

# Five Polymorphisms and Breast Cancer Risk: Results from the Breast Cancer Association Consortium

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## Abstract

Previous studies have suggested that minor alleles for ERCC4 rs744154, TNF rs361525, CASP10 rs13010627, PGR rs1042838, and BID rs8190315 may influence breast cancer risk, but the evidence is inconclusive due to their small sample size. These polymorphisms were genotyped in more than 30,000 breast cancer cases and 30,000 controls, primarily of European descent, from 30 studies in the Breast Cancer Association Consortium. We calculated odds ratios (OR) and 95% confidence intervals (95% CI) as a measure of association. We found that the minor alleles for these polymorphisms were not related to invasive breast cancer risk overall in women of European descent: ECCR4 per-allele OR (95% CI) = 0.99 (0.97-1.02), minor allele frequency = 27.5%; TNF 1.00 (0.95-1.06), 5.0%; CASP10 1.02 (0.98-1.07), 6.5%; PGR 1.02 (0.99-1.06), 15.3%; and BID 0.98 (0.86-1.12), 1.7%. However, we

observed significant between-study heterogeneity for associations with risk for single-nucleotide polymorphisms (SNP) in CASP10, PGR, and BID. Estimates were imprecise for women of Asian and African descent due to small numbers and lower minor allele frequencies (with the exception of BID SNP). The ORs for each copy of the minor allele were not significantly different by estrogen or progesterone receptor status, nor were any significant interactions found between the polymorphisms and age or family history of breast cancer. In conclusion, our data provide persuasive evidence against an overall association between invasive breast cancer risk and ERCC4 rs744154, TNF rs361525, CASP10 rs13010627, PGR rs1042838, and BID rs8190315 genotypes among women of European descent. (Cancer Epidemiol Biomarkers Prev 2009;18(5):1610-6)

## Introduction

Results from genome-wide scans have confirmed common genetic variants with very small effects on breast cancer risk; however, this approach likely misses clinically and functionally important cancer susceptibility alleles because of false negatives and poor coverage in some regions. Therefore, examination of candidate genes still provides a useful, complementary approach. The Breast Cancer Association Consortium, a collaborative

data pooling effort of more than 60,000 subjects, was initiated to validate reported genetic associations with breast cancer risk. At least one member group had reported associations between breast cancer risk and ERCC4 rs744154 (1), TNF rs361525 (2), and CASP10 rs13010627 (3). Here, we also follow up on the Breast Cancer Association Consortium's previously published evidence of a suggestive association between PGR rs1042838 and breast cancer risk based on five studies that are also included in this report (4). In addition, BID rs8190315 was significantly associated with elevated breast cancer risk [per minor G allele, odds ratio (OR), 1.91; 95% confidence intervals (95% CI), 1.17-3.19] in the Sheffield Breast Cancer Study of 1,021 cases and 1,115 controls.<sup>31</sup> To precisely estimate the hypothesized weak effects of these polymorphisms, we assessed their association with breast cancer risk using more than 30,000 breast cancer cases and 30,000 controls in the Breast Cancer Association Consortium.

Received 8/10/08; revised 1/7/09; accepted 2/23/09.

Note: Supplementary data for this article are available at Cancer Epidemiology Biomarkers and Prevention Online (<http://cebp.aacrjournals.org/>).

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doi:10.1158/1055-9965.EPI-08-0745

<sup>31</sup> Unpublished data.

**Table 1. Summary ORs and 95% CIs for five polymorphisms and invasive breast cancer risk among women of European descent, based on data from ~30,000 cases and 30,000 controls from the Breast Cancer Association Consortium**

