Gaudet, MM; Kuchenbaecker, KB; Vijai, J; Klein, RJ; Kirchhoff, T; McGuffog, L; Barrowdale, D; Dunning, AM; Lee, A; Dennis, J; +90 more... Healey, S; Dicks, E; Soucy, P; Sinilnikova, O; Pankratz, VS; Wang, XS; Eldridge, RC; Tessier, DC; Vincent, D; Bacot, F; Hogervorst, FBL; Peock, S; Stoppan-Lyonnet, D; Peterlongo, P; Schmutzler, RK; Nathanson, KL; Piedmonte, M; Singer, CF; Thomassen, M; Hansen, TVO; Neuhausen, SL; Blanco, I; Greene, MH; Garber, J; Weitzel, JN; Andrulis, IL; Goldgar, DE; D’Andrea, E; Caldes, T; Nevanlinna, H; Osorio, A; van Rensburg, EJ; Arason, A; Rennert, G; van Den Ouweland, AMW; van der Hout, AH; Kets, CM; Aalfs, CM; Wijnen, JT; Ausems, Mgem; Frost, D; Ellis, S; Fineberg, E; Platte, R; Evans, DG; Jacobs, C; Adlard, J; Tischkowitz, M; Porteous, ME; Damila, F; Golmard, L; Barjhoux, L; Longy, M; Belotti, M; Ferrer, SF; Mazoyer, S; Spurdle, AB; Manoukian, S; Barile, M; Genuardi, M; Arnold, N; Meindl, A; Sutter, C; Wappenschmidt, B; Domchek, SM; Pfeiler, G; Friedman, E; Jensen, UB; Robson, M; Shah, S; Lazaro, C; Mai, PL; Benitez, J; Southey, MC; Schmidt, MK; Fasching, PA; Peto, J; Humphreys, MK; Wang, Q; Michailidou, K; Sawyer, EJ; Burwinkel, B; Guenel, P; Bojesen, SE; Milne, RL; Brenner, H; Lochmann, M; Aittomaki, K; Dork, T; Margolin, S; (2013) Identification of a BRCA2-Specific Modifier Locus at 6p24 Related to Breast Cancer Risk. PLoS genetics, 9 (3). ISSN 1553-7390 DOI: https://doi.org/10.1371/journal.pgen.1003173

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DOI: https://doi.org/10.1371/journal.pgen.1003173

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Identification of a BRCA2-Specific Modifier Locus at 6p24 Related to Breast Cancer Risk


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Abstract

Common genetic variants contribute to the observed variation in breast cancer risk for BRCA2 mutation carriers; those known to date have all been found through population-based genome-wide association studies (GWAS). To cross-validate previously identified breast cancer risk variants, we conducted a deep replication step of an ongoing GWAS discovery study. Using the ranked P-values of the breast cancer associations with the imputed known to date have all been found through population-based genome-wide association studies (GWAS). To Common genetic variants contribute to the observed variation in breast cancer risk for BRCA2 mutation carriers; those known to date have all been found through population-based genome-wide association studies (GWAS). To cross-validate previously identified breast cancer risk variants, we conducted a deep replication step of an ongoing GWAS discovery study. 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Using the ranked P-values of the breast cancer associations with the imputed known to date have all been found through population-based genome-wide association studies (GWAS). To cross-validate previously identified breast cancer risk variants, we conducted a deep replication step. The locus lies within a region containing TFAP2A, which encodes a transcriptional activation protein that interacts with several tumor suppressor genes. This report identifies the first breast cancer risk locus specific to a BRCA2 mutation carriers. This panel may have clinical utility for women with BRCA2 mutations weighing options for medical prevention of breast cancer.
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### Funding:

This work was supported by the following institutions: ICOSG: The creation of the custom Illumina multiplex chip and the genotyping of the BRCA2 carriers in CMIB was made possible by grants from the Starr Cancer Consortium A4-A02 (PI: K Offit), the Sandra Taub Memorial Fund of the Breast Cancer Research Foundation (PI: K Offit), the Norman and Carol Stone Cancer Genetics Fund (PI: K Offit), and the European Commission’s Seventh Framework Programme grant agreement 232175 (HEALTH-F2-2009-232175). AC Antoniou is a Cancer Research UK Senior Cancer Research Fellow. O Cheveaux-Trench is an NHMRC Senior Principal Research Fellow. Consortium of Modifiers of BRCA1/2 Associations: The CMIB data management and data analysis were supported by Cancer Research UK grants C12292/A11174 and C1287/A10118. S Healey is supported by an NHMRC Program Grant to G Cheveaux-Trench. AC Antoniou is a Cancer Research UK Senior Cancer Research Fellow. G Cheveaux-Trench is an NHMRC Principal Research Fellow. Amsterdam Breast Cancer Study: The ABCS study was supported by the Dutch Cancer Society (grants NKI 2007-3839; 2009-4363; BBMRI-NL, which is a Research Infrastructure financed by the Dutch government (NWO 104.021.007); and the Dutch National Genomics Initiative. Bavarian Breast Cancer Cases and Controls: The work of the BBCS was partly funded by ELAN-Fund of the University Hospital of Erlangen. British Breast Cancer Study: The BBCS is funded by Cancer Research UK and Breakthrough Breast Cancer and acknowledges NHF funding to the NIHR Biomedical Research Centre, and the National Cancer Research Network (NCRN). Breast Cancer Family Registry Studies: The Australian Breast Cancer Family Study (ABCFS), New York City (New York Breast CFR), Northern California Breast Cancer Family Registry (NC-BCFR), and Utah (Utah Breast CFR) work was supported by the United States National Cancer Institute, National Institutes of Health (NIH), under RFA-CA-06-003 (P30 CA13696 and P30 ES009089), and through cooperative agreements with members of the BCFR and Principal Investigators, including Cancer Care Ontario (U01 CA96476), Columbia University (U01 CA69398), Cancer Prevention Institute of California (U01 CA69417), Fox Chase Cancer Center (U01 CA69313), Huntsman Cancer Institute (U01 CA94644), and University of Miami (CA69386). The ABCFS was also supported by the National Health and Medical Research Council of Australia, the Victorian Health Promotion Foundation (Australia), and the Victorian Breast Cancer Research Consortium. The New York BCFR site was also supported by NIH grants P30 CA13696 and P30 ES009089. MC Soutemy is an NHMRC Senior Research Fellow and a Victorian Breast Cancer Research Consortium Group Leader. Baltic Familial Breast Ovarian Cancer Consortium: BFBOCC is partly supported by: Lithuania: BFBOCC-LT, Research Council of Lithuania grant LIG-19-2010, and Hereditary Cancer Association (Paveldzi na va, asociacija). Latvia: BFBOCC-LV is partly supported by: LIE LEADER project 0902-010.001.00.10.88 and in part by a grant act LP08-13 (0122060), Breast Cancer in Galway Genetic Study: Guy’s & St. Thomas’ NHS Foundation Trust in partnership with King’s College London, United Kingdom. BRCA-gene mutations and breast cancer in South African women: BMBSA was supported by grants from the Cancer Association of South Africa (Cansa) to EJ van Rensburg NIH R01CA74415 and P30 CA033752. Beckman Research Institute of the City of Hope: SL Neuhausen was partially supported by the Morris and Horowitz Families Endowed Professorship. BRICOC was supported by: the Israeli Cancer Society, the Israel Cancer Association, the Israel Cancer Registry and the Israel Cancer Hospital: The Hopp Fonds, the Helmholtz Society and the German Cancer Research Center (DKFZ). Richgospitalet: The CBS study was supported by the NEYE Foundation. CECEL Breast Cancer Study: The CECEL study was funded by Fondation de France, Institut National du cancer (INCa), Ligue Nationale contre le Cancer, Ligue contre le Cancer Grand Ouest, Agence Nationale de Sécurité Sanitaire (ANSES), Agence Nationale de la Recherche (ANR). Copenhagen General Population Study: The CGPS was supported by the Chief Physician Johan Boserup and Lise Boserup Fund, the Danish Medical Research Council and Herlev Hospital. The Cancer Center Karolinska: The KBCP study was supported by the Swedish Cancer Society, the Gustav V Jubilee Foundation, and the Bert von Kantzow Foundation. Kuopio Breast Cancer Project: The KBCP study was financially supported by the special Government Funding (EVO) of Kuopio University
Introduction

