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


1 Epidemiology Research Program, American Cancer Society, Atlanta, Georgia, United States of America, 2 Centre for Cancer Genetic Epidemiology, Department of Public Health and Primary Care, University of Cambridge, Cambridge, United Kingdom, 3 Clinical Genetics Service, Memorial Sloan-Kettering Cancer Center, New York, New York, United States of America, 4 Program in Cancer Biology and Genetics, Memorial Sloan-Kettering Cancer Center, New York, New York, United States of America, 5 Division of Epidemiology, Department of Environmental Medicine, New York University School of Medicine, New York, New York, United States of America, 6 Centre for Cancer Genetic Epidemiology, Department of Oncology, University of Cambridge, Cambridge, United Kingdom, 7 Genetics and Population Health Division, Queensland Institute of Medical Research, Brisbane, Australia, 8 Cancer Genomics Laboratory, Centre Hospitalier Universitaire de Quebec and Laval University, Quebec City, Quebec, Canada, 9 Unité Mètie de Génétique Constitutionnelle des Cancers Fréquents, Hospices Civils de Lyon–Centre Léon Bérard, Lyon, France, 10 INSERM U1052, CNRS UMR5286, Université Lyon 1, Centre de Recherche en Cancérologie de Lyon, Lyon, France, 11 Department of Health Sciences Research, Mayo Clinic, Rochester, Minnesota, United States of America, 12 Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota, United States of America, 13 Department of Epidemiology, Rollins School of Public Health, Emory University, Atlanta, Georgia, United States of America, 14 Centre d’Innovation Génomique Quebec et Université McGill, Montreal, Quebec, Canada, 15 Family Cancer Clinic, Netherlands Cancer Institute, Amsterdam, The Netherlands, 16 Institut Curie, Department of Tumour Biology, Paris, France, 17 Institut Curie, INSERM U830, Paris, France, 18 Université Paris Descartes, Sorbonne Paris Cité, Paris, France, 19 Kathleen Cunningham Consortium for Research into Familial Breast Cancer–Peter MacCallum Cancer Center, Melbourne, Australia, 20 Unit of Molecular Bases of Genetic Risk and Testing, Department of Preventive and Predictive Medicine, Fondazione IRCCS Istituto Nazionale Tumori, Milan, Italy, 21 IFOM, Fondazione Istituto FIRC di Oncologia Molecolare, Milan, Italy, 22 University Hospital of Cologne, Cologne, Germany, 23 Abramson Cancer Center, The University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania, United States of America, 24 Department of Medicine, The University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania, United States of America, 25 Gynecologic Oncology Group Statistical and Data Center, Roswell Park Cancer Institute, Buffalo, New York, United States of America, 26 Department of Obstetrics and Gynecology and Comprehensive Cancer Center, Medical University of Vienna, Vienna, Austria, 27 Department of Clinical Genetics, Odense University Hospital, Odense, Denmark, 28 Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Ontario, Canada, 29 Center for Genomic Medicine, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark, 30 Department of Population Sciences, Beckman Research Institute of City of Hope, Duarte, California, United States of America, 31 Genetic Counseling Unit, Hereditary Cancer Program, IDIBELL–Catalan Institute of Oncology, Barcelona, Spain, 32 Clinical Genetics Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Rockville, Maryland, United States of America, 33 Department of Medical Oncology, Dana-Farber/Partners CancerCare, Boston, Massachusetts, United States of America, 34 Clinical Cancer Genetics (for the City of Hope Clinical Cancer Genetics Community Research Network), City of Hope, Duarte, California, United States of America, 35 Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Ontario, Canada, 36 Departments of Molecular Genetics and Laboratory Medicine and Pathobiology, University of Toronto, Ontario, Canada, 37 Department of Dermatology, University of Utah School of Medicine, Salt Lake City, Utah,
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 confirm whether previously identified breast cancer susceptibility alleles are replicated in BRCA2 mutation carriers, we conducted a deep replication of an ongoing GWAS discovery study. Using the ranked P-values of the breast cancer associations with the imputed mutation carriers. This panel may have clinical utility for women with BRCA2 background. This comprehensive update of novel and previously reported breast cancer susceptibility loci contributes to BRCA2 with several tumor suppressor genes. This report identifies the first breast cancer risk locus specific to a BRCA2 of an ongoing GWAS discovery study. Using the ranked P-values of the breast cancer associations with the imputed
This is an open-access article, free of all copyright, and may be freely reproduced, distributed, transmitted, modified, built upon, or otherwise used by anyone for any lawful purpose. The work is made available under the Creative Commons CC0 public domain dedication.

