backes92, Gustavo Rodríguez93, Wendy Brewster94, Katie Wakeley95, Thomas Rutherford96, Trinidad Caldés, Heli Nevanlinna97, Kristiina Aittomäki97, Matti A. Rookus98, Theo A. M. van Os99, Lizet van der Kolk100, J. L. de Lange101, Hanne E. J. Meijers-Heijboer102, A. H. van der Hout103, Christi J. van Asperen104, Encarna B. Gómez García105, Nicoline Hoogerbrugge106, J. Margriet Collée107, Carolien H. M. van Deurzen108, Rob B. van der Luijt109, Peter Devilee110, HEBON111, Edith Olah112, Conxi Lázaro113, Alex Teule7, Mireia Menéndez113, Anna Jakubowska114, Cezary Cybulski114, Jacek Gronwald114, Jan Lubinski114, Katarzyna Durda114, Katarzyna Jaworska-Bieniek114,115, Oskar Th. Johannsson116, Christine Maugard117, Marco Montagna118, Silvia Tognazz118, Manuel R. Teixeira119, Sue Healey120, kConFab Investigators121, Curtis Olswold122, Luca Guidugli123, Noralane Lindor124, Susan Slager125, Csilla I. Szabo126, Joseph Vijai127,128, Mark Robson127, Noah Kauff127, Liying Zhang129, Rohini Rau-Murthy127, Anneliese Fink-Retter130, Christian F. Singer130, Christine Rappaport130, Daphne Geschwintler Kaulich130, Georg Pfeifer130, Muy-Kheng Tea130, Andreas Berger130, Catherine M. Phelan131, Mark H. Greene132, Phuong L. Mai132, Flavio Lejbkowicz133, Irene Andrusli134, Anna Marie Mulligan135, Gord Glendon136, Amanda Ewart Toland137, Anders Bojesen138, Inge Sokilde Pedersen139, Lone Sunde140, Mads Thomassen141, Torben A. Kruse141, Uffe Birk Jensen140, Eitan Friedman142, Yael Laitman142, Shani Paluch-Shimon142, Jacques Simard143, Douglas F. Easton4, Kenneth Offit127,128, Fergus J. Couch122,123, Georgia Chenevix-Trench120, Antonis C. Antoniou4, Javier Benitez12,5
Abstract

Single Nucleotide Polymorphisms (SNPs) in genes involved in the DNA Base Excision Repair (BER) pathway could be associated with cancer risk in carriers of mutations in the high-penetration susceptibility genes BRCA1 and BRCA2, given the relation of synthetic lethality that exists between one of the components of the BER pathway, PARP1 (poly ADP ribose polymerase), and both BRCA1 and BRCA2. In the present study, we have performed a comprehensive analysis of 18 genes involved in BER using a tagging SNP approach in a large series of BRCA1 and BRCA2 mutation carriers. 144 SNPs were analyzed in a two stage study involving 23,463 carriers from the CIMBA consortium (the Consortium of Investigators of Modifiers of BRCA1 and BRCA2). Eleven SNPs showed evidence of association with breast and/or ovarian cancer at p<0.05 in the combined analysis. Four of the five genes for which strongest evidence of association was observed were DNA glycosylases. The strongest evidence was for rs1466785 in the NEIL2 (endonuclease VIII-like 2) gene (HR: 1.09, 95% CI (1.03–1.16), p = 2.7×10^{-3}) for association with breast cancer risk in BRCA2 mutation carriers, and rs2304277 in the OGG1 (8-guanine DNA glycosylase) gene, with ovarian cancer risk in BRCA1 mutation carriers (HR: 1.12 95%CI: 1.03–1.21, p = 4.8×10^{-3}). DNA glycosylases involved in the first steps of the BER pathway may be associated with cancer risk in BRCA1/2 mutation carriers and should be more comprehensively studied.