The lifetime risk of breast cancer associated with carrying a BRCA2 mutation varies from 40 to 84% [1]. To determine whether common genetic variants modify breast cancer risk for BRCA2 mutation carriers, we previously conducted a GWAS of BRCA2 mutation carriers from the Consortium of Investigators of Modifiers of BRCA1/2 (CIMBA) [2]. Using the Affymetrix 6.0 platform, the discovery stage results were based on 899 young (<40 years) affected and 804 unaffected carriers of European ancestry. In a rapid replication stage wherein 85 discovery stage SNPs with the smallest P-values were genotyped in 2,486 ancestry. In a rapid replication stage wherein 85 discovery stage SNPs with the smallest P-values were genotyped in 2,486
mutation carriers. Two other loci, in 6p24 (rs16917302) on 10q21 and a locus on 20q13 (rs311499), were also associated with breast cancer risk in BRCA2 mutation carriers with P-values<10^{-4} (P = 3.8 \times 10^{-5} and 6.6 \times 10^{-5}, respectively). A nearby SNP in 6p24 was also associated with breast cancer risk in a study of unselected cases [3] and in a study of mammographic density [4]. Additional follow-up replicated the findings for rs16917302, but not rs311499 [5] in a larger set of BRCA2 mutation carriers. Combining this BRCA2-specific SNP with 13 other breast cancer risk SNPs also known to modify risk in BRCA2 mutation carriers, we were able to derive a risk prediction model that could be useful in helping women with BRCA2 mutations weigh their risk-reduction strategy options.

GWAS discovery stage samples. Details of these samples have been described previously [2]. Data from 899 young (<40 years) and 804 older (>40 years) unaffected carriers of European ancestry from 14 countries were used to select SNPs for inclusion on the iCOGS array.

Samples genotyped in the extended replication set. Forty-seven studies from 24 different countries (including two East-Asian countries) provided DNA from a total of 10,048 BRCA2 mutation carriers. All eligible samples were genotyped using COGs, including those from the discovery stage.

Genotyping and quality control

BRCA2 SNP selection for inclusion on iCOGS. The Collaborative Oncological Gene-Environment Study (COGS) consortium developed a custom genotyping array (referred to as the iCOGS array) to provide efficient genotyping of common and rare genetic variants to identify novel loci that are associated with risk of breast, ovarian, and prostate cancers as well as to fine-map known cancer susceptibility loci. SNPs were selected for inclusion on iCOGS separately by each participating consortium: Breast Cancer Association Consortium (BCAC) [6], Ovarian Cancer Association Consortium (OCAC) [7], Prostate Cancer Association Group to Investigate Cancer Associated Alterations in the Genome (PRACTICAL) [8], and CIMBA. SNP lists from a BRCA4 GWAS and SNPs in candidate regions were used together with the BRCA2 GWAS lists to generate a ranked CIMBA SNP list that included SNPs with the following nominal proportions: 55.5% from the BRCA1 GWAS, 41.6% from the BRCA2 GWAS and fine mapping, 2.9% for CIMBA candidate SNPs. Each consortium was given a share of the array: nominally 25% of the SNPs each for BCAC, PRACTICAL and OCAC; 17.5% for CIMBA and 7.5% for SNPs from commonly researched pathways (e.g., inflammation). For the CIMBA BRCA2 GWAS, we used the iCOGS array as the platform to genotype the extended replication set of the discovery GWAS stage [2]. SNPs were selected on the basis of the strength of their associations with breast cancer risk in the discovery stage [2], using imputed genotype data for 1.4 M SNPs identified through CEU+TSI samples on HapMap3, release 2. A ranked list of SNPs was based on the 1-df trend test statistic, after excluding highly correlated SNPs (r^2>0.4). The final list included the 39,015 SNPs with the smallest p-values. An additional set of SNPs were selected for fine mapping of the regions surrounding the SNPs found to be associated with breast cancer in the discovery GWAS stage: rs16917302 on 10q21 and rs311499 on 20q13, including SNPs with a MAF >0.05 located 500 kb in both directions of the SNP, based on HapMap 2 data. The final combined list of SNPs for the iCOGS array comprised 220,123 SNPs. Of these, 211,155 were successfully manufactured onto the array. The present analyses are based on the 19,029 SNPs selected on the basis of BRCA2 GWAS and fine mapping that were included on the iCOGS array.

Materials and Methods

Ethics statement
Each of the host institutions (Table S1) recruited under ethically-approved protocols. Written informed consent was obtained from all subjects.

Study subjects
The majority of BRCA2 mutation carriers were recruited through cancer genetics clinics and some came from population or community-based studies. Studies contributing DNA samples to these research efforts were members of the Consortium of Investigators of Modifiers of BRCA1/2 (CIMBA) with the exception of one study (NICCC). Eligible subjects were women of European descent who carried a pathogenic BRCA2 mutation, had complete phenotype information, and were at least 18 years of age. Harmonized phenotypic data included year of birth, age at breast cancer diagnosis, age at bilateral prophylactic mastectomy and oophorectomy, age at interview or last follow-up, BRCA2 mutation description, self-reported ethnicity, and breast cancer estrogen receptor status.