Funding: This work was supported by the following institutions: ICOCG: The creation of the custom Illumina multiplex chip and the genotyping of the BRCa2 carriers in CIMBA was made possible by grants from the Starr Cancer Consortium H4-A02 (PI: K Ofti), the Sandra Taub Memorial Fund of the Breast Cancer Research Foundation (PI: K Ofti), the Norman and Carol Stone Cancer Genetics Fund (PI: K Ofti), and the European Commission’s Seventh Framework Programme grant agreement 223175 (HEALTH-F2-2009-223175). AC Antoniou is a Cancer Research UK Senior Cancer Research Fellow. G Chevenix-Trench is an NHMRC Senior Principal Research Fellow. Consortium of Modifiers of BRCa1/2: The CIMBA data management and data analysis were supported by Cancer Research UK grants U1229/2/A1174 and C1287/A10118. S Healey is supported by an NHMRC Program Grant to G Chevenix-Trench. AC Antoniou is a Cancer Research UK Senior Cancer Research Fellow. G Chevenix-Trench is an NHMRC Senior Principal Research Fellow. Amsterdam Breast Cancer Study: The ABCs study was supported by the Dutch Society Cancer (grants NKI 2007-3839; 2004.363); BBMRI-NL, which is a Research Infrastructure financed by the Dutch government (NWO 100.021.007); and the Dutch National Genomics Initiative. Bavarian Breast Cancer Cases and Controls: The work of the BBCC was partly funded by ELAN-Fond of the University Hospital of Erlangen. British Breast Cancer Study: The BBCS is funded by Cancer Research UK and Breakthrough Breast Cancer and acknowledges NHS 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), Ontario Familial Breast Cancer Registry (DFCRC), and Utah (Utah Breast CFR) work was supported by the United States National Cancer Institute, National Institutes of Health, under RFA-CA-06-003 (P30 CA13696 and P30 ES009089), and through cooperative agreements with members of the BCRF and Principal Investigators, including Cancer Care Ontario (U01 CA 94674), Columbia University (U01 CA69398), Cancer Prevention Institute of California (U01 CA69417), Fox Chase Cancer Center (U01 CA66301), Huntsman Cancer Institute (U01 CA49446), and University of Miami (CA6938). The ABCFS was also supported by the National Health and Medical Research Council of Australia, the New South Wales Cancer Council, 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 Soutey is an NHMRC Senior Breast Cancer Research Fellow and a Victorian Breast Cancer Research Consortium Group Leader. Baltic Familial Breast Ovarian Cancer Consortium: BFOCC is partly supported by: Lithuania: (BFBOCC-LT), Research Council of Lithuania grant LG 19-2010, and Hereditary Cancer Association (Paveldžio vėžo asociacija). Latvia: (BFBOCC-LV) is partly supported by grant 09.0001.06.000 1/2/2010. Sweden: Cancer Grant no 2009/0202/IDP/1.1.2.0/09/001/001. Genexa 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 E Van Rensburg NIH R01CA47411 and P30 CA033752. Beckman Research Institute of the City of Hope: SL Neuhausen was partially supported by the Morris and Horowitz Families Endowed Professorship. BRCIC was supported by the Morris and Horowitz Clinic Heidelberg: The KM Gruber Breast Cancer Study was supported by the Maximilian Foundation Hopp Fonds, the Helmholtz Society and the German Cancer Research Center (DKFZ). Righospitaleit: The CBS study was supported by the NEYE Foundation. CECILE Breast Cancer Study: The CECILE study was funded by Fondation de Frante, 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 COGS was supported by the Chief Physician Johan Boserup and Lise Boserup Fund, the Danish Medical Research Council and Herlev Hospital. Spanish National Cancer Centre Breast Cancer Study: The CNIO-BCS was supported by the Genome Spain Foundation, the Red Tematicana de Investigación Cooperativa en cáncer and grants from the Asociación Española Contra el Cáncer and the Fondo de Investigación Sanitario (PI11/00523 and PI11B10). City of Hope Cancer Center: The City of Hope Clinical Cancer Genetics Community Research Network is supported by Award Number RC4153828 (PI: JW Neitzel) from the National Cancer Institute and the Office of the Director, National Institutes of Health. CONSORZIO Studi Italiani sui Tumori Ereditari Alla Mammella: CONSIT TEAM was funded by grants from Fondazione Italiana per la Ricerca sul Cancro (Special Project “Hereditary tumours”), Italian Association for Cancer Research (AIRC, IG 8713), Italian Ministry of Health (Extraordinary National Cancer Program 2006, “Alleanza contro il Cancro” and “Progetto Tumori Femminili”), Italian Ministry of Education, University and Research (Prin 2008) Centro di Ascolto Donne Operate al Seno (CADS) association and from funds by italian citizens who allocated the 5 x 1000 share of their tax payment in support of the Fondazione RIRCS Istituto Nazionale Tumori, according to Italian laws (INT-Institutional strategic projects “S x 1000”). German Cancer Research Center: The DKFZ study was supported by the DKFZ. Genen omgeving studie van de werkgroep Hereditair Borstkanker Onderzoek Nederland. The DNA HEBON study is supported by the Dutch Cancer Society grants N011998-1824, N02060-3088, NK0207-3756, the NWO grant 91109024, the Pink Ribbon grant 110005, and the BBMRI grant CP46/NWO. Epidemiological study of BRCA1 & BRCa2 mutation carriers: EMBRACE is supported by Cancer Research UK Grants C1287/A10118 and C1287/A11990. DG Evans is supported by an NIH grant to the Biomedical Research Centre, Manchester. ESTHER Breast Cancer Study: The ESTHER study was supported by a grant from the Baden Württemberg Ministry of Science, Research and Arts. Additional support is required in the context of the VERDI study, which was supported by a grant from the German Cancer Aid (Deutscher Krebsfonds). German Consortium for Breast and Ovarian Cancer: GC-HBOC is supported by the German Cancer Aid (grant no 109876); by the Center for Molecular Medicine Cologne (CMMC), and by Deutsche Krebshilfe (107 352). GC-HBOC is supported by Deutsche Krebshilfe. Genetic Modifiers of cancer risk in BRCA1/2 mutation carriers: The GEMO study was supported by the Ligue Nationale Contre le Cancer; the Association “Le cancer du sein, parlons-en!” Award and the Canadian Institutes of Health Research for the “CIHR Team in Familial Risks of Breast Cancer” program. Gene Environment Interaction and Breast Cancer in Germany: The GENICA was funded by the Federal Ministry of Education and Research (BMBF) Germany grants 01KWK975/5, 01KWK976/6, 01KWK977/0 and 01KW0114, the Robert Bosch Foundation, Stuttgart, Deutsches Krebsforschungszentrum (DKFZ), Heidelberg, Institute for Prevention and Occupational Medicine of the German Social Accident Insurance (IFA), Bochum, as well as the Department of Internal Medicine, Evangelische Kliniken Bonn gGmbH, Johanniter Krankenhau, Bonn, Germany, Gynecologic Oncology Group. This study was supported by National Cancer Institute grants to the Gynecologic Oncology Group (GOG) Administrative Office and Tissue Bank (CA 27469), the GOG Statistical and Data Center (CA 35717), and GOG’s Cancer Prevention and Control Committee (CA 101165). MH Greene and PL Mai are supported by funding from the Intramural Research Program, NCI. Hospital Clinicino San Carlos: HCSC was supported by a grant RD06/0020/0021 from RTICCC (ISCII), Spanish Ministry of Economy and Competitiveness. Helsinki Breast Cancer Study: The HEBCS was financially supported by the Helsinki University Central Hospital Research Fund, Academy of Finland (132473) the Finnish Cancer Society, the Nordic Cancer Union, and the Santander Bank Foundation. Hannover-Minck Breast Cancer Study: The HMBCS was supported by a grant from the Friends of Hannover Medical School and by the Rudolf Bartling Foundation. Study of Genetic Mutations in Breast and Ovarian Cancer patients in Hong Kong and Asia: HERBCP is supported by The Hong Kong Hereditary Breast Cancer Family Registry and the Dr. Ellen Li Charitable Foundation, Hong Kong. Molecular Genetics Study of Breast and Ovarian Cancer in Hungary: Hungarian Breast Cancer Grant KTA-OTKA CK-80745 and the Hungarian EAA Financial Mechanism HU115/NA/2008-3/OP-9. Instituto Catalán de Oncología: The ICO study was supported by the Asociacion Española Contra el Cancer, Spanish Health Research Foundation, Ramón Areces Foundation, Carlos III Health Institute, Catalan Health Institute, and Autonomous Government of Catalonia and contract grant numbers ISCIII, RETIC RD06/0020/1051, PI09/02483, P101/01422, P101/00748, 2009SG2920, and 2009SG283. Iceland Landsdísptali-University Hospital: The ILUH group was supported by grants from the Landspítali University Hospital Research Fund. Interdisciplinary Health Research Internal Team Breast Cancer susceptibility: INHERIT work was supported by the Canadian Institutes of Health Research for the “CIHR Team in Familial Risks of Breast Cancer” program, the Canadian Breast Cancer Research Alliance grant 019511 and the Ministry of Economic Development, Innovation and Export Trade grant PSI-SIRI-701. J Simard is Chairholder of the Canada Research Chair in Oncogenetics. Istituto Oncologico Venezoto: The IOVHBCS study was supported by Ministero dell’Istruzione, dell’University e della Ricerca, Italia, University of Genova and the Roberto and Maria Luisa Carella Foundation (Project PON 000269, Ricerca Finalizzata, project N° 1C02/86.9). Karolinska Breast Cancer Study: The KARBAC study was supported by the Swedish Cancer Society, the Gustav V Jubileum Foundation, and the Bert von Kantzow Foundation. Kuopio Breast Cancer Project: The KBCP was supported financially by the special Government Funding (EVO) of Kuopio University
Introduction