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Introduction

Carrying an inherited mutation in the \(\text{BRCA1}\) or \(\text{BRCA2}\) gene increases a woman’s lifetime risk of developing breast, ovarian, and other cancers. The estimated cumulative risk of developing breast cancer by the age of 70 in \(\text{BRCA1}\) and \(\text{BRCA2}\) mutation carriers varies between 43% to 86%; similarly, between 11% to 59% of mutation carriers will develop ovarian cancer by the age of 70 [1–3]. These considerable differences in disease manifestation suggest the existence of other genetic or environmental factors that modify the risk of cancer development. The Consortium of Investigators of Modifiers of \(\text{BRCA1}\) and \(\text{BRCA2}\) (CIMBA), was established in 2006 [4] and with more than 40,000 mutation carriers currently provides the largest sample size for reliable evaluation of even modest associations between single-nucleotide polymorphisms (SNPs) and cancer risk. CIMBA studies have so far demonstrated that more than 25 SNPs are associated with the risk of developing breast or ovarian cancer for \(\text{BRCA1}\) or \(\text{BRCA2}\) carriers. These were identified through genome-wide association studies (GWAS) of breast or ovarian cancer in the general population or through \(\text{BRCA1}\) and \(\text{BRCA2}\)-specific GWAS [5–8]. Cells harboring mutations in \(\text{BRCA1}\) or \(\text{BRCA2}\) show impaired homologous recombination (HR) [9–11] and are thus critically dependent on other pathways of DNA repair machinery such as poly ADP ribosepolymerase (PARP1) involved in the Base Excision Repair (BER) pathway. The BER pathway is crucial for the replacement of aberrant bases generated by different causes [12]. A deficiency in BER can give rise to a further accumulation of double-strand DNA breaks which, in the presence of a defective \(\text{BRCA1}\) or \(\text{BRCA2}\) background, could persist and lead to cell cycle arrest or cell death; this makes \(\text{BRCA}\)-deficient cells extremely sensitive to PARP inhibitors, as previously demonstrated [13]. We hypothesize that SNPs in PARP1 and other members of BER may be associated with cancer risk in \(\text{BRCA1}\) and \(\text{BRCA2}\) mutation carriers. SNPs in \(\text{XRCC1}\), one of the main components of BER, have been recently evaluated within the CIMBA consortium [14], however a comprehensive study has not yet been performed of either \(\text{XRCC1}\) or the other genes participating in BER.

In the present study, we used a tagging SNP approach to evaluate whether the common genetic variation in the genes involved in the BER pathway could be associated with cancer risk in a large series of \(\text{BRCA1}/2\) mutation carriers using a two-stage approach. The first stage involved an analysis of 144 tag SNPs in 1,787 Spanish and Italian \(\text{BRCA1}/2\) mutation carriers. In stage II, the 36 SNPs showing the strongest evidence of association in stage I, were evaluated in a further 23,463 CIMBA mutation carriers included in the Collaborative Oncological Gene-environment Study (COGS) and genotyped using the iCOGS custom genotyping array.

Results

Breast cancer association

In stage I, 144 selected Tag SNPs covering the 18 selected BER genes were genotyped in 968 \(\text{BRCA1}\) and 819 \(\text{BRCA2}\) mutation carriers from five CIMBA centres (Spanish National Cancer ResearchCentre (CNIO), Hospital Clínic San Carlos (HCSG), Catalan Institute of Oncology (ICO), Demokritos and Milan Breast Cancer Study Group (MBCSG)). Of those, 50 were excluded because of low call-rates, minor allele frequency (MAF)<0.05, evidence of deviation from Hardy Weinberg Equilibrium (p-value<10^-3) or monomorphism. Associations with
breast cancer risk were assessed for 94 SNPs, as summarized in Table S1. The 36 SNPs that showed evidence of association at \( p \leq 0.05 \) were selected for analysis in stage II. Of the 36 SNPs successfully genotyped in the whole CIMBA series comprising 15,252 \textit{BRCA1} and 8211 \textit{BRCA2} mutation carriers, consistent evidence of association with breast cancer risk \( (p\text{-trend}<0.05) \) was observed for six SNPs (Table 1). The strongest evidence of association was observed for rs1466785 in the \textit{NEIL2} gene \( \text{(HR: 1.09, 95% CI (1.03–1.16), } p = 2.7 \times 10^{-5}) \). The SNP was primarily associated with ER-negative breast cancer \( \text{(HR: 1.20, 95% CI (1.06–1.37), } p = 4 \times 10^{-5}) \), although the difference in HRs for ER-positive and ER-negative disease was not statistically significant. The evidence of association in Stage II was somewhat stronger when considering the genotype-specific models, with the dominant being the best fitting \( \text{(HR: 1.20 95% CI: 1.09–1.37, } p = 1 \times 10^{-4}) \). The associations remained significant and the estimated effect sizes remained consistent with the overall analysis when the data were reanalyzed excluding samples used in stage I of the study (data not shown). Imputation using the 1000 genomes data showed that there were several SNPs in strong linkage disequilibrium (LD) with rs1466785 showing more significant associations \( (p<10^{-5}) \) (Figure 1).

Ovarian cancer association

Due to lack of power we did not perform analysis of associations with ovarian cancer in stage I. However, we performed this analysis for the 36 SNPs tested in stage II. Although they had been selected based on their evidence of association with breast cancer risk, under the initial hypothesis they are also plausible modifiers of ovarian cancer risk for \textit{BRCA1} and \textit{BRCA2} mutation carriers. We found four SNPs associated with ovarian cancer risk with a \( p\text{-trend}<0.01 \) in \textit{BRCA1} or \textit{BRCA2} mutation carriers (Table 1). The strongest association was found for rs2304277 in \textit{OGG1} in \textit{BRCA1} mutation carriers \( \text{(HR: 1.12, 95% CI: 1.03–1.21, } p = 4.8 \times 10^{-5}) \), and 82 other SNPs were found to be associated with ovarian cancer risk in \textit{BRCA2} mutation carriers \( (p\text{-trend}<10^{-5}) \), these results were based on a relatively small number of ovarian cancer cases. Imputed data did not show any SNPs with substantially more significant associations with ovarian cancer risk except for rs3093926 in \textit{PARP2}; associated with ovarian cancer risk in \textit{BRCA2} mutation carriers for which there was a SNP, rs61995542, with a stronger association \( \text{(HR: 0.67, } p = 4.6 \times 10^{-5}) \) (Figure S1).