Author Summary
Women who carry BRCA2 mutations have an increased risk of breast cancer that varies widely. To identify common genetic variants that modify the breast cancer risk associated with BRCA2 mutations, we have built upon our previous work in which we examined genetic variants across the genome in relation to breast cancer risk among BRCA2 mutation carriers. Using a custom genotyping platform with 211,155 genetic variants known as single nucleotide polymorphisms (SNPs), we genotyped 3,881 women who had breast cancer and 4,330 women without breast cancer, which represents the largest possible, international collection of BRCA2 mutation carriers. We identified that a SNP located at 6p24 in the genome was associated with lower risk of breast cancer. Importantly, this SNP was not associated with breast cancer in BRCA1 mutation carriers or in a general population of women, indicating that the breast cancer association with this SNP might be specific to BRCA2 mutation carriers. Combining this BRCA2-specific SNP with 13 other breast cancer risk SNPs also known to modify risk in BRCA2 mutation carriers, we were able to derive a risk prediction model that could be useful in helping women with BRCA2 mutations weigh their risk-reduction strategy options.
consortia, and each major ethnicity. Only plates with a consistent high call rate in the initial calling were used. We also included 390 samples of European, African, and Asian ethnicity genotyped as part of the Hapmap and 1000 Genomes project, and 160 samples that were known positive controls for rare variants on the array. This subset was used to generate a cluster file that was then applied to call the genotypes for the remaining samples.

**Quality control of SNPs.** Of the 211,155 SNPs on the iCOGS array, we excluded SNPs for the following reasons (Table S2): on the Y-chromosome, call rate < 95%, deviations from Hardy-Weinberg equilibrium (P < 10^{-7}), using a stratified 1-d.f. test [10], and monomorphic. SNPs that gave discrepant genotypes among known duplicates were also excluded. After quality control filtering, 200,908 SNPs were available for analysis (Table S2); 18,066 of which were selected on the basis of the discovery BRCA2 GWAS [2]. Cluster plots of all reported SNPs were inspected manually for quality (Figure S1).

**Description of imputation.** Genotypes for SNPs identified through the 1000 Genomes Phase I data (released Jan 2012) [11] were imputed using SNPs on the iCOGS chip in a region of 500 kb around the novel modifier locus at 6p24. The boundaries were determined according to the linkage disequilibrium (LD) structure in the region based on HapMap data. The imputation was carried out using IMPUTE 2.2 [12]. SNPs with imputation information/accuracy r^2 < 0.30 were excluded in the analyses.

**Quality control of DNA samples.** Of 10,048 genotyped samples (Table S2), 742 were excluded because they did not meet the phenotypic eligibility criteria or had self-reported non-CEU ethnicity. Samples were then excluded for the following reasons: not female (XXY, XY), call rate < 95%, low or high heterozygosity (P < 10^{-5}), discordant genotypes from previous CIMP8 genotyping efforts, or discordant duplicate samples. For duplicates with concordant phenotypic data, or in cases of cryptic monozygotic twins, only one of the samples was included. Cryptic duplicates for which phenotypic data indicated different individuals were all excluded. Samples of non-European ancestry were identified using multi-dimensional scaling, after combining the BRCA2 mutation carrier samples with the HapMap2 CEU, CHB, JPT and YRI samples using a set of 37,120 uncorrelated SNPs from the iCOGS array. Samples with >19% non-European ancestry were excluded (Figure S2). A total of 4,330 affected and 3,881 unaffected BRCA2 mutation carrier women of European ancestry from 42 studies remained in the analysis (Table S1), including 3,234 breast cancer cases and 3,490 unaffected carriers that were not in the discovery set.

**BRCA1 and BCAC samples.** Details of the sample collection, genotyping and quality control process for the BRCA1 and BCAC samples, are reported elsewhere [13,14].

**Statistical methods**

The associations between genotype and breast cancer risk were analyzed within a retrospective cohort framework with time to breast cancer diagnosis as the outcome [15]. Each BCAC carrier was followed until the first event: breast or ovarian cancer diagnosis, bilateral prophylactic mastectomy, or age at last observation. Only those with a breast cancer diagnosis were considered as cases in the analysis. The majority of mutation carriers were recruited through genetic counseling centers where genetic testing is targeted at women diagnosed with breast or ovarian cancer and in particular to those diagnosed with breast cancer at a young age. Therefore, these women are more likely to be sampled compared to unaffected mutation carriers or carriers diagnosed with the disease at older ages. As a consequence, sampling was not random with respect to disease phenotype and standard methods of survival analysis (such as Cox regression) may lead to biased estimates of the associations [16]. We therefore conducted the analysis by modeling the retrospective likelihood of the observed genotypes conditional on the disease phenotypes. This has been shown to provide unbiased estimates of the associations [15]. The implementation of the retrospective likelihoods has been described in detail elsewhere [15,17]. The associations between genotype and breast cancer risk were assessed using the 1 degree of freedom score test statistic based on the retrospective likelihood [15]. In order to account for non-independence between relatives, an adjusted version of the score test was used in which the variance of the score was derived taking into account the correlation between the genotypes [18]. P-values were not adjusted using genomic control because there was little evidence of inflation. Inflation was assessed using the genomic inflation factor, λ. Since this estimate is dependent on sample size, we also calculated λ adjusted to 1000 affected and 1000 unaffected samples. Per-allele and genotype-specific hazard-ratios (HR) and 95% confidence intervals (CI) were estimated by maximizing the retrospective likelihood. Calendar-year and cohort-specific breast cancer incidences for BRCA2 were used [1]. All analyses were stratified by country of residence. The USA and Canada strata were further subdivided by self-reported Ashkenazi Jewish ancestry. The assumption of proportional hazards was assessed by fitting a model that included a genotype-by-age interaction term. Between-country heterogeneity was assessed by comparing the results of the main analysis to a model with country-specific log-HRs. A possible survival bias due to inclusion of prevalent cases was evaluated by re-fitting the model after excluding affected carriers that were diagnosed ≥5 years prior to study recruitment. The associations between genotypes and tumor subtypes were evaluated using an extension of the retrospective likelihood approach that models the association with two or more subtypes simultaneously [19]. To investigate whether any of the significant SNPs were associated with ovarian cancer risk for BRCA2 mutation carriers and whether the inclusion of ovarian cancer patients as unaffected subjects biased our results, we also analyzed the data within a competing risks framework and estimated HR simultaneously for breast and ovarian cancer using the methods described elsewhere [15]. Analyses were carried out in R using the GenABEL libraries [20] and custom-written software. The retrospective likelihood was modeled in the pedigree-analysis software MENDEL [21], as described in detail elsewhere [15].

**TCGA analysis.** Affymetrix SNP 6.0 genotype calls for normal (non-tumor) breast DNA were downloaded for all available individuals from The Cancer Genome Atlas in September 2011. Analyses were limited to the 401 individuals of European ancestry based on principal component analysis. Expression levels in breast tumor tissue were adjusted for the top two principal components, age, gender (there are some male breast cancer cases in TCGA), and average copy number across the gene in the tumor. Linear regression was then used to test for association between the SNP and the adjusted gene expression level for all genes within one megabase.