Gene, SNP description, and rs no.	No. of studies	Genotype	No. of cases	No. of controls	MAF	Pooled OR* (95% CI)	$P^\dagger$	Between-study heterogeneity <sup>‡</sup>
<i>ERCC4</i> , IVS1-807G>C, rs744154	27	GG	13,585	15,267	27.5	1.00	0.62	0.36
		GC	10,280	11,616		1.00 (0.97-1.04)		
		CC	1,878	2,191		0.97 (0.91-1.04)		
<i>TNF</i> , -417A>G, rs361525	28	per allele			5.0	0.99 (0.97-1.02)	0.93	0.65
		GG	25,623	28,815		1.00		
		GA	2,616	2,993		0.99 (0.94-1.05)		
<i>CASP10</i> , Ex9-188G>A, rs13010627	28	AA	94	93	6.5	1.14 (0.85-1.52)	0.33	0.008
		per allele				1.00 (0.95-1.06)		
		GG	23,456	26,597		1.00		
<i>PGR</i> , Ex4+72G>T, rs1042838	24	GA	3,352	3,706	15.3	1.03 (0.98-1.09)	0.26	0.04
		AA	109	126		0.94 (0.72-1.22)		
		per allele				1.02 (0.98-1.06)		
<i>BID</i> , S56G, rs8190315	11	GG	16,506	19,746	1.7	1.02 (0.98-1.06)	0.81	0.05
		GT	6,040	7,097		1.04 (0.93-1.17)		
		TT	583	664		1.04 (0.93-1.17)		
		per allele				1.02 (0.99-1.06)		
		AA	13,211	13,711	1.00			
		AG	452	478	0.99 (0.87-1.14)			
		GG	1	4		N/E		

Abbreviation: N/E, not estimated.

\*Unconditional logistic regression models were adjusted for study and included genotypes using indicator variables with homozygotes of the major allele as the reference category.

† We assumed a log-linear association with the number of minor alleles to calculate the  $P$  value for a linear trend.

‡ The  $P$  value for "between study heterogeneity" was calculated using an interaction variable with study and genotypes, which was tested for deviation from a multiplicative interaction model using the log likelihood ratio test to compare the fit of logistic models with and without an interaction term.

## Materials and Methods

**Study Populations.** Thirty case-control studies (described in Supplementary Table S1) contributed data to these analyses. Studies were conducted in Europe, the United States, and Australia, among women of primarily European descent, with the exception of the two Southeast Asian studies. Most studies (exceptions listed in Supplementary Table S2) provided information on disease status, age at diagnosis/enrollment, ethnic group (European, Asian, other), and first-degree history of breast cancer. All studies received approval from their institutional review committees and participants provided informed consent or were analyzed under specific coding procedures (Amsterdam Breast Cancer Study).

**Genotyping.** Genotyping platforms are detailed in Supplementary Table S1. Case and control counts, minor allele frequencies, tests for Hardy-Weinberg equilibrium, and completion proportions by study and locus can be found in Supplementary Table S3. Data on specific genotypes from studies with gross deviations from Hardy-Weinberg equilibrium ( $P > 10^{-4}$ ) were excluded from analyses (Norwegian Breast Cancer Study for *ERCC4* rs744154, Bavarian Breast Cancer Cases and Controls for *TNF* rs361525, Norwegian Breast Cancer Study and Taiwanese Breast Cancer Study for *CASP10* rs13010627; data not shown). A total of 4,819 subjects with >20% failed genotypes for the five single-nucleotide polymorphisms (SNP) in this analysis (6.2% overall—7.2% of invasive cases, 16.6% of *in situ* cases, and 5.0% of controls) were excluded from analyses (data not shown). Although numbers for each SNP varied based on study participation in genotyping efforts, 34,239 invasive