Discussion

Based on the interaction of synthetic lethality that has been described between \textit{PARP1} and both \textit{BRCA1} and \textit{BRCA2}, we hypothesize that this and other genes involved in the BER pathway could potentially be associated with cancer risk in \textit{BRCA1} or \textit{BRCA2} mutation carriers. Several studies have recently investigated the association of some of the BER genes with breast cancer; however, no definitive conclusions can be drawn, given that some publications suggest that SNPs in these genes can be associated with breast cancer risk with marginal p-values while others rule out a major role of these genes in the disease [15–21]. There is only one study from the CIMBA consortium which has evaluated the role of three of the most studied SNPs in the \textit{XRCC1} gene, -77C>T \( \text{(rs3213245 Arg280His (rs25489) and } p\text{.Gln399Arg (rs25487), ruling out associations of these variants with cancer risk in } \text{BRCA1} \text{ and } \text{BRCA2} \text{ mutation carriers [14]. However, a comprehensive analysis of neither } \text{XRCC1} \text{ nor the other genes involved in the pathway in the context of } \text{BRCA} \text{ mutation carriers has been performed. In the present study we have assessed the common genetic variation of 18 genes participating in } \text{BRCA} \text{ by using a two stage strategy.}

Eleven SNPs showed evidence of association with breast and/or ovarian cancer at \( p<0.05 \) in stage II of the experiment (Table 1). Of those, six showed a \( p\text{-trend}<0.01 \) and were therefore considered the best candidates for further evaluation. Only one of those six, rs1466785 in the \textit{NEIL2} gene \( \text{(HR: 1.09, 95% CI: 1.03–1.16, } p = 2.7 \times 10^{-5}) \) showed an association with breast cancer risk while the other five, rs2304277 in \textit{OGG1} \( \text{(8-guanine DNA glycosylase), rs167715 and } rs4135087 \text{ in } \text{TDG} \text{ (thymine-DNA glycosylase), rs3093926 in } \text{PARP2 (Poly(ADP-ribose) polymerase 2) and } rs43259 \text{ in } \text{UNG} \text{ (uracil-DNA glycosylase) were associated with ovarian cancer risk.}

The minor allele of \textit{NEIL2}-rs1466785 was associated with increased breast cancer risk in \textit{BRCA2} mutation carriers; moreover, when considering the genotype-specific risks observed that the best fitting model was the dominant one. \textit{NEIL2} is one of the oxidized base-specific DNA glycosylases that participate in the initial steps of BER and specifically removes oxidized bases from transcribing genes [22]. By imputing using the 1000 genome data we found six correlated SNPs in strong LD with rs1466785 \( (r^2>0.8) \), located closer or inside the gene and showing slightly stronger and more significant associations with the disease and therefore being better candidate causal variants. From those, we considered rs804276 and rs804271 as the best candidates given that they showed the most significant associations \( (p = 6 \times 10^{-4} \text{ and } p = 8 \times 10^{-4} \text{ respectively}) \) and there were available epidemiological or functional data supporting their putative role in cancer. SNP rs804276 has been associated with disease recurrence in patients with bladder cancer treated with Bacillus Calmette-Guérin (BCG) \( \text{(HR: 2.71, 95% CI (1.75–4.20), } p = 9 \times 10^{-5}) \) [23]. SNP rs804271 is located in a positive regulatory region in the promoter of the gene, between two potential cis-binding sites for reactive oxygen species responsive transcription factors in which sequence variation has
### Table 1. Associations with breast and ovarian cancer risk for SNPs observed at p-trend < 0.05 in stage II of the experiment.

| SNP name  | Gene     | Unaffected (Number) | Affected (Number) | p-trend | p-het | p-hom |
|-----------|----------|---------------------|-------------------|---------|-------|-------|
| rs3847954 | UNG      | 7455                | 7797              | 0.04    | 0.011 | 0.713 |
| rs2072668 | OGG1     | 12786               | 2461              | 0.016   | 3 x 10^-3 | 0.77 |
| rs2304277 | OGG1     | 12783               | 2462              | 0.013   | 0.014 | 0.268 |
| rs10161263| SMUG1    | 12790               | 2462              | 0.024   | 9 x 10^-3 | 0.49 |
| rs2072668 | OGG1     | 3879                | 4328              | 0.018   | 0.098 | 7 x 10^-3 |
| rs3136811 | POLB     | 3873                | 4321              | 0.035   | 0.083 | 0.054 |
| rs2304277 | OGG1     | 3880                | 4330              | 0.032   | 0.019 | 0.715 |
| rs1466785 | NEIL2    | 3879                | 4330              | 0.013   | 0.058 | 0.01  |
| rs167715  | TDG      | 7577                | 631               | 7.4 x 10^-3 | 4.1 x 10^-3 | 0.866 |
| rs3093926 | PARP2    | 7580                | 631               | 1.5 x 10^-3 |     |      |
| rs4135087 | TDG      | 7580                | 631               | 2.8 x 10^-3 | 3.8 x 10^-3 | 0.185 |
| rs34259   | UNG      | 7580                | 631               | 7.6 x 10^-3 | 0.25 | 0.028 |