**Gene set enrichment analysis.** To investigate enrichment of genes associated with breast cancer risk, the gene-set enrichment approach was implemented using Versatile Gene-based Association Study [22] based on the ranked P-values from retrospective likelihood analysis. Association List Go Annotator was also used to prioritize gene pathways using functional annotation from gene ontology (GO) [23] to increase the power to detect association to a pathway, as opposed to individual genes in the pathway. Both analyses were corrected for LD between SNPs, variable gene size, and interdependence of GO categories.
where applicable, based on imputation. 100,000 Monte Carlo simulations were performed in VEGAS and 5000 replicate gene lists using random sampling of SNPs and 5000 replicate studies (sampling with replacement) were performed to estimate P-values.

**Predicted absolute breast cancer risks by combined SNP profile.** We estimated the absolute risks of developing breast cancer based on the joint distribution of SNPs associated with breast cancer for *BRCA2* mutation carriers. The methods have been described elsewhere [24]. To construct the SNP profiles, we considered the single SNP from each region with the strongest evidence of association in the present dataset. We included all loci that had previously been found to be associated with breast cancer risk through GWAS in the general population and demonstrated associations with breast cancer risk for *BRCA2* mutation carriers, and loci that had GWAS level of significance in the current study. We assumed that all loci in the profile were independent (i.e., they interact multiplicatively on *BRCA2* breast cancer risk). Genotype frequencies were obtained under the assumption of Hardy-Weinberg Equilibrium. For each SNP, the effect of each allele was assumed to be consistent with a multiplicative model (log-additive). We assumed that the average, age-specific breast cancer incidences, over all associated loci, agreed with published breast cancer risk estimates for *BRCA2* mutation carriers [1].

**Results**

The genomic inflation factor (λ) based on the 18,086 *BRCA2* GWAS SNPs in the 6,724 *BRCA2* mutation carriers who were not used in the SNP discovery set was 1.034 (λ adjusted to 1000 affected and 1000 unaffected: 1.010, Figure S3). Multiple variants were associated with breast cancer risk in the combined discovery and replication datasets (Figure S4). SNPs in three independent regions had P-values < 5 x 10^-8; one was a region not previously associated with breast cancer.

The most significant associations were observed for known breast cancer susceptibility regions, rs2420946 (per allele P = 2 x 10^-14) in **FGFR2** and rs3803662 (P = 5.4 x 10^-11) near **TOX3** (Table 1). Breast cancer risk associations with other SNPs reported previously for *BRCA2* mutation carriers are summarized in Table 1. In this larger set of *BRCA2* mutation carriers, we also identified novel SNPs in the 12p11 (**PTHLH**, 5q11 (**MAP3K1**), and 9p21 (**CDK2A1/B**)) regions with smaller P-values for association than those of previously reported SNPs. These novel SNPs were not correlated with the previously reported SNPs (r^2 < 0.14). For one of the novel SNPs identified in the discovery GWAS [2], ZNF365 rs16917302, there was weak evidence of association with breast cancer risk (P = 0.01); however, an uncorrelated SNP, rs17221319 (r^2 < 0.01), 54 kb upstream of rs16917302 had stronger evidence of association (P = 6 x 10^-6).

One SNP, rs9348512 at 6p24 not known to be associated with breast cancer, had a combined P-value of association of 3.9 x 10^-9 amongst all *BRCA2* samples (Table 2), with strong evidence of replication in the set of *BRCA2* samples that were not used in the discovery stage (P = 5.2 x 10^-6). The minor allele of rs9348512 (MAF = 0.35) was associated with a 15% decreased risk of breast cancer among *BRCA2* mutation carriers (per allele HR = 0.85, 95% CI 0.80–0.90) with no evidence of between-country heterogeneity (P = 0.78, Figure S5). None of the genotyped (n = 68) or imputed (n = 3,507) SNPs in this region showed a stronger association with risk (Figure 1; Table S3), but there were 40 SNPs with P < 10^-4 (pairwise r^2 > 0.38 with rs9348512, with the exception of rs11526201 for which r^2 = 0.01, Table S3). The association with rs9348512 did not differ by 6174delT mutation status (P for difference = 0.33), age (P = 0.39), or estrogen receptor (ER) status of the breast tumor (P = 0.41). Exclusion of prevalent breast cancer cases (n = 1,732) produced results (HR = 0.83, 95% CI 0.77–0.89, P = 3.4 x 10^-7) consistent with those for all cases.

SNPs in two additional regions had P-values < 10^-7 for breast cancer risk associations for *BRCA2* mutation carriers (Table 2). The magnitude of associations for both SNPs was similar in the discovery and second stage samples. In the combined analysis of all samples, the minor allele of rs619373, located in **FGF13** (Xq26.3), was associated with higher breast cancer risk (HR = 1.30, 95% CI 1.17–1.43, P = 3.1 x 10^-6). The minor allele of rs184577, located in **CIP11-AS1** (3p22–p21), was associated with lower breast cancer risk (HR = 0.93, 95% CI 0.79–0.91, P = 3.6 x 10^-6). These findings were consistent across countries (P for heterogeneity between country strata = 0.39 and P = 0.30, respectively; Figure S6). There was no evidence that the HR estimates for rs619373 and rs184577 change with age of the *BRCA2* mutation carriers (P for the genotype-age interaction = 0.80 and P = 0.40, respectively) and no evidence of survival bias for either SNP (rs619373: HR = 1.35, 95% CI 1.20–1.53, P = 1.5 x 10^-6 and rs184577: HR = 0.86, 95% CI 0.79–0.93, P = 2.0 x 10^-4, after excluding prevalent cases). The estimates for risk of ER-negative and ER-positive breast cancer were not significantly different (P for heterogeneity between tumor subtypes = 0.79 and 0.67, respectively). When associations were evaluated under a competing risks model, there was no evidence of association with ovarian cancer risk for SNPs rs9348512 at 6p24, rs619373 in **FGF13** or rs184577 at 2p22 and the breast cancer associations were virtually unchanged (Table S4).

Gene set enrichment analysis confirmed that strong associations exist for known breast cancer susceptibility loci and the novel loci identified here (gene-based P < 1 x 10^-5). The pathways most strongly associated with breast cancer risk that contained statistically significant SNPs included those related to ATP binding, organ morphogenesis, and several nucleotide bindings (pathway-based P < 0.05).

To begin to determine the functional effect of rs9348512, we examined associations of expression levels of any nearby gene in breast tumors with the minor A allele. Using data from The Cancer Genome Atlas, we found that the A allele of rs9348512 was strongly associated with mRNA levels of **GCNT2** in breast tumors (P = 7.3 x 10^-5).