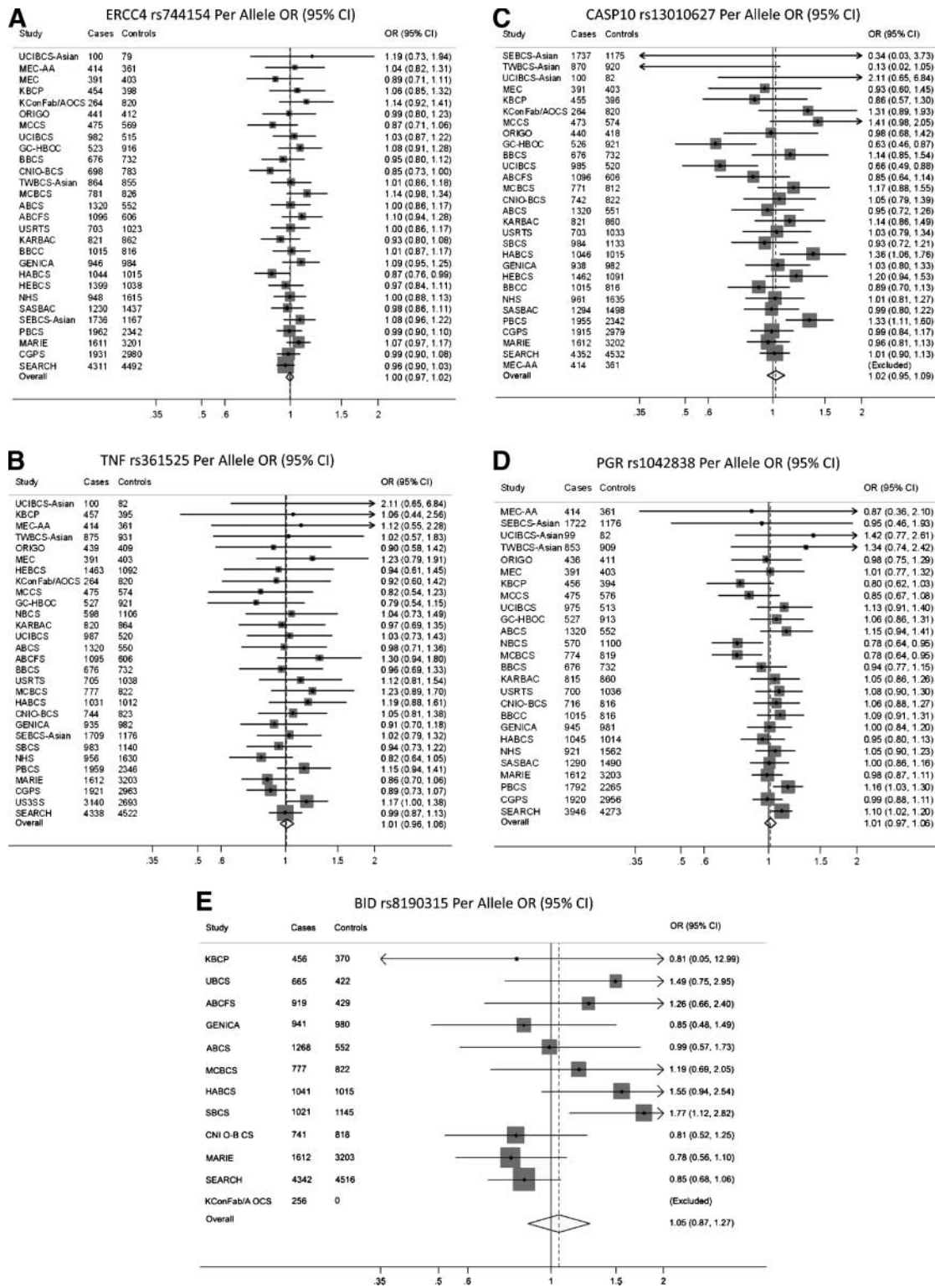
breast cancer cases, 1,092 *in situ* cases, and 37,214 controls were the maximum numbers of subjects available for statistical analysis. Call rates for these cases and controls were >94% for all assays in each study (Supplementary Table S3).

**Statistical Analyses.** Multivariate, random-effects models were weighted for each study by the within-study and between-study variances (5). For *BID* rs8190315, a Cochran-Mantel-Haenszel meta-analysis was done using Genie (6, 7) to account for familial relationships present in the Utah Breast Cancer Study. The presence of between-study heterogeneity was assessed by the  $Q$  test (5). Women of Asian and African descent, comprising  $\geq 10\%$  of the study population, were treated separately in statistical models.

We also used unconditional logistic regression models to estimate pooled ORs and 95% CIs for the association between individual SNPs and breast cancer risk for all studies combined (8). All models were adjusted for race and study, but not age, because age was missing for more than half of participants from seven studies (Supplementary Table S2). However, the inclusion of age did not alter OR estimates in analyses of data from studies with available information (data not shown). Exclusion of studies with Hardy-Weinberg equilibrium  $P$  values between  $10^{-4}$  and 0.05 did not substantially alter overall ORs (data not shown). All analyses were done with STATA (version 10.0) or Genie (6, 7) when the Utah Breast Cancer Study was included.

## Results

Most invasive breast cancer cases were diagnosed with ductal carcinomas (73.1%), were stage I (50.5%) or stage



**Figure 1.** Per-allele ORs and 95% CIs for breast cancer risk by study are shown for *ERCC4* rs744154 (A), *TNF* rs361525 (B), *CASP10* rs13010627 (C), *PGR* rs1042838 (D), and *BID* rs8190315 (E), based on data from ~ 30,000 cases and 30,000 controls from the Breast Cancer Association Consortium. Studies are weighted and ranked according to the inverse of the variance of the log OR estimate. The size of the box is inversely proportional to the variance of the log OR estimate. The solid line is drawn where OR is equal to 1.0, and the dotted line is at the OR estimate for all studies combined. Estimates were calculated separately for women of European, Asian (labeled as “Asian”), and African (labeled as “AA”) descent. Study acronyms are defined in Supplementary Table S1.