**BRCA1 carriers**

| SNP name  | Gene     | Unaffected (Number) | Affected (Number) | HR per allele | HR heterozygote | HR homozygote |
|-----------|----------|---------------------|-------------------|--------------|----------------|--------------|
| rs3847954 | UNG      | 7455                | 7797              | 1.05 (1.00-1.11) | 1.09 (1.02-1.16) | 0.99 (0.84-1.16) |
| rs2072668 | OGG1     | 12786               | 2461              | 1.09 (1.01-1.18) | 1.16 (1.05-1.27) | 1.03 (0.82-1.28) |
| rs2304277 | OGG1     | 12783               | 2462              | 1.12 (1.03-1.21) | 1.19 (1.08-1.3) | 1.01 (0.79-1.30) |
| rs10161263| SMUG1    | 12790               | 2462              | 0.92 (0.86-0.99) | 0.88 (0.80-0.97) | 0.90 (0.78-1.04) |

**BRCA2 carriers**

| SNP name  | Gene     | Unaffected (Number) | Affected (Number) | HR per allele | HR heterozygote | HR homozygote |
|-----------|----------|---------------------|-------------------|--------------|----------------|--------------|
| rs2269112 | OGG1     | 3880                | 4329              | 0.91 (0.85-0.98) | 0.95 (0.87-1.04) | 0.75 (0.62-0.91) |
| rs3136811 | POLB     | 3873                | 4321              | 1.12 (1.05-1.25) | 1.17 (1.03-1.32) | 0.86 (0.49-1.48) |
| rs2304277 | OGG1     | 3880                | 4330              | 0.91 (0.84-0.97) | 0.94 (0.85-1.03) | 0.74 (0.60-0.91) |
| rs1466785 | NEIL2    | 3879                | 4330              | 1.09 (1.03-1.16) | 1.20 (1.09-1.37) | 1.16 (1.03-1.31) |
| rs4135087 | TDG      | 7577                | 631               | 0.76 (0.62-0.94) | 0.72 (0.58-0.90) | 0.89 (0.41-1.89) |
| rs3093926 | PARP2    | 7580                | 631               | 0.64 (0.49-0.84) | 1.5 x 10^-3 |     |
| rs34259   | UNG      | 7580                | 631               | 0.80 (0.69-0.94) | 0.84 (0.70-1.01) | 0.51 (0.29-0.90) |

**Ovarian cancer**

| SNP name  | Gene     | Unaffected (Number) | Affected (Number) | HR per allele | HR heterozygote | HR homozygote |
|-----------|----------|---------------------|-------------------|--------------|----------------|--------------|
| rs2269112 | OGG1     | 12789               | 2461              | 1.11 (1.02-1.21) | 1.11 (1.01-1.23) | 1.21 (0.92-1.58) |
| rs2269112 | OGG1     | 12783               | 2462              | 0.21         | 1.12 (1.03-1.21) | 1.19 (1.08-1.3) |
| rs2269112 | OGG1     | 3880                | 4330              | 0.93 (0.85-0.99) | 0.76 (0.58-0.99) | 0.035 |
| rs2269112 | OGG1     | 3880                | 4330              | 0.91 (0.84-0.97) | 0.94 (0.85-1.03) | 0.74 (0.60-0.91) |
| rs1466785 | NEIL2    | 3879                | 4330              | 0.43         | 1.09 (1.03-1.16) | 1.20 (1.09-1.37) |
| rs1466785 | NEIL2    | 3879                | 4330              | 0.43         | 1.09 (1.03-1.16) | 1.20 (1.09-1.37) |