The hazard ratios for the percentiles of the combined genotype distribution of loci associated with breast cancer risk in *BRCA2* mutation carriers were translated into absolute breast cancer risks under the assumption that SNPs interact multiplicatively. Based on our results for SNPs in **FGFR2**, **TOX3**, 12p11, 5q11, **CDK2A1/B**, **LSP1**, 8q24, **ESR1**, 2qF365, 3p24, 12q24, 5p12, 11q13 and also the 6p24 locus, the 5% of the *BRCA2* mutation carriers at lowest risk were predicted to have breast cancer risks by age 80 in the range of 21–47% compared to 83–100% for the 5% of mutation carriers at highest risk on the basis of the combined SNP profile distribution (Figure 2). The breast cancer risk by age 50 was predicted to be 4–11% for the 5% of the carriers at lowest risk compared to 29–81% for the 5% at highest risk.

**Discussion**

In the largest assemblage of *BRCA2* mutation carriers, we identified a novel locus at 6q24 that is associated with breast cancer risk, and noted two potential SNPs of interest at Xq26 and 2p22. We also replicated associations with known breast cancer susceptibility SNPs previously reported in the general population and in *BRCA2* mutation carriers. For the 12p11 (**PTHLH**, 5q11 (**MAP3K1**), and 9p21 (**CDK2A1/B**), we found uncorrelated SNPs...
<table>
<thead>
<tr>
<th>Chr (Nearby Gene)</th>
<th>SNP</th>
<th>Ref</th>
<th>Affected N</th>
<th>Unaffected N</th>
<th>Per Allele HR (95%CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>10q26 (FGFR2)</td>
<td>rs2981575</td>
<td>G</td>
<td>2,155</td>
<td>2,016</td>
<td>1.28 (1.18, 1.39)</td>
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<tr>
<td>10q26 (FGFR2)</td>
<td>rs2420946</td>
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<td>4,328</td>
<td>3,877</td>
<td>0.40</td>
<td>1.30</td>
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<td>10q26 (FGFR2)</td>
<td>rs1071399</td>
<td>G</td>
<td>3,314</td>
<td>2,900</td>
<td>1.01 (0.94, 1.08)</td>
<td>0.60</td>
</tr>
<tr>
<td>12p11 (PTHLH)</td>
<td>rs10771399</td>
<td>A</td>
<td>4,330</td>
<td>3,880</td>
<td>0.11 (0.08, 0.14)</td>
<td>0.10</td>
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<tr>
<td>5q11 (MAP3K1)</td>
<td>rs889312</td>
<td>C</td>
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<td>2,282</td>
<td>1.10 (1.01, 1.19)</td>
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</tr>
<tr>
<td>9p21 (CDKN2A/B)</td>
<td>rs1011970</td>
<td>A</td>
<td>3,807</td>
<td>3,316</td>
<td>1.09 (1.00, 1.18)</td>
<td>0.05</td>
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<tr>
<td>11p15 (LSP1)</td>
<td>rs3817198</td>
<td>G</td>
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<td>2,636</td>
<td>1.14 (1.06, 1.23)</td>
<td>0.08</td>
</tr>
<tr>
<td>8q24 (ESR1)</td>
<td>rs13281615</td>
<td>G</td>
<td>3,266</td>
<td>2,636</td>
<td>1.14 (1.06, 1.23)</td>
<td>0.08</td>
</tr>
<tr>
<td>5p12 reported</td>
<td>rs10941679</td>
<td>G</td>
<td>3,263</td>
<td>2,591</td>
<td>1.09 (1.01, 1.19)</td>
<td>0.03</td>
</tr>
<tr>
<td>11q13 reported</td>
<td>rs614367</td>
<td>A</td>
<td>3,789</td>
<td>3,307</td>
<td>1.03 (0.95, 1.11)</td>
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<tr>
<td>1p11 (NOTCH2)</td>
<td>rs11249433</td>
<td>G</td>
<td>3,423</td>
<td>2,891</td>
<td>1.09 (0.95, 1.23)</td>
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<tr>
<td>17q23 (STXBP4, COX11)</td>
<td>rs6504950</td>
<td>A</td>
<td>3,401</td>
<td>2,813</td>
<td>1.03 (0.95, 1.11)</td>
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<td>19p13 (MERIT40)</td>
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<td>A</td>
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<td>G</td>
<td>3,300</td>
<td>2,646</td>
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<tr>
<td>9q31 reported</td>
<td>rs865686</td>
<td>C</td>
<td>3,799</td>
<td>3,312</td>
<td>0.99 (0.91, 1.08)</td>
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Table 2. Breast cancer hazard ratios (HR) and 95% confidence intervals (CI) of novel breast cancer loci with P-values of association <10^{-5} among BRCA2 mutation carriers.

<table>
<thead>
<tr>
<th>SNP rs No.</th>
<th>Chr (Nearby Genes)</th>
<th>Genotype</th>
<th>Discovery Stage</th>
<th>Stage 2</th>
<th>Combined</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs9348512</td>
<td>Chr6 (TFAP2A, C6orf218)</td>
<td>CC</td>
<td>390 (46.4)</td>
<td>1392 (43.0)</td>
<td>1640 (42.3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CA</td>
<td>368 (43.8)</td>
<td>1515 (43.4)</td>
<td>1883 (43.5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AA</td>
<td>82 (9.8)</td>
<td>368 (10.5)</td>
<td>450 (10.4)</td>
</tr>
<tr>
<td>rs619373</td>
<td>ChrX (FGF13)</td>
<td>GG</td>
<td>693 (75.8)</td>
<td>2882 (82.7)</td>
<td>3575 (82.6)</td>
</tr>
<tr>
<td>rs184577</td>
<td>Chr2 (C2orf58)</td>
<td>GG</td>
<td>520 (61.9)</td>
<td>2104 (60.3)</td>
<td>2624 (60.6)</td>
</tr>
</tbody>
</table>

1P-value was calculated based on the 1-degree of freedom score test.

doi:10.1371/journal.pgen.1003173.t002
that had stronger associations than the originally identified SNP in the breast cancer susceptibility region that should be replicated in the general population. In BRCA2 mutation carriers, evidence for a breast cancer association with genetic variants in PTHLH has been restricted previously to ER-negative tumors [25]; however, the novel susceptibility variant we reported here was associated with risk of ER+ and ER- breast cancer.

