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Evidence for *SMAD3* as a modifier of breast cancer risk in *BRCA2* mutation carriers

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ABSTRACT

Introduction: Current attempts to identify genetic modifiers of *BRCA1* and *BRCA2* associated risk have focused on a candidate gene approach, based on knowledge of gene functions, or the development of large genome wide association studies. In this study, we evaluated 24 single nucleotide polymorphisms (SNPs) tagged to 14 candidate genes derived through a novel approach that analysed gene expression differences to prioritize candidate modifier genes for association studies.

Methods: We successfully genotyped 24 SNPs in a cohort of up to 4,724 *BRCA1* and 2,693 *BRCA2* female mutation carriers from 15 study groups and assessed whether these variants were associated with risk of breast cancer in *BRCA1* and *BRCA2* mutation carriers.

Results: SNPs in five of the 14 candidate genes showed evidence of association with breast cancer risk for *BRCA1* or *BRCA2* carriers ($P<0.05$). Notably, the minor alleles of two SNPs (rs7166081 and rs3825977) in high linkage disequilibrium (LD) ($r^2=0.78$), located at the *SMAD3* locus (15q22), were each associated with increased breast cancer risk for *BRCA2* mutation carriers (Relative risk [RR] = 1.25, 95% CI: 1.07 – 1.45, $P_{trend} = 0.004$ and RR = 1.20, 95% CI: 1.03 – 1.40, $P_{trend} = 0.018$).

Conclusions: This study provides evidence that the *SMAD3* gene, which encodes a key regulatory protein in the transforming growth factor beta (TGF-beta) signalling pathway and is known to interact directly with BRCA2, may contribute to increased risk of breast cancer in *BRCA2* mutation carriers. This finding suggests that genes with expression associated with *BRCA1* and *BRCA2* mutation status are enriched for the presence of common genetic modifiers of breast cancer risk in these populations.

INTRODUCTION

BRCA1 and *BRCA2* mutation carriers are at increased risk for developing breast and/or ovarian cancer. Estimates of the cumulative risk of breast cancer by age 70 years range from 46% to 87% for *BRCA1* mutation carriers and from 43% to 84% for *BRCA2* mutation carriers [1-6]. Evidence from these studies suggests that breast cancer risks in mutation carriers are modified by environmental or genetic factors. A number of large studies, facilitated through the Consortium of Investigators of Modifiers of *BRCA1*/*BRCA2* (CIMBA), have evaluated associations between genetic polymorphisms and breast cancer risk in *BRCA1* and *BRCA2* mutation carriers [7-15].

The candidate gene (or candidate SNP) approach for identifying potential risk modifiers has been successfully used to identify a SNP in the 5' untranslated region of *RAD51*. Until recently, this finding has provided the most reliable evidence for a genetic modifier in *BRCA2* mutation carriers [7]. However, a major disadvantage of using this approach to identify common genetic modifiers of breast cancer is the limited understanding of mechanisms and pathways that underlie breast cancer development in families carrying mutations in *BRCA1* or *BRCA2*.An alternative and powerful approach that can overcome such issues is the use of genome wide association (GWA) studies to identify candidate SNPs. Analysis of breast cancer risk-associated SNPs identified by a large population-based GWA study of breast cancer [16] has shown that several of these SNPs also appear to modify risk in *BRCA1* and/or *BRCA2* mutation carriers [8]. However, not all of the breast cancer-associated SNPs assessed have been found to modify risk in carriers, and some of the risk associations are specific for *BRCA2* mutation carriers only and not *BRCA1* [8]. While GWA studies specifically addressing risk for *BRCA1* and/or *BRCA2* carriers is a more direct approach to identifying modifiers of these genes using an agnostic approach, GWA studies require large sample sizes to identify genetic modifiers with confidence. To address the problem of inadequate sample size, the Consortium of Investigators of Modifiers of *BRCA1* and *BRCA2* (CIMBA) was established in 2005 to link clinical and epidemiological data from many groups from around the world [17].

However, the GWA approach is still limited in that study designs involve pre-defined stringent selection criteria for which SNPs identified from the initial whole genome scan are going to be analyzed in subsequent replication studies, a study design enforced by current genotyping costs. Moreover, GWA studies are often limited in information about exogenous risk factors, such as environmental exposures, which confounds any effort to explore the effect of environmental factors in modifying gene-disease associations. Global gene expression analysis as a means to agnostically identify candidate genetic modifiers has the potential to prioritize SNPs for candidate genes for association studies. This may be particularly valuable given recent observations that SNPs associated with risk of cancer in the general population appear to reside in non-coding regions that may modulate gene expression.

An alternative approach to prioritizing SNPs and candidate genes for association studies in *BRCA1* and *BRCA2* mutation carriers could rely on the selection of genes displaying associations with *BRCA1* or *BRCA2* mutation status at the expression level in response to DNA damage. In a previous study, we used a novel combinatorial approach to identify a subset of 20 irradiation responsive genes as high priority candidate *BRCA1* and/or *BRCA2* modifier genes [18]. The expression levels of these genes were shown to be associated with *BRCA1* and/or *BRCA2* mutation status in irradiated lymphoblastoid cell lines (LCLs) from female carriers when compared against irradiated LCLs from healthy controls. Furthermore, each of the genes were tagged with one or more SNPs shown to be associated with breast cancer risk from the Cancer Genetic Markers of Susceptibility (CGEMS) Phase 1 Breast Cancer Whole Genome Association Scan [19, 20]. In this study we investigated the association of these polymorphisms, tagged to genes demonstrated *in vitro* to be involved in irradiation response, with risk of breast cancer for *BRCA1* and *BRCA2* mutation carriers.

MATERIALS AND METHODS

Study Participants

Eligibility of study participants was restricted to female *BRCA1* or *BRCA2* pathogenic mutation carriers who were aged 18 years or older. Fifteen clinic and population-based research studies from USA, Canada, Australia, United Kingdom and Europe submitted data to this study (Table 1). Information collected included year of birth, age at diagnosis of breast or ovarian cancer, age at last observation, family membership, ethnicity and information on bilateral prophylactic mastectomy and oophorectomy. All centres have obtained informed consent from study participants and institutional review board approved protocols. In total, this study included of up to 4724 *BRCA1* and 2693 *BRCA2* eligible female mutation carriers. Of the 2193 and 1189 unaffected *BRCA1* and *BRCA2* carriers, respectively, 972 (44.3%) and 589 (49.5%) had a relative that was in the affected group.

SNP Selection and Genotyping

In a previous report, we proposed 13 genes (*ARHGEF2, HNRPDL, IL4R, JUND, LSM2, MAGED2, MLF2, MS4A1, SMAD3, STIP1, THEM2, TOMM40, VNN2*) as candidate modifiers of breast cancer risk for *BRCA1* mutation carriers, and 14 genes (*ARHGEF2, JUND, MLF2, SMAD3, STIP1, THEM2, TOMM40, ABL1, ELMO1, EPM2AIP1, PER1, PLCG2, PLD3, SLC20A1*) as candidate modifiers of breast cancer risk for *BRCA2* mutation carriers (Supplementary table