**Hazard Ratio per allele (1 df) estimated from the retrospective likelihood analysis.**

**Hazard Ratio under the genotype specific models (2 df) estimated from the retrospective likelihood analysis.**

**p-values were based on the score test.**

**HR per allele of 1.69 and p-trend of 1 x 10^-4 for BRCA2 mutation carriers in stage I of the study.**

**HR per allele of 1.43 and p-trend of 0.01 for BRCA1 mutation carriers in stage I of the study.**

**HR per allele of 1.30 and p-trend of 0.03 for BRCA2 mutation carriers in stage I of the study.**

**HR per allele of 0.64 and p-trend of 0.057 for BRCA2 mutation carriers in stage I of the study.**

**HR per allele of 1.25 and p-trend of 0.04 for BRCA1 mutation carriers in stage I of the study.**

**HR per allele of 1.25 and p-trend of 0.058 for BRCA2 mutation carriers in stage I of the study.**

**rs3093926 did not yield results under the genotype specific model due to the low minor allele frequency.**

**Highlighted in bold are those SNPs showing strongest associations with breast or ovarian cancer risk (p < 0.05).**

**Highlighted are those SNPs showing strongest associations with breast or ovarian cancer risk (p < 0.01).**
been proven to alter the transcriptional response to oxidative stress [24]. Moreover, this SNP has been proposed to partly explain the inter-individual variability observed in NEIL2 expression levels in the general population and has been proposed as a potential risk modifier of disease susceptibility [25].

Several studies have been published showing associations between SNPs in NEIL2 and lung or oropharyngeal cancer risk [26,27] but to our knowledge, no association with breast cancer risk has been reported. We hypothesize that the potential association observed in the present study could be explained by the interaction between NEIL2 and BRCA2, each of them causing a deficiency in the BER and HR DNA repair pathways, respectively. This would explain why the breast cancer risk modification due to rs1466785 would only be detected in the context of BRCA2 mutation carriers and not in the general population.

The strongest evidence of association found in BRCA1 carriers was between rs2304277 in the OGG1 gene and ovarian cancer risk. The association was more significant when considering the dominant model. OGG1 removes 8-oxodeoxyguanosine which is generated by oxidative stress and is highly mutagenic, and it has been suggested that SNPs in the gene could be associated with cancer risk [28–31]. This is an interesting result, given that to date only one SNP, rs4691139 in the 4q35.3 region, also identified through the iCOGS effort, did not show better results for a more plausible causal SNP.

We have identified four SNPs associated with ovarian cancer risk in BRCA2 mutation carriers, rs167715 and rs4135087 in the TDG gene, rs34259 in the UNG gene and rs3093926 in PARP2. However, these last results should be interpreted with caution given that the number of BRCA2 carriers affected with ovarian cancer is four-fold lower than for BRCA1 carriers and the statistical power was therefore more limited, increasing the possibility of false-positives. In the case of PARP2, imputed data showed a lower p-value of association ($4 \times 10^{-5}$) for another SNP, rs61995542, that had a slightly higher MAF than rs3093926 (0.074 vs. 0.067) (Figure S1). However, it must still be interpreted with caution due to small number of ovarian cancer cases in the BRCA2 group.

It is worth noting that, four of the five genes for which strongest evidence of association was observed, are all DNA glycosylases participating in the initiation of BER by removing damaged or mismatched bases. Apart from the already mentioned NEIL2 and OGG1, TDG initiates repair of G/T and G/U mismatches commonly associated with CpG islands, while UNG removes uracil in DNA resulting from deamination of cytosine or replicative incorporation of dUMP. We have not found strong associations with SNPs in genes involved in any other parts of the pathway, such as strand incision, trimming of ends, gap filling or ligation. It has been suggested that at least in the case of uracil repair, base removal is the major rate-limiting step of BER [33].

This is consistent with our findings, suggesting that SNPs causing impairment in the function of these specific DNA glycosylases

![Figure 1. p-values of association (−\log_{10} scale) with breast cancer risk in BRCA2 carriers for genotyped and imputed SNPs in the NEIL2 gene. SNP rs1466785 is indicated with a purple arrow and the best causal imputed SNPs, rs804276 and rs804271 are indicated with a red arrow. Colors represent the pairwise r^2. Plot generated with LocusZoom [42] (http://csg.sph.umich.edu/locuszoom/). doi:10.1371/journal.pgen.1004256.g001](http://csg.sph.umich.edu/locuszoom/)
could give rise to accumulation of single strand breaks and subsequently DNA double strand breaks that, in the HR defective context of BRCA1/2 mutation carriers would increase breast and ovarian cancer risk.

The fact that the SNPs tested are located in genes participating in the same DNA repair pathway as PARP1, make them especially interesting; not only as risk modifiers but also because they could have an impact on patients’ response to treatment with PARP inhibitors. BRCA1/2 mutation carriers harboring a potential modifier SNP in DNA glycosylases could be even more sensitive to PARP1 due to a constitutional slight impairment of the BER activity. This is a hypothesis that should be confirmed in further studies. The design of this study in two stages, the hypothesis-based approach adopted to select genes, and that it is based on the largest possible series of BRCA1 and BRCA2 carriers available nowadays, mean that the results obtained are quite solid. However, the study still has some limitations such as the possible existence of residual confounding due to environmental risk factors for which we did not have information.