The novel SNP rs9348512 (6p24) is located in a region with no known genes (Figure 1). C6orf218, a gene encoding a hypothetical protein LOC221718, and a possible tumor suppressor gene, TFAP2A, are within 100 kb of rs9348512. TFAP2A encodes the AP-2α transcription factor that is normally expressed in breast ductal epithelium nuclei, with progressive expression loss from normal, to ductal carcinoma in situ, to invasive cancer [26,27]. AP-2α also acts as a tumor suppressor via negative regulation of MYC [28] and augmented p53-dependent transcription [29]. However, the minor allele of rs9348512 was not associated with gene expression changes of TFAP2A in breast cancer tissues in The Cancer Genome Atlas (TCGA) data; this analysis might not be informative since expression of TFAP2A in invasive breast tissue is low [26,27]. Using the TCGA data and a 1 Mb window, expression changes with genotypes of rs9348512 were observed for GCNT2, the gene encoding the enzyme for the blood group I antigen glucosaminyl (N-acetyl) transferase 2. GCNT2, recently found to be overexpressed in highly metastatic breast cancer cell lines [30] and basal-like breast cancer [31], interacts with TGF-β to promote epithelial-to-mesenchymal transition, enhancing the metastatic potential of breast cancer [31]. An assessment of alterations in expression patterns in normal breast tissue from BRCA2 mutation carriers by genotype are needed to further evaluate the functional implications of rs9348512 in the breast tumorigenesis of BRCA2 mutation carriers.

To determine whether the breast cancer association with rs9348512 was limited to BRCA2 mutation carriers, we compared results to those in the general population genotyped by BCAC and to BRCA1 mutation carriers in CIMBA. No evidence of an association between rs9348512 and breast cancer risk was observed in the general population (OR = 1.00, 95% CI 0.98–1.02, P = 0.74) [14], nor in BRCA1 mutation carriers (HR = 0.99, 95% CI 0.94–1.04, P = 0.75) [13]. Stratifying cases by ER status, there was no association observed with ER-subtypes in either the general population or among BRCA1 mutation carriers (BCAC: ER positive P = 0.89 and ER negative P = 0.60; CIMBA BRCA1: P = 0.49 and P = 0.99, respectively). For the two SNPs associated with breast cancer with P < 10^{-5}, neither rs619373, located in FGF13 (Xq26.3), nor rs184577, located in CYP1B1-AS1 (2p22-p21), was associated with breast cancer risk in the general population [14] or among BRCA1 mutation carriers [13]. The narrow CIs for the overall associations in the general population and in BRCA1 mutation carriers rule out associations of magnitude similar to those observed for BRCA2 mutation carriers. The consistency of the association in the discovery and replication stages and by country, the strong quality control measures and filters, and the clear cluster plot for rs9348512 suggest that our results constitute the discovery of a novel breast cancer susceptibility locus specific to BRCA2 mutation carriers rather than a false positive finding. Replicating this SNP in an even larger population of BRCA2 mutation carriers would be ideal, but not currently.

**Figure 1. Associations between SNPs in the region surrounding rs9348512 on chromosome 6 and breast cancer risk.** Results based on imputed and observed genotypes. The blue spikes indicate the recombination rate at each position. Genotyped SNPs are represented by diamonds and imputed SNPs are represented by squares. Color saturation indicates the degree of correlation with the SNP rs9348512. doi:10.1371/journal.pgen.1003173.g001
ZNF365 mutation carriers. Knowledge of the 6p24 locus might associate with breast cancer risk in the general population and/or that are specific modifiers of breast cancer risk in BRCA2 development in provide further insights into the biology of breast cancer BRCA2 may yet be discovered; their detection would require assembling risks at the 5th and 95th percentiles of the combined genotyped BRCA2 newly identified CDKN2A/B, LSP1 known breast cancer susceptibility loci at FGFR2, TOX3, 12p11, 5q11, CDKN2A/B, LSP1, 8q24, ESR1, ZNF365, 3p24, 12q24, 5p12, 11q13 and the newly identified BRCA2 modifier locus at 6p24. The figure shows the risks at the 5th and 95th percentiles of the combined genotyped distribution as well as minimum, maximum and average risks. doi:10.1371/journal.pgen.1003173.g002

Figure S1 Cluster plots for SNPs (A.) rs9348512, (B.) rs619373, and (C.) rs184577. (TIF)

Figure S2 Multidimensional scaling plots of the top two principal components of genomic ancestry of all eligible BRCA2 iCOGS samples plotted with the HapMap CEU, ASI, and YRI samples: (A.) samples from Finland and BRCA2 617delIT carriers highlighted, and (B.) samples, indicated in red, with >19% non-European ancestry were excluded. (TIF)

Figure S3 Quantile–quantile plot comparing expected and observed distributions of P-values. Results displayed (A) for the complete sample, (B) after excluding samples from the GWAS discovery stage, and (C) for the complete sample and a set of SNPs from the iCOGS array that were selected independent from the results of the BRCA2 mutation carriers. (TIF)

Figure S4 Manhattan plot of P-values by chromosomal position for 18,086 SNPs selected on the basis of a previously published genome-wide association study of BRCA2 mutation carriers. Breast cancer associations results based on 4,330 breast cancer cases and 3,881 unaffected BRCA2 carriers. (TIF)

Figure S5 Forest plot of the country-specific, per-allele hazard ratios (HR) and 95% confidence intervals for the association between breast cancer and rs9348512 genotypes. (TIF)

Figure S6 Forest plot of the country-specific, per-allele hazard ratios (HR) and 95% confidence intervals for the association with breast cancer for (A.) rs619373 and (B.) rs184577 genotypes. (TIF)

Table S1 Quality control filtering steps for BRCA2 mutation carriers and SNPs on the COGs array. (DOC)

Table S2 Description of breast cancer affected and unaffected BRCA2 carriers included in the final analysis of the COGs array SNPs. (DOC)

Table S3 Breast cancer hazards ratios (HR) and 95% confidence intervals (CI) for all SNPs with P<10^-5 in a 500 Mb region around rs9348512 on 6p24 among BRCA2 mutation carriers. (DOC)

Table S4 Associations with SNPs at 6p24, FGF13 and 2p22 and breast and ovarian cancer risk using a competing risk analysis model. (DOC)

Acknowledgments

iCOGS: We acknowledge the contributions of Kyriaki Michailidou, Jonathan Tyrer, and Ali Amin Al Olama to the iCOGS statistical analyses and Shahana Ahmed, Melanie J. Maranian, and Catherine S. Healey for their contributions to the iCOGS genotyping quality control process.

Consortium of Modifiers of BRCA1/2 Associations (CIMBA): The authors would like to acknowledge the contribution of the staff of the genotyping unit under the supervision of Dr. Sylvie LaBoissi`ere as well as Frédéric Robidoux from the McGill University and G´enome Que´bec Innovation Centre.

Breast Cancer Association Consortium (BCAC): We thank all the individuals who took part in these studies and all the researchers, possible because we know of no investigators with appropriate data and germline DNA from BRCA2 mutation carriers who did not contribute their mutation carriers to iCOGS. However, CIMBA studies continue to recruit individuals into the consortium.
	rs9348512 (6p24) is the first example of a common susceptibility variant identified through GWAS that modifies breast cancer risk specifically in BRCA2 mutation carriers. Previously reported BRCA2-modifying alleles for breast cancer, including those in FGFR2, TOX3, MAP3K1, LSP1, 2q35, SLC4A7, 5p12, 1p11.2, ZNF365, and 19p13.1 (ER-negative only) [18,32,33], are also associated with breast cancer risk in the general population and/or BRCA1 mutation carriers. Knowledge of the 6p24 locus might provide further insights into the biology of breast cancer development in BRCA2 mutation carriers. Additional variants that are specific modifiers of breast cancer risk in BRCA2 carriers may yet be discovered; their detection would require assembling larger samples of BRCA2 mutation carriers in the future.

While individually each of the SNPs associated with breast cancer in BRCA2 mutation carriers are unlikely to be used to guide breast cancer screening and risk-reducing management strategies, the combined effect of the general and BRCA2-specific breast cancer susceptibility SNPs might be used to tailor manage subsets of BRCA2 mutation carriers. Taking into account all loci associated with breast cancer risk in BRCA2 mutation carriers from the current analysis, including the 6p24 locus, the 5% of the BRCA2 mutation carriers at lowest risk were predicted to have breast cancer risks by age 80 in the range of 21–47% compared to 83–100% for the 5% of mutation carriers at highest risk on the basis of the combined SNP profile distribution. These results might serve as a stimulus for prospective trials of the clinical utility of such modifier panels.
clinicians, technicians, and administrators who have enabled this work to be carried out.

Amsterdam Breast Cancer Study (ABCs): We thank Annejen Broeks, Sten Cornelissen, Richard van Hien, Linde Braaf, Senno Verhoef, Laura van ’t Veer, Emiel Rutgers, Ellen van der Schout, and Femke Atsma.

Bavarian Breast Cancer Cases and Controls (BBCC): We thank Lothar Haeberle, Sonja Oeser, Silke Landrith, and Reiner Strick.

British Breast Study (BBS): We thank Eileen Williams, Elaine Ryder-Mills, and Kara Sarges.

Breast Cancer Family Registry (BCFR) Studies: Samples from the NC-BCFR were processed and distributed by the Coriell Institute for Medical Research. We wish to thank members and participants in the Breast Cancer Family Registry for their contributions to the study. The ABCFS would like to also thank Maggie Angelakos, Judi Maskiell, and Angela Jones.

BRCA-gene mutations and breast cancer in South African women (BMBSA): We wish to thank the families who contribute to the BMBSA study.

Beckman Research Institute of the City of Hope (BRICOH): We thank Frank Greg Wilhite, Yuan Chun Ding, Linda Steele, and Marie Pinto for their work in participant enrollment and biospecimen and data management.

Breast Cancer Study of the University Clinic Heidelberg (BUSCH): We thank Peter Bugert, Medical Faculty Mannheim.

Copenhagen General Population Study (CGPS): We appreciate the staff and participants of the Copenhagen General Population Study. For the excellent technical assistance, we thank Dortele Uhall Andeles, Maria Birna Arnadottir, Anne Bank, and Dorthe Kjeldgaard Hansen.

Spanish National Cancer Centre (CNIO): We wish to thank Alicia Barroso, Rosario Alonso, and Guillermo Pita for their assistance.

Spanish National Cancer Centre Breast Cancer Study (CNIO-BCS): We thank Charo Alonso, Guillermo Pita, Nuria Alvarez, Daniel Herrero, Primitiva Menendez, Jose Ignacio Arias Perez, Filar Zamora, the Human Genotyping-CEGEN Unit (CNIO), and the BFCOC-LT for providing DNA samples.


Breast Cancer in Galway Genetic Study (BIGGS): We thank Niall McInerney, Gabrielle Colleran, Andrew Rowan, and Angela Jones.

British Breast Cancer Study (BBCS): We thank Annegien Jobson. Institut Gustave Roussy, Villejuif: Brigitte Bressac-de-Boisredon, Dominique Stoppa-Lyonnet, Marion Gauthier-Villars, Bruno Buecher, Le´one; and Service de Ge ´ne´tique Oncologique, Institut Curie, Paris: Equipe «Ge ´ne´tique du cancer du sein», Centre de Recherche en des Cancers Fre´quents, Hospices Civils de Lyon - Centre Le´on Be´rard, & des Cancers Rares, Hospices Civils de Lyon - Centre Jean Dausset: Bernard Peissel, Daniela Zaffaroni, and Giulia Savarese and Aline Martayain of the Istituto Nazionale Tumori Regina Margherita, Fondazione IRCCS, Aviano (PN); Liliana Varesco of the IRCCS Human Genotyping-CEGEN Unit (CNIO).

Additional cases were recruited in the context of the VERDI study. We thank Hartwig Ziegler, Sonja Wolf, and Volker Hermann.

German Consortium of Hereditary Breast and Ovarian Cancer (GC-HBOC): We are very thankful to all family members who participated in this study; Wolfram Heinritz, Center Leipzig, and Dieter Schäfer, Center Frankfurt, for providing DNA samples; and Juliane Kohler for excellent technical assistance; as well as Heide Hellebrand, Sabine Euerger, and GC-HBOC.

Genetic Modifiers of Cancer Risk in BRCA1/2 Mutation Carriers (GEMO): National Cancer Genetics Network «UNICANCER Genetic Groups», France. We wish to thank all the GEMO collaborating groups for their contribution to this study. GEMO Collaborating Centers are: Coordinating Centres, Unité Mixte de Génétique Constitutionnelle des Cancers Fréquents, Hospices Civils de Lyon - Centre Léon Bérard, & Equipe «Génétique du cancer du sein», Centre de Recherche en Cancérologie: C. Seynave, O. Obana, J. Gélot, S. Mounier, M. Alamdari, Laura Barjhoux, Carole Verny-Pierre, Sophie Giraud, Mélanie Léonce; and Service de Génétique Oncologique, Institut Curie, Paris: Dominique Stoppa-Lyonnet, Marion Gauthier-Villars, Bruno Buecher, Claude Houl ścian, Virginie Moncier, Muriel Belotti, Carole Tirapó, Antoine de Pauw. Institut Gustave Roussy, Villejuif: Brigitte Bressac-de-
contribute to kConFab.


Gene Environment Interaction and Breast Cancer in Germany (GENICA): The GENICA network: Dr. Margarete Fischer-Bosch-Institute of Clinical Pharmacology, Stuttgart, and University of Tubingen, Germany; [CJ, Hiltrud Brauch], Department of Internal Medicine, Evangelische Krankenhaus Bonn, anHB, Johanniter Krankenhaus, Bonn, Germany; [Jon-Ducher, Ko, Christiane], Institute of Pathology, University of Bonn, Bonn, Germany [Hand-Peter Fischer], Molecular Genetics of Breast Cancer, Deutsches Krebsforschungszentrum (DKFZ), Heidelberg, Germany [UH]; and Institute for Prevention and Occupational Medicine of the German Social Accident Insurance (lPA), Bochum, Germany [Thomas Bruning, Beate Pesch, Sylvia Rabstein, Anne Spieckermann, VH].