The lifetime risk of breast cancer associated with carrying a \textit{BRCA2} mutation varies from 40 to 84\% [1]. To determine whether common genetic variants modify breast cancer risk for \textit{BRCA2} mutation carriers, we previously conducted a GWAS of \textit{BRCA2} mutation carriers from the Consortium of Investigators of Modifiers of \textit{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 young and healthy women, we replicated 21 SNPs with P-values less than 1.0x10\(^{-7}\). These include three SNPs at 10q26 (rs2981575; \(P = 1.2 \times 10^{-8}\)). 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 additional \textit{BRCA2} mutation carriers, only published loci associated with breast cancer risk in the general population, including \textit{FGFR2} (10q26; rs2981575; \(P = 1.2 \times 10^{-8}\)), were associated with breast cancer risk at the genome-wide significance level among \textit{BRCA2} mutation carriers.
breast cancer risk in **BRCA2** in CIMBA, which represents the largest, international collection of mutation carriers, we conducted an extended replication of the **BRCA2** genotype association with breast cancer. ZNF365 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 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.

**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 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.

**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. SNPs lists from a **BRCA1/2** 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+TSI3 samples on HapMap3, release 2. A ranked list of SNPs was based on the 1-df trend test statistic, after excluding highly correlated SNPs (r2 > 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 850 kb in both directions of the SNP, based on HapMap2 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.

**Genotyping.** The genotyping was performed on DNA samples from 10,048 **BRCA2** mutation carriers at the McGill University and Génomique Québec Innovation Centre (Montreal, Canada). As a quality control measure, each plate included DNA samples from six individuals who were members of two CEPI1 trios. Some plates also contained three duplicate pairs of quality control samples. Genotypes were called using GenCall [9]. Initial calling was based on a cluster file generated using 270 samples from Hapmap2. To generate the final calls, we first selected a subset of 3,018 individuals, including samples from each of the genotyping centers in the iCOGS project, each of the participating
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^{-5}), 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); 18,086 of which were selected on the basis of the discovery 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 CIMBA 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 BRCA2 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 modelling 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 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<5×10^{-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 = 2×10^{-14}) in FGFR2 and rs3803662 (P = 5.4×10^{-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 (CDK2A1/1B) 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 = 6×10^{-5}).

One SNP, rs9348512 at 6p24 not known to be associated with breast cancer, had a combined P-value of association of 3.9×10^{-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 = 5.2×10^{-9}). The minor allele of rs9348512 (MAF = 0.35) was associated with a 15% decreased risk of breast cancer within BRCA2 mutation carriers (per allele HR = 0.85, 95% CI 0.80-0.90) with no evidence of heterogeneity between country strata (P = 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 = 1.5×10^{-6} and rs184577: HR = 0.86, 95% CI 0.79-0.93, P = 2.0×10^{-4}, after excluding prevalent cases). The estimates for the HRs were virtually unchanged (Table S4).

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 tumors (p = 7.3×10^{-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, CDK2A1/1B, LSP1, 8q24, ESRI, 2q13, 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 8q26 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 (CDK2A1/1B), we found uncorrelated SNPs
Table 1. Per allele hazard ratios (HR) and 95% confidence intervals (CI) of previously published breast cancer loci among BRCA2 mutation carriers from previous reports and from the iCOGS array, ordered by statistical significance of the region.