In summary, we have identified at least two SNPs, rs1466785 and rs2304277, in the DNA glycosylases NEIL2 and OGG1, potentially associated with increased breast and ovarian cancer risks in BRCA2 and BRCA1 mutation carriers, respectively. Our results suggest that glycosylases involved in the first steps of the BER pathway may be cancer risk modifiers in BRCA1/2 mutation carriers and should be more comprehensively studied. If confirmed, these findings could have implications not only for risk assessment, but also for treatment of BRCA1/2 mutation carriers with PARP inhibitors.

Materials and Methods

Subjects

Eligible subjects were female carriers of deleterious mutations in BRCA1 or BRCA2 aged 18 years or older [6]. A total of 55 collaborating CIMBA studies contributed genotypes for the study. Numbers of samples included from each are provided in Table S2. A total of 1,787 mutation carriers (968 with mutations in BRCA1 and 819 with mutations in BRCA2) were genotyped for the 36 selected SNPs. After the quality control process a total of 23,463 carriers were genotyped in stage II. A final number of 144 SNPs was included in the DNA Repair Genes.html as at the 31st December, 2009. Tag information available at http://www.cgal.icnet.uk/

Methods stage I

Selection and genotyping of SNPs. Eighteen genes (UNG, SMUG1, MBD4, TDG, OGG1, MUTYH, NTHL1, MPG, NEIL1, NEIL2, APEX1, APEX2, LIG3, XRCC1, PNP, POLB, PARP1 and PARP2) involved in the BER pathway were selected, based on the information available at http://www.cgal.icnet.uk/DNA_Repair_Genets.html as at the 31st December, 2009. Tag SNPs for the selected genes were defined using Haploview v.4.0 (http://www.broad.mit.edu/mpg/haploview) with an r^2 threshold of 0.8 and a minimum minor allele frequency of 0.05. In addition, SNPs with potentially functional effects already described in the literature were selected. A final number of 144 SNPs was included in an oligonucleotide pool assay for genotyping using the Illumina Veracode technology (Illumina Inc., San Diego, CA). Three hundred nanograms of DNA from each sample were genotyped using the GoldenGate Genotyping Assay with Veracode technology according to the published Illumina protocol. Genotype clustering and calling were carried out using the GenomeStudio software. SNPs with a call rate <0.95 were excluded from further analysis. Duplicate samples and CEPH trios (Coriell Cell Repository, Camden, NJ) were genotyped across the plates. SNPs showing Mendelian allele-transmission errors or showing discordant genotypes across duplicates were excluded.

Statistical analysis. To test for departure from Hardy-Weinberg equilibrium (HWE), a single individual was randomly selected from each family and Pearson’s X^2 Test (1df) was applied to genotypes from this set of individuals. The association of the SNPs with breast cancer risk was assessed by estimating hazard ratios (HR) and their corresponding 95% confidence intervals (CI) using weighted multivariable Cox proportional hazards regression with robust estimates of variance [34]. For each mutation carrier, we modeled the time to diagnosis of breast cancer from birth, censoring at the first of the following events: bilateral prophylactic mastectomy, breast cancer diagnosis, ovarian cancer diagnosis, death or date last known to be alive. Subjects were considered affected if their age at censoring corresponded to their age at diagnosis of breast cancer and unaffected otherwise. Weights were assigned separately for carriers of mutations in BRCA1 and BRCA2, by age and affection status, so that the weighted observed incidences in the sample agreed with established estimates for mutation carriers [1]; [34].

We considered log-additive and co-dominant genetic models and tested for departure from HR = 1 by applying a Wald test based on the log-HR estimate and its standard error. Additional independent variables included in all analyses were year of study, centre and country. All statistical analyses were carried out using Stata: Release 10 (StataCorp. 2007. Stata Statistical Software: Release 10.0. College Station, TX: Stata Corporation LP). Robust estimates of variance were calculated using the cluster subcommand, applied to an identifier variable unique to each family.

Methods stage II

iCOGS SNP array. Stage II of the experiment was performed as part of the iCOGS genotyping experiment. The iCOGS custom array was designed in collaboration between the Breast Cancer Association Consortium (BCAC), the Ovarian Cancer Association Consortium (OCAC), the Prostate Cancer Association Group to Investigate Cancer Associated in the Genome (PRACTICAL) and CIMBA. The final design comprised 211,155 successfully manufactured SNPs of which approximately 17.5% had been proposed by CIMBA. A total of 43 SNPs were nominated for inclusion on iCOGS based on statistical evidence of association in stage I of the present study (p≤0.05). Of these, 36 were successfully manufactured and genotyped in CIMBA mutation carriers.

iCOGS genotyping and quality control. Genotyping was performed at Mayo Clinic and the McGill University and Génome Québec Innovation Centre (Montreal, Canada). Genotypes were called using Illumina’s GenCall algorithm. Sample and quality control process have been described in detail elsewhere [32,35]. After the quality control process a total of 23,463 carriers were genotyped for the 36 selected SNPs.