II (42.3%) disease, and were moderately differentiated (49.2%). The mean ( $\pm$  SD) age was 54.9 ( $\pm$  11.6) years for invasive cases, 55.7 ( $\pm$  10.7) years for *in situ* cases, and 56.5 ( $\pm$  12.5) years for controls. A larger proportion of invasive and *in situ* cases (16.3% and 23.7%, respectively) had a family history of breast cancer than controls (5.0%). Most subjects were of European descent (90.0% of invasive cases, 96.3% of *in situ* cases, and 92.4% of controls). Other women were of African descent (1.9%, 1.4%, and 1.5%, respectively), Asian descent (8.0%, 2.0%, and 5.9%, respectively), and unknown ancestry (0.1%, 0.3%, and 0.1%, respectively). Among controls of European descent, pooled estimates for the minor allele frequencies of *ERCC4* rs744154, *TNF* rs361525, *CASP10* rs13010627, *PGR* rs1042838, and *BID* rs8190315 ranged from 1.7% to 27.5% (Table 1). Minor allele frequencies were slightly lower for controls of African and Asian descent with the exception of *CASP10* rs13010627 (Supplementary Table S4).

Figure 1 displays the random effects OR for the association between genotype and overall breast cancer risk by study and ethnic group, whereas Table 1 displays the ORs for the association between genotype and invasive breast cancer risk among women of European descent only. In both models, none of the SNPs were associated with invasive breast cancer risk with per-allele ORs close to unity (Table 1; Fig. 1). Among women of European descent (Table 1), there was some evidence for between-study differences for *CASP10* rs13010627 (between-study heterogeneity,  $P = 0.008$ ), *PGR* rs1042838 ( $P = 0.04$ ), and *BID* rs8190315 ( $P = 0.05$ ), but not for *ERCC4* rs744154 ( $P = 0.36$ ) and *TNF* rs361525 ( $P = 0.65$ ). Genotypes were also not associated with *in situ* breast cancer risk with the possible exception of *BID* rs8190315 (Supplementary Table S5).

The genotype-invasive breast cancer risk association was not modified by age (Supplementary Table S6) or family history of breast cancer (Supplementary Table S7). Given the compelling association previously found for *CASP8* rs1045485 and breast cancer risk (9) and the functional interaction of *CASP8* and *BID* in the apoptosis pathway, we also tested for an interaction between *CASP8* rs1045485 and *BID* rs8190315. Assuming dominance for the high-risk allele at each locus, we found no evidence of departure from multiplicative effects ( $P = 0.75$ ).

## Discussion

In a pooled study of more than 30,000 cases and 30,000 controls, the minor alleles of *ERCC4* rs744154, *TNF* rs361525, *CASP10* rs13010627, *PGR* rs1042838, and *BID* rs8190315 were not associated with risk of invasive breast cancer. We observed significant between-study heterogeneity for results of *CASP10* rs13010627, *PGR* rs1042838, and *BID* rs8190315. Outlier studies for the three SNPs did not differ from other studies with respect to genotyping quality, study population characteristics, or study design. The source of observed between study heterogeneity is thus unknown and could be due to chance.

Despite previous epidemiologic results (1-4, 10-17) and, in some cases, biological plausibility (18-20), it is unlikely that there are weak associations between the

genetic polymorphisms under study and breast cancer risk. The narrow confidence intervals excluded log-additive relative risks  $>1.1$  for "risk" variants (or  $<0.9$  for "protective" variants) and even smaller risks for some variants. Larger sample sizes would be needed to evaluate moderate recessive effects, particularly for *TNF* rs361525. It is possible that other genetic variations conferring elevated breast cancer risk might exist in or around these genes. Further, although we did not comprehensively examine interaction between these SNPs and other breast cancer risk factors, the identification of strong, common modifiers is unlikely based on the persuasive lack of main effects. We found no evidence of interaction with age, family history of breast cancer, or, in the case of *BID* rs8190315, with *CASP8* rs1045485.