Hospital Clinico San Carlos (HCSC): We acknowledge Alicia Tosar for her technical assistance.

Helsinki Breast Cancer Study (HEBCS): HEBCS would like to thank Drs. Kristiina Aittomäki, Carl Blomqvist and Kirsimari Aaltonen, and Taru A. Muronen and RN Irja Erkikila for their help with the HEBCS data and samples.

Hannover-Minsk Breast Cancer Study (HMBCS): We thank Natalia Bogdanova, Natalia Antonenkov, Hans Christiansen, and Peter Hillersen.

Study of Genetic Mutations in Breast and Ovarian Cancer patients in Hong Kong and Asia (HRBCP): We wish to thank Hong Kong Sanatorium and Hospital for their continual support.

Molecular Genetic Studies of Breast- and Ovarian Cancer in Hungary (HUNBOSC): We wish to thank the Hungarian Breast and Ovarian Cancer Study Group members [Janos Papp, Aniko Bozík, Kristof Arvai, Judith Franko, Maria Balogh, Gabriella Varga, Judith Ferenczi, Department of Molecular Genetics, National Institute of Oncology, Budapest, Hungary], and the clinicians and patients for their contributions to this study.

University Hospital Vall d’Hebron (HV1): We thank the study staff and participants.

Interdisciplinary HEalth Research Internal Team BReast Cancer Susceptibility (INHERIT): We would like to thank Dr. Martine Dumontier, Martine Tranchant for sample management and skillful technical assistance.

Kuopio Breast Cancer Project (KBCP): We thank Eija Myohanen and Helena Kemilainen.

Kathleen Cunningham Consortium for Research into Familial Breast Cancer ([kComFab/AOCS]: We thank Heather Thorne, Eveline Niedermayr, all the kComFab research nurses and staff, the heads and staff of the Family Cancer Clinics, and the Clinical Follow Up Study for their contributions to this resource, and the many families who contribute to kComFab.

Leuven Multidisciplinary Breast Centre (LMBC): We thank Gillian Peuteman, Dominiek Smeets, Thomas Van Brussel, and Kathleen Corthouts.

Mammary Carcinoma Risk Factor Investigation (MARIE): We thank Dieter Flech-Jans, Rebecca Hein, Stefan Nikels, Muhabbett Gelik, Sabine Behrens, and Ursula Elber.

Milan Breast Cancer Study Group (MBCSG): We thank Daniela Zaffaroni of the Fondazione Istituto Nazionale Tumori, Milan, Italy and the personnel of the CGT laboratory at IFOM-IEO Campus, Milan, Italy.


Gene Environment Interaction and Breast Cancer in Germany (GENICA): The GENICA network: Dr. Margarete Fischer-Bosch-Institute of Clinical Pharmacology, Stuttgart, and University of Tubingen, Germany; [CJ, Hiltrud Brauch], Department of Internal Medicine, Evangelische Krankenhaus Bonn, anHB, Johanniter Krankenhaus, Bonn, Germany; [Jon-Ducher, Ko, Christiane], Institute of Pathology, University of Bonn, Bonn, Germany [Hand-Peter Fischer], Molecular Genetics of Breast Cancer, Deutsches Krebsforschungszentrum (DKFZ), Heidelberg, Germany [UH]; and Institute for Prevention and Occupational Medicine of the German Social Accident Insurance (lPA), Bochum, Germany [Thomas Bruning, Beate Pesch, Sylvia Rabstein, Anne Spieckermann, VH].

Hospital Clinico San Carlos (HCSC): We acknowledge Alicia Tosar for her technical assistance.

Helsinki Breast Cancer Study (HEBCS): HEBCS would like to thank Drs. Kristiina Aittomäki, Carl Blomqvist and Kirsimari Aaltonen, and Taru A. Muronen and RN Irja Erkikila for their help with the HEBCS data and samples.

Hannover-Minsk Breast Cancer Study (HMBCS): We thank Natalia Bogdanova, Natalia Antonenkov, Hans Christiansen, and Peter Hillersen.

Study of Genetic Mutations in Breast and Ovarian Cancer patients in Hong Kong and Asia (HRBCP): We wish to thank Hong Kong Sanatorium and Hospital for their continual support.

Molecular Genetic Studies of Breast- and Ovarian Cancer in Hungary (HUNBOSC): We wish to thank the Hungarian Breast and Ovarian Cancer Study Group members [Janos Papp, Aniko Bozík, Kristof Arvai, Judith Franko, Maria Balogh, Gabriella Varga, Judith Ferenczi, Department of Molecular Genetics, National Institute of Oncology, Budapest, Hungary], and the clinicians and patients for their contributions to this study.

University Hospital Vall d’Hebron (HV1): We thank the study staff and participants.

Interdisciplinary HEalth Research Internal Team BReast Cancer Susceptibility (INHERIT): We would like to thank Dr. Martine Dumontier, Martine Tranchant for sample management and skillful technical assistance.

Kuopio Breast Cancer Project (KBCP): We thank Eija Myohanen and Helena Kemilainen.

Kathleen Cunningham Consortium for Research into Familial Breast Cancer ([kComFab/AOCS]: We thank Heather Thorne, Eveline Niedermayr, all the kComFab research nurses and staff, the heads and staff of the Family Cancer Clinics, and the Clinical Follow Up Study for their contributions to this resource, and the many families who contribute to kComFab.

Leuven Multidisciplinary Breast Centre (LMBC): We thank Gillian Peuteman, Dominiek Smeets, Thomas Van Brussel, and Kathleen Corthouts.
Cecilia Zvocić, Qun Niu, physicians, genetic counselors, research nurses and staff of the Cancer Risk Clinic for their contributions to this resource, and the many families who contribute to our program.

University of California Los Angeles (UCLA): We thank Joyce Seldon MSGC and Lorna Kwan MPH for assembling the data for this study.

University of California San Francisco (UCSF): We would like to thank Ms. Salina Chan for her data management and the following genetic counselors for participant recruitment: Beth Crawford, Nicola Stewart, Julie Mak, and Kate Lamvik.

United Kingdom Breakthrough Generations Study (UKBGS): We thank Breakthrough Breast Cancer and the Institute of Cancer Research for support of the Breakthrough Generations Study, and the study participants, study staff, and the doctors, nurses, and other health care providers and health information sources who have contributed to the study.

United Kingdom Familial Ovarian Cancer Registries (UKFOCR): We thank Simen Yang, Susan O’Connor, Carole Pye, Patricia Harrington, and Eva Wozniak for their contributions towards the UKFOCR.

Victorian Familial Cancer Trials Group (VFCTG): We acknowledge Geoffrey Lindeman, Marion Harris, Martin Delatycki of the Victorian Familial Cancer Trials Group. We thank Sarah Sawyer and Rebecca Driessen for assembling this data and Ella Thompson for performing all DNA amplification.

Author Contributions


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