Statistical analysis. Both breast and ovarian cancer associations were evaluated in stage II. Censoring for breast cancer followed the same approach as in stage I. Censoring for ovarian cancer risk occurred at risk-reducing salpingo-oophorectomy or last follow-up.

The genotype-disease associations were evaluated within a survival analysis framework, by modelling the retrospective likelihood of the observed genotypes conditional on the disease phenotypes [9,34,36,37]. The associations between genotype and breast or ovarian cancer risk were assessed using the 1 d.f. score test statistic based on this retrospective likelihood. To allow for the
non-independence among related individuals, we accounted for the correlation between the genotypes by estimating the kinship coefficient for each pair of individuals using the available genomic data [34,38,39]. These analyses were performed in R using the GenABEL libraries and custom-written functions in FORTRAN and Python.

To estimate the magnitude of the associations (HRs), the effect of each SNP was modeled either as a per-allele HR (multiplicative model) or as genotype-specific HRs, and was estimated using the logistic scale by maximizing the retrospective likelihood. The retrospective likelihood was fitted using the pedigree-analysis software MENDEL. The variances of the parameter estimates were obtained by robust variance estimation based on reported family membership. All analyses were stratified by country of residence and based on calendar-year and cohort-specific breast cancer incidence rates for mutation carriers. Countries with small number of mutation carriers were combined with neighbouring countries to ensure sufficiently large numbers within each stratum. USA and Canada were further stratified by reported Ashkenazi Jewish (AJ) ancestry.

**Imputation.** Genotypes were imputed separately for BRCA1 and BRCA2 mutation carriers using the v3 April 2012 release (Genomes Project et al., 2012) as reference panel. To improve computation efficiency we used a two-step procedure which involved pre-phasing in the first step and imputation of the phased computation. We used a two-step procedure which involved pre-phasing in the first step and imputation of the phased panel and in the graph with a purple arrow. For PARP2 gene, the imputed SNP with the strongest association, rs61995542, is indicated with a red arrow. Colors represent the pairwise r2.

**Supporting Information**

**Figure S1** p-values of association (~log10 scale) with breast and ovarian cancer risk in BRCA1 and BRCA2 carriers for genotyped and imputed SNPs considering 15 kb upstream and downstream the genes in which SNPs described in Table 1 were located. rs numbers of SNPs from Table 1 are indicated at the top of each panel and in the graph with a purple arrow. For PARP2 gene, the imputed SNP with the strongest association, rs61995542, is indicated with a red arrow. Colors represent the pairwise r2.

**Table S1** Association with breast cancer for the 94 SNPs selected for analysis in stage I.

**Table S2** number of BRCA1 and BRCA2 carriers by study.

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Author Contributions

Conceived and designed the experiments: ARO LGM JPJ. Performed the experiments: AO TV RA BH LTM JS KO FJC. Analyzed the data: RLM KKu ACA. Contributed reagents/materials/analysis tools: AO RLM Kku GPJ. Wrote the paper: ARO LGM JPJ. Revised the paper: LEGD AEJH ZJ MR TK LF HE LLK KD JKB OTJ CM MMo ST MRT SHe CO LG NLi SS CIS JV MR NK LZ RRM AFS CRS DFK MGK ABC MPJ MPIL FLX IA AMM GG AET ABO ISP LsMT TAK UB EFJ YLPS JS IDF KF JC GCT ACA JB. Wrote the paper: ARO LGM KKu ACA JB.