This analysis of selected candidate polymorphisms and breast cancer risk in 30 studies further supports the utility of large consortia. We have shown compelling evidence of no overall association with breast cancer risk for *ERCC4* rs744154, *TNF* rs361525, *CASP10* rs13010627, *PGR* rs1042838, and *BID* rs8190315, although they may be associated with breast cancer risk for small subgroups of women.

## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

## Acknowledgments

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We thank all the participants for taking part in this research. The MCCS is supported by the Australian National Health and Medical Research Council (grants 209057, 251533, 396414, 504711). Cohort recruitment and follow up is funded by The Cancer Council Victoria. The MCBCS was supported by the National Institutes of Health grant and the U.S. medical research and materiel command breast cancer IDEA award W81XWH-04-1-0588. ELG is a Fraternal Order of the Eagles Cancer Research Fellow. The MARIE study is supported by the Deutsche Krebshilfe e.V. (Project number 70-2892-Br I). We thank Tracy Slang and Elke Mutschelknauss for supervision of patient recruitment and clinical data collection and Ursula Eilber, Sabine Behrens, Nicole Knese-Yanning, and Belinda Kasperreit for excellent technical support. The KBCP was supported by the special Government Funding (EVO) of Kuopio University Hospital grants, Finnish Cancer Organisations and Cancer Fund of North Savo. We are grateful to Eija Myöhänen for technical assistance. The KARBAC was supported by the Swedish Cancer Society, the Gustav the V Jubileé Foundation, the FoU Foundation and the Bert von Kantzows Foundation. The HEBCS was supported by The Academy of Finland (project 110663), Helsinki University Central Hospital Research Funds, The Sigrid Juselius Foundation and The Finnish Cancer Society. We thank Drs. Carl Blomqvist and Kristiina Aittomöki and Research Nurse Kirsi Leinonen for help with patient contacts and data collection. The HABCS was supported by an intramural grant of Hannover Medical School. NB was generously supported by the Friends of Hannover Medical School. We gratefully acknowledge the technical assistance of Marion Haidukiewicz in DNA sample preparation. We furthermore thank Peter Hillemanns, Christof Sohn, Alexander Scharf, Michael Bremer and Johann Hinrich Karstens for their invaluable support in terms of infrastructure and patient samples. The GC-HBOC study was supported by Deutsche Krebshilfe (107054), by the Center of Molecular