<table>
<thead>
<tr>
<th>Chr (Nearby Genes)</th>
<th>Report Status¹</th>
<th>SNP</th>
<th>r²</th>
<th>Minor Allele</th>
<th>Previously Reported Results</th>
<th>iCOGS Results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Ref</td>
<td>Affected N</td>
</tr>
<tr>
<td>10q26  (FGFR2)</td>
<td>reported</td>
<td>n2981575</td>
<td>G</td>
<td>[2]</td>
<td>2,155 2,016</td>
<td>1.28 (1.18, 1.39)</td>
</tr>
<tr>
<td></td>
<td>novel</td>
<td>n2420946</td>
<td>0.96</td>
<td>A</td>
<td></td>
<td>4,328</td>
</tr>
<tr>
<td>16q12  (TOX3)</td>
<td>reported</td>
<td>n3803662</td>
<td>A</td>
<td>[2]</td>
<td>2,162 2,026</td>
<td>1.20 (1.10, 1.31)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>n10771399</td>
<td>G</td>
<td>[34]</td>
<td>3,798 3,314</td>
<td>0.93 (0.84, 1.04)</td>
</tr>
<tr>
<td></td>
<td>novel</td>
<td>n27633</td>
<td>0.05</td>
<td>C</td>
<td></td>
<td>4,252</td>
</tr>
<tr>
<td>5q11  (MAF3K1)</td>
<td>reported</td>
<td>n889312</td>
<td>C</td>
<td>[24]</td>
<td>2,840 2,282</td>
<td>1.10 (1.01, 1.19)</td>
</tr>
<tr>
<td></td>
<td>novel</td>
<td>n1688611</td>
<td>0.14</td>
<td>A</td>
<td></td>
<td>4,330</td>
</tr>
<tr>
<td>9p21  (CDKN2A/B)</td>
<td>reported</td>
<td>n1019970</td>
<td>A</td>
<td>[34]</td>
<td>3,807 3,316</td>
<td>1.09 (1.00, 1.18)</td>
</tr>
<tr>
<td></td>
<td>novel</td>
<td>n1095163</td>
<td>0.00</td>
<td>A</td>
<td></td>
<td>4,329</td>
</tr>
<tr>
<td>11p15 (LSPI1)</td>
<td>reported</td>
<td>n3817198</td>
<td>G</td>
<td>[24]</td>
<td>3,266 2,636</td>
<td>1.14 (1.06, 1.23)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>n13281615</td>
<td>G</td>
<td>[24]</td>
<td>3,338 2,723</td>
<td>1.06 (0.98, 1.13)</td>
</tr>
<tr>
<td>8q24</td>
<td>reported</td>
<td>n4733664</td>
<td>0.00</td>
<td>A</td>
<td></td>
<td>4,329</td>
</tr>
<tr>
<td>20q13</td>
<td>reported</td>
<td>n311498³</td>
<td>A³</td>
<td>[5]</td>
<td>3,808 3,318</td>
<td>0.95 (0.84, 1.07)</td>
</tr>
<tr>
<td></td>
<td>novel</td>
<td>n13039229</td>
<td>0.00</td>
<td>C</td>
<td></td>
<td>4,326</td>
</tr>
<tr>
<td>6q25  (ESR1)</td>
<td>reported</td>
<td>n59397435</td>
<td>G</td>
<td>[35]</td>
<td>3,809 3,316</td>
<td>1.14 (1.01, 1.27)</td>
</tr>
<tr>
<td></td>
<td>novel</td>
<td>n2253407</td>
<td>0.01</td>
<td>C</td>
<td></td>
<td>4,330</td>
</tr>
<tr>
<td>10q21  (ZNF365)</td>
<td>reported</td>
<td>n16917302</td>
<td>C</td>
<td>[5]</td>
<td>3,807 3,315</td>
<td>0.83 (0.75, 0.93)</td>
</tr>
<tr>
<td></td>
<td>novel</td>
<td>n17221319</td>
<td>0.00</td>
<td>A</td>
<td></td>
<td>4,330</td>
</tr>
<tr>
<td>3p24  (SLC4A2, NEX10)</td>
<td>reported</td>
<td>n4973768</td>
<td>A</td>
<td>[24]</td>
<td>3,370 2,783</td>
<td>1.10 (1.03, 1.18)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>n1290514³</td>
<td>A³</td>
<td>[34]</td>
<td>2,530 2,342</td>
<td>0.94 (0.87, 1.01)</td>
</tr>
<tr>
<td>5p12</td>
<td>reported</td>
<td>n10941679³</td>
<td>G</td>
<td>[24]</td>
<td>3,263 2,591</td>
<td>1.09 (1.01, 1.19)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>n614367</td>
<td>A</td>
<td>[34]</td>
<td>3,789 3,307</td>
<td>1.03 (0.95, 1.13)</td>
</tr>
<tr>
<td>11q13</td>
<td>reported</td>
<td>n51249433</td>
<td>G</td>
<td>[35]</td>
<td>3,423 2,827</td>
<td>1.09 (1.02, 1.17)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>n168170</td>
<td>A</td>
<td>[5]</td>
<td>3,655 3,086</td>
<td>0.98 (0.90, 1.07)</td>
</tr>
<tr>
<td>17q23  (STXBP4, COX11)</td>
<td>reported</td>
<td>n6504950</td>
<td>A</td>
<td>[24]</td>
<td>3,401 2,813</td>
<td>1.03 (0.95, 1.11)</td>
</tr>
<tr>
<td>19p13  (MERITAO)</td>
<td>reported</td>
<td>n8170</td>
<td>A</td>
<td>[5]</td>
<td>3,655 3,086</td>
<td>0.98 (0.90, 1.07)</td>
</tr>
<tr>
<td>2q35</td>
<td>reported</td>
<td>n1338704³</td>
<td>G</td>
<td>[24]</td>
<td>3,300 2,646</td>
<td>1.05 (0.98, 1.13)</td>
</tr>
<tr>
<td>9q31</td>
<td>reported</td>
<td>n865686</td>
<td>C</td>
<td>[34]</td>
<td>3,799 3,312</td>
<td>0.95 (0.89, 1.01)</td>
</tr>
<tr>
<td>10q22  (ZMIZ1)</td>
<td>reported</td>
<td>n704010</td>
<td>A</td>
<td>[34]</td>
<td>3,761 3,279</td>
<td>1.01 (0.95, 1.08)</td>
</tr>
</tbody>
</table>

¹Reporting status of the SNP is either previously reported or novel to this report.
²p-value was calculated based on the 1-degree of freedom score test statistic.
³rs311499 could not be designed onto the iCOGS array. A surrogate (r² = 1.0), rs311498, was included, however, and reported here.
⁴Stronger associations were originally reported for the SNP, assuming a dominant or recessive model of the 'risk allele'.

doi:10.1371/journal.pgen.1003173.t001
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. Chr. (Nearby Genes)</th>
<th>Genotype</th>
<th>Discovery Stage</th>
<th>Stage 2</th>
<th>Combined</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Affected No. (%)</td>
<td>Unaffected No. (%)</td>
<td>HR (95% CI)</td>
</tr>
<tr>
<td>rs9346512 Chr6 (TFAP2A, C6orf218)</td>
<td>CC</td>
<td>390 (46.4)</td>
<td>248 (38.3)</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>CA</td>
<td>368 (43.8)</td>
<td>299 (46.2)</td>
<td>0.81 (0.67-0.96)</td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>82 (9.8)</td>
<td>100 (15.5)</td>
<td>0.55 (0.42-0.74)</td>
</tr>
<tr>
<td></td>
<td>per allele</td>
<td>0.76 (0.67-0.87)</td>
<td>2.6×10^-5</td>
<td>0.87 (0.81-0.93)</td>
</tr>
<tr>
<td>rs619373 ChrX (FGF13)</td>
<td>GG</td>
<td>693 (75.8)</td>
<td>568 (87.8)</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>GA</td>
<td>143 (15.7)</td>
<td>78 (12.1)</td>
<td>1.43 (1.13-1.80)</td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>4 (8.5)</td>
<td>1 (0.1)</td>
<td>2.01 (0.50-8.06)</td>
</tr>
<tr>
<td></td>
<td>per allele</td>
<td>1.43 (1.15-1.78)</td>
<td>3.0×10^-3</td>
<td>1.27 (1.12-1.44)</td>
</tr>
<tr>
<td>rs184577 Chr2 (C2orf58)</td>
<td>GG</td>
<td>520 (61.9)</td>
<td>368 (56.9)</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>GA</td>
<td>278 (33.1)</td>
<td>234 (36.2)</td>
<td>0.86 (0.71-1.03)</td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>42 (5.0)</td>
<td>45 (7.0)</td>
<td>0.67 (0.46-0.96)</td>
</tr>
<tr>
<td></td>
<td>per allele</td>
<td>0.84 (0.73-0.97)</td>
<td>1.5×10^-2</td>
<td>0.86 (0.79-0.93)</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-2a 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-2a 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 associations 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
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 FGF2, TOX3, MAP3K1, LSP1, ESRI, 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 2. Predicted breast cancer risks for BRCA2 mutation carriers by the combined SNP profile distributions. Based on the known breast cancer susceptibility loci at FGF2, TOX3, 12p11, 5q11, CDKN2A/B, LSP1, 8q24, ESRI, 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.

Supporting Information

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<10−7 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 LaBoissière as well as Frédéric Robidoux from the McGill University and Génome Québec Innovation Centre.

Breast Cancer Association Consortium (BCAC): We thank all the individuals who took part in these studies and all the researchers,
clinicians, technicians, and administrators who have enabled this work to be carried out.

Amsterdam Breast Cancer Study (ABCs): We thank Anneegje Broeks, Sten Cornelissen, Richard van Hien, Linde Braat, Senno Verhoeef, Laura van ’t Veer, Emiel Rutgers, Ellen van der Schoot, and Femke Atsum.

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

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

Breast Cancer Family Registry (BCFR) Studies: Samples from the NC-BCFR were processed and distributed by the Correll 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 Elaine Ryder-Mills 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 Dorthe Uldall Andersen, Maria Birna Arnadottir, Anne Bank, and Dorte Kyeldahl Hansen.

Spanish National Cancer Centre (CNIO): We 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, Pilar Zamora, the Human Genotyping-CEGEN Unit (CNG), the Spanish Familial Breast Ovarian Cancer Consortium (BFBOCC), and the Spanish Breast Cancer Study of the University Clinic Heidelberg (BBS). We thank Niall McInerney, Gabrielle Colleran, Andrew Rowan, and Angela Jones.

Breast Cancer in Galway Genetic Study (BIGGS): We thank Marie Pinto for their work in participant enrollment and biospecimen and product management.

Breast Cancer Study of the University Clinic Heidelberg (BUSA): 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 Dorthe Uldall Andersen, Maria Birna Arnadottir, Anne Bank, and Dorte Kyeldahl Hansen.

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

Spanish National Cancer Centre Breast Cancer Study (CNIO-BCS): We thank Chaoqan Li, Gilina Dallinger, Santiago Jaramillo, and Giulia Pellenz for their work in participant enrollment and biospecimen and data management.

Breast Cancer Study of the University Clinic Heidelberg (BUSA): 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 Dorthe Uldall Andersen, Maria Birna Arnadottir, Anne Bank, and Dorte Kyeldahl Hansen.

Spanish National Cancer Centre (CNIO): We 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, Pilar Zamora, the Human Genotyping-CEGEN Unit (CNG), the Spanish Familial Breast Ovarian Cancer Consortium (BFBOCC), and the Spanish Breast Cancer Study of the University Clinic Heidelberg (BBS). We thank Niall McInerney, Gabrielle Colleran, Andrew Rowan, and Angela Jones.

Breast Cancer in Galway Genetic Study (BIGGS): We thank Marie Pinto for their work in participant enrollment and biospecimen and product management.

Breast Cancer Study of the University Clinic Heidelberg (BUSA): 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 Dorthe Uldall Andersen, Maria Birna Arnadottir, Anne Bank, and Dorte Kyeldahl Hansen.

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

Spanish National Cancer Centre Breast Cancer Study (CNIO-BCS): We thank Chaoqan Li, Gilina Dallinger, Santiago Jaramillo, and Giulia Pellenz for their work in participant enrollment and biospecimen and data management.
We thank Drs. Kristiina Aittomäki, Carl Blomqvist and Kirsimari Aaltonen, Department of Molecular Genetics, National Institute of Oncology, Helsinki, Finland (FINBOCS) for their data management support.

We thank E. Krol-Warmerdam, and J. Blom for patient accrual, administering questionnaires, and managing clinical information. The LUMC survival data were retrieved from the Leiden hospital-based cancer registry system (ONCODC) with the help of Dr. J. Mellenar.

The Ohio State University Comprehensive Cancer Center (OSUCCG): Kevin Sweet, Caroline Craven, and Michelle O’Connor were instrumental in accrual of study participants, ascertainment of medical records and database management. Samples were processed by the OSU Human Genetics Sample Bank.

We thank the NICCC National Familial Cancer Consultation Service team led by Sara Dishon, the lab team led by Dr. Flavio Lejbkowicz, and the research field operations team led by Dr. Milla Pinesch.

Oulu Breast Cancer Study (OBCS): We thank Katri Pykäs, Arja Jukkola-Vuorinen, Mervi Gripp, Saara Kaupulainen, Meri Otuskka, and Kari Mononen.

The National Israeli Cancer Control Center (NICCC): We wish to thank the NICCC National Familial Cancer Consultation Service team led by Sara Dishon, the lab team led by Dr. Flavio Lejbkowicz, and the research field operations team led by Dr. Milla Pinesch.

We thank Katri Pykäs, Arja Jukkola-Vuorinen, Mervi Gripp, Saara Kaupulainen, Meri Otuskka, and Kari Mononen.

Ontario Cancer Genetics Network (OCGN): We thank the study staff and participants.

We acknowledge Alicia Tosar for her technical assistance.

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.

We thank Natasha Bogdanova, Natalia Antonenkova, Hans Christiansen, and Peter Hilleurstensson.

Study of Genetic Mutations in Breast and Ovarian Cancer in Hungary (HUNBOCS): We wish to thank the Hungarian Breast and Ovarian Cancer Study Group members [Janos Papp, Aniko Bozsik, Kristof Arvai, Judit Franko, Maria Balogh, Gabriella Varga, Judit 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 (HVH): We thank the study staff and participants.

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

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

Kathleen Cuningham Consortium for Research into Familial Breast Cancer (kConFab/AOCS): We thank Heather Thorne, Eveline Niedermayr, all the kConFab 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 kConFab.

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


Gene Environment Interaction and Breast Cancer in Germany (GENICA): The GENICA network: Dr. Margarete Fischer-Bosch-Institute of Clinical Pharmacology, Stuttgart, and University of Tübingen, Germany; [CJ, Hiltrud Brauch], Department of Internal Medicine, Evangelische Krankenhaus Bonn eGmbH, Johanniter Krankenhaus, Bonn, Germany; [Yon-Dschun Ko, Christian Baisch], 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 (lP), Bochum, Germany [Thomas Bruening, Beate Pesch, Sylvia Rabstein, Anne Spichiger-Reichenbecher, 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. Muranen and RN Irja Erkkila for their help with the HEBCS data and samples.

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

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 (HUNBOCS): We wish to thank the Hungarian Breast and Ovarian Cancer Study Group members [Janos Papp, Aniko Bozsik, Kristof Arvai, Judit Franko, Maria Balogh, Gabriella Varga, Judit 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 (HVH): We thank the study staff and participants.

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

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

Kathleen Cuningham Consortium for Research into Familial Breast Cancer (kConFab/AOCS): We thank Heather Thorne, Eveline Niedermayr, all the kConFab 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 kConFab.

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

Mammary Carcinoma Risk Factor Investigation (MARIÉ): We thank Dieter Flesch-Janys, Rebecca Hein, Stefan Nickels, Muhabbel Gëkic, Sabine Behrens, and Ursula Elbert.

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.

Montreal Gene-Environment Breast Cancer Study (MTGEBCS): We thank Martine Tranchant (Cancer Genomics Laboratory, CRCHUQ), Marc-Yvan Layseca, Cécile Tourgeon, and Lea Heguy (McGill University Health Center, Royal Victoria Hospital; McGill University) for DNA extraction, sample management, and skillful technical assistance.

General Hospital Vienna (MUV): We thank the study staff and participants.

National Israeli Cancer Control Center (NCC): We wish to thank the NICCC National Familial Cancer Consultation Service team led by Sara Dishon, the lab team led by Dr. Flavio Lejbkowicz, and the research field operations team led by Dr. Milla Pinesch.

Oulu Breast Cancer Study (OBCS): We thank Katri Pykäs, Arja Jukkola-Vuorinen, Mervi Gripp, Saara Kaupulainen, Meri Otuskka, and Kari Mononen.

Ontario Cancer Genetics Network (OCGN): We thank the study staff and participants.

University Medical Centre Medical Breast Cancer Study (ORIGO): We thank E. Krol-Warmerdam, and J. Blom for patient accrual, administering questionnaires, and managing clinical information. The LUMC survival data were retrieved from the Leiden hospital-based cancer registry system (ONCODC) with the help of Dr. J. Mellenar.

The Ohio State University Comprehensive Cancer Center (OSUCCG): Kevin Sweet, Caroline Craven, and Michelle O’Connor were instrumental in accrual of study participants, ascertainment of medical records and database management. Samples were processed by the OSU Human Genetics Sample Bank.

Odense University Hospital (OUH): We thank the study staff and participants.

Università di Pisa (PBCS): We thank the study staff and participants.

The U.S. National Cancer Institute Polish Breast Cancer Study (OSUCCG): We thank the study collaborators Drs. Louise Brinton, Mark Sherman, Stephen Chanock, Neonila Szeszenia-Dabrowska, Beata Peplonska, and Witold Zatonski, as well as Pei Chao and Michael Stagner, for their data management support.

Rotterdam Breast Cancer Study (RBSC): We thank Petra Bos, Janmet Blom, Ellen Crepin, Elianthe Huisjens, Annette Heemskerk, and the Erasmus MC Family Cancer Clinic.

Sheffield Breast Cancer Study (SBCS): We thank Sue Higham, Helen Cranp, and Dan Corrigan.

Source East Asian Breast Cancer Association Study (SABASS): We would like to thank Yip Cheng Har, Nur Aishah Mohd Taib, Phuah Sze Yee, Norhashimah Hassan, and all the research nurses, research assistants, and doctors involved in the MyBcCa Study for assistance in patient recruitment, data collection, and sample preparation. In addition, we thank Philip Isu, Sze Yee, Norhashimah Hassan, and Sharifah Nor Akmal for contributing samples from the Singapore Breast Cancer Study and the HUKM-HKL Study respectively.

Study of Epidemiology and Risk Factors in Cancer Heredity (SEARCH): We thank the SEARCH and EPIC teams.

Sheba Medical Centre (SMC): SMC team wishes to acknowledge the assistance of the Meirav Comprehensive breast cancer center team at the Sheba Medical Center for assistance in this study.

Swedish Breast Cancer Study Group (SWE-BRCA): We thank the Swedish BRCA collaborators: from Lund University and University Hospital: Åke Borg, Håkan Olsson, Helena Jernström, Karin Henriksson, Katja Harbst, María Soller, Niklas Loman, Ulf Kristofferson; from Gothenburg Sahlgrenska University Hospital: Anna Olofverham, Margreta Nordling, Per Karlsson, Zakaria Einbicgi; from Stockholm and Karolinska University Hospital: Anna von Wachenfeldt, Amelie Liljenberg, Anika Lindholm, Brita Arver, Gisela Barbany Bustinza, Johanna Rantala; from Umeå University Hospital: Beatrice Melin, Christina Ehredolter Ardnon, Monica Emanuelsson; from Uppsala University: Hans Ehren- crona, Maritta Hellström Pigg, Richard Rosenquist; from Linköping University Hospital: Marie Stemmark-Aksamul, Sigrun Liedgren.

The University of Chicago Center for Clinical Cancer Genetics and Global Health (UCHICAGO): We wish to thank
References