References

1. Antoniou A, Pharoah PD, Narod S, Risch HA, Eyfjord JE, et al. (2003) Average risks of breast and ovarian cancer associated with BRCA1 or BRCA2 mutations detected in case series unselected for family history: a combined analysis of 22 studies. Am J Hum Genet 72: 1117–1130.
2. Chen S, Iversen ES, Friebel T, Finkelnberg D, Weber BL, et al. (2006) Characterization of BRCA1 and BRCA2 mutations in a large U.S. population sample. J Clin Oncol 24: 863–871.
3. Milne RL, Oostra A, Cajal TR, Vega A, Llort G, et al. (2008) The average cumulative risks of breast and ovarian cancer for carriers of mutations in BRCA1 and BRCA2 attending genetic counseling units in Spain. Clin Cancer Res 14: 2896–2899.
4. Chenevix-Trench G, Milne RL, Antoniou AC, Couch FJ, Easton DF, et al. (2007) An international initiative to identify genetic modifiers of cancer risk in BRCA1 and BRCA2 carrier mutation carriers: the Consortium of Investigators of Modifiers of BRCA1 and BRCA2 (CIMBA). Breast Cancer Res 9: 104.
5. Antoniou AC, Kuchenbaecker BK, Souey P, Besley J, Chen X, et al. (2012) Common variants at 12p11.1, 12p24, 9q31.2 and ZNF365 are associated with breast cancer risk for BRCA1 and/or BRCA2 carrier mutations. Breast Cancer Res 14: R33.
6. Antoniou AC, Spurdle AB, Sinilnikova OM, Healey S, Pooley KA, et al. (2008) Common breast cancer-predisposition alleles are associated with breast cancer risk in BRCA1 and BRCA2 mutation carriers. Am J Hum Genet 82: 937–948.
7. Antoniou AC, Sinilnikova OM, McGuffog I, Healey S, Nevanlinna H, et al. (2009) Common variants in LSP1, 2q35 and 8q24 and breast cancer risk for BRCA1 and BRCA2 mutation carriers. Hum Mol Genet 18: 4449–4456.
8. Antoniou AC, Wang X, Frederickson ZS, McGuffog I, Larrer R, et al. (2010) A locus on 19p13 modifies risk of breast cancer in BRCA1 mutation carriers and is associated with hormone receptor-negative breast cancer in the general population. Nat Genet 42: 883–892.
9. Antoniou AC, Sinilnikova OM, Simard J, Leone M, Dumont M, et al. (2007) RAD51 1356G→C modifies breast cancer risk among BRCA2 mutation carriers: results from a combined analysis of 19 studies. Am J Hum Genet 81: 1186–1200.
10. Miller MA, Healy S, Nevanlinna H, Powles T, et al. (1999) 2q35 allelic imbalance of chromosome band 3p25.3 and TP53 mutations in breast and ovarian cancer. Cancer Epidemiol Biomarkers Prev 8: 1191–1195.
11. Patel KJ, Yu VP, Lee H, Corcoran A, Thistlethwaite FC, et al. (1998) Mutations in the XRCC1 gene as a phenotypic modifier in BRCA1/2 mutation carriers. Nature Genet 20: 400–402.
12. Zhai X, Zhao H, Liu Z, Wang LE, El-Naggar AK, et al. (2008) Functional characterization of a novel OGG1 variant, allelic imbalance of chromosome band 3p25.3 and TP53 mutations in ovarian cancer. Int J Oncol 27: 1315–1320.
13. Kjeldsen SL, Valsbro P, Nielsen J, Lund Nielsen U, Christiansen J, et al. (2007) The contribution of high-risk BRCA1 and/or BRCA2 mutation carriers to the Danish Breast Cancer Family Registry: A family-based case-control study. J Carcinog 6: 9.
14. Farmer H, McCabe N, Lord CJ, Tutt AN, Johnson DA, et al. (2005) Targeting DNAdamaging agents to cancer cells using nucleotide excision repair. Nature Biotechnol 23: 556–561.
15. Kinslow CJ, El-Zein RA, Rondelli CM, Hill CE, Wickliffe JK, et al. (2010) Regulatory regions responsive to oxidative stress in the promoter of the human DNA glycosylase gene NEIL2. Mutagenesis 25: 171–177.
16. Kinslow CJ, El-Zein RA, Hill CE, Wickliffe JK, Abdel-Rahman SZ (2008) Single nucleotide polymorphisms 5′ upstream of the coding region of the NEIL2 gene influence transcription levels and alter levels of genetic damage. Genes Chromosomes Cancer 47: 923–932.
17. Moynahan ME, Chiu JW, Koller BH, Jasin M (1999) Brca1 controls homology-directed DNA repair. Mol Cell 4: 511–518.
18. Stine RB, Kemp RD, Brocker BS, Auerbach AD, et al. (1998) Obligatory involvement of Brca2 in breast cancer DNA repair. Mol Cell 1: 349–358.
19. Xi X, Herzig M, Rotrekl V, Walter CA (2008) Base excision repair, aging and health span. Mech Ageing Dev 129: 366–382.
20. Farmer H, McCabe N, Lord CJ, Tutt AN, Johnson DA, et al. (2005) Targeting DNA-damaging agents to cancer cells using nucleotide excision repair. Nature Biotechnol 23: 556–561.
37. Barnes DR, Antoniou AC (2012) Unravelling modifiers of breast and ovarian cancer risk for BRCA1 and BRCA2 mutation carriers: update on genetic modifiers. J Intern Med 271: 331–343.
38. Amin N, van Duijn CM, Aulchenko YS (2007) A genomic background based method for association analysis in related individuals. PLoS One 2: e1274.
39. Leutenegger AL, Prum B, Genin E, Verny C, Lemainque A, et al. (2003) Estimation of the inbreeding coefficient through use of genomic data. Am J Hum Genet 73: 516–523.
40. Delaneau O, Zagury JF (2012) Haplotype inference. Methods Mol Biol 888: 177–196.
41. Howie BN, Donnelly P, Marchini J (2009) A flexible and accurate genotype imputation method for the next generation of genome-wide association studies. PLoS Genet 5: e1000529.
42. Pruim RJ, Welch RP, Sanna S, Teslovich TM, Chines PS, et al. (2010) LocusZoom: regional visualization of genome-wide association scan results. Bioinformatics 26: 2336–2337.