Medicine, Cologne, and the Helmholtz society. We are thankful to Bernd Frank for participating in genotyping. The *GENICA* study was supported by the German Human Genome Project and funded by the Federal Ministry of Education and Research (BMBF) Germany grants 01KW9975/5, 01KW9976/8, 01KW9977/0 and 01KW0114. Genotyping analyses were supported by Deutsches Krebsforschungszentrum, Heidelberg and the Robert Bosch Foundation of Medical Research, Stuttgart, Germany. Yon Ko was involved in the design of the *GENICA* study and was responsible for patient recruitment and collection of clinical data. Beate Pesch and Thomas Bruning were involved in the design of the *GENICA* study and responsible for recruitment of the study subjects as well as collection of epidemiological data. The *CNIO-BCS* was supported by the Genome Spain Foundation. We thank Anna González-Neira, Charo Alonso and Tais Moreno for their technical support. The *CGPS* was supported by Chief Physician Johan Boserup and Lise Boserup Fund, the Danish Medical Research Council and Copenhagen County. The *BBCS* and the Mammography Oestrogens and Growth Factors study are funded by CR-UK and Breakthrough Breast Cancer. Funding of the *ABCS* study was provided by the Dutch Cancer Society (grants NKI 2001-2423; 2007-3839) and the Dutch National Genomics Initiative. *ABCS* acknowledges Richard van Hien, Linde Braaf, Rob Tollenaar and other contributors to the 'BOSOM' study, and the support of dr. H.B. Bueno-de-Mesquita for organizing the release of control DNA. The *ABCFS* was funded by the National Health and Medical Research Council, the Victorian Health Promotion Foundation, the New South Wales Cancer Council, and as part of the Breast Cancer Family Registry funded by the National Cancer Institute (USA) under RFA # CA-95-003. We want to thank Letitia Smith for her contribution to the genotyping for *ABCFS*. John Hopper is an Australia Fellow of the National Health and Medical Research Council (NHMRC) and Melissa Southey is a NHMRC Senior Research Fellow. From the University of Southern California, we thank Loreall Pooler and David Wong for their laboratory assistance for the *MEC* study. The *MEC* Study was supported by National Cancer Institute (NCI) grants CA63464 and CA54281. *NHS* acknowledges knowledge Pati Soule and Hardeep Ranu of the DF/HCC High Throughput Polymorphism Detection Core. *NHS* was supported by NCI grants CA 0657245 and CA098233. The *ORIGO* study was funded by the Dutch Cancer Society; we thank Jannet Blom, Elly Krol-Warmerdam, and Petra Huijts for patient recruitment and collection of clinical data. The *PBCS* was funded by Intramural Research Funds of the National Cancer Institute, Department of Health and Human Services, USA. The authors would like to thank Dr. Neonila Szeszenia-Dabrowska of the Nofer Institute of Occupational Medicine (Lodz, Poland) and Witold Zatonski of the M.Sklodowska-Curie Institute of Oncology and Cancer Center (Warsaw, Poland) for their contribution to the *PBCS*. The *SASBAC* study was supported by funding from the Agency for Science, Technology and Research of Singapore (A\*STAR), the United States National Institute of Health (NIH) and the Susan G. Komen Breast Cancer Foundation. The *SBCS* was supported by Yorkshire Cancer Research and the Breast Cancer Campaign. We would like to thank Sue Higham, Helen Cramp, Dan Connley, Ian Brock, Gordon MacPherson, Nitai Bhattacharyya and Mark Meuth for their contribution to the *SBCS*. *SEARCH* was funded by Cancer Research-UK (CR-UK); contributing authors, PDPP is a Senior Clinical Research Fellow. The *SEBCS* was supported by a grant from the National Research and Development Program for Cancer Control, Ministry of Health and Welfare, Republic of Korea (0620410-1). The *TWBCS* was supported by the Department of Health, Taiwan. *UCIBCS* is supported by grants from the NCI (CA2 R01 CA58860-14) and the Lon v smith Foundation (LVSF-41027, 3834, 42344). The *USRT* study was supported in part by the Intramural Research Program of the Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, USA. The authors are grateful to the radiologic technologists who participated in the *USRT* Study; Jerry Reid of the American Registry of Radiologic

Technologists for continued support of this study; Diane Kampa and Allison Iwan of the University of Minnesota for data collection and study coordination; Chris McClure of Research Triangle International, Inc. for tracing and data management; and, Laura Bowen of Information Management Services for biomedical computing. For the *UBCS*, genotype data and analysis was supported by a Susan G. Komen Foundation grant (BCTR0706911) and an NIH grant (CA98364). Data collection was supported by NCI, assisted by the Utah Cancer Registry, which is funded by contract number N01-PC-35141 with additional support from the Utah State Department of Health and the University of Utah. Partial support for all datasets with the Utah Population Database was provided by the University of Utah Huntsman Cancer Institute. The authors are grateful to Study Coordinators, Laboratory Specialist Kim Nguyen, and Computer Specialist Jathine Wong. We wish to 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 (funded by NHMRC grants 145684, 288704 and 454508) for their contributions to this resource, and the many families who contribute to *kConFab*. *kConFab* is supported by grants from the National Breast Cancer Foundation, the National Health and Medical Research Council (NHMRC) and by the Queensland Cancer Fund, the Cancer Councils of New South Wales, Victoria, Tasmania and South Australia, and the Cancer Foundation of Western Australia. *ACS/AOCS*: The AOCS Management Group (D Bowtell, G Chenevix-Trench, A deFazio, D Gertig, A Green, P Webb) gratefully acknowledges the contribution of all the clinical and scientific collaborators (see <http://www.aocstudy.org/>), AOCS and the ACS Management Group (A Green, P Parsons, N Hayward, P Webb, D Whiteman) thank all of the project staff, collaborating institutions and study participants. Financial support was provided by: U.S. Army Medical Research and Materiel Command under DAMD17-01-1-0729, the Cancer Council Tasmania and Cancer Foundation of Western Australia (AOCS study); The National Health and Medical Research Council of Australia (199600) (ACS study). The content of this manuscript does not necessarily reflect the views or policies of the National Cancer Institute or any of the collaborating centres in the CFRs, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government or the CFRs Centers. The genotyping and analysis were supported by grants from the National Health and Medical Research Council (NHMRC). *ABS* was funded by an NHMRC Career Development Award, and GC-T and JLH are NHMRC Senior Principal Research Fellows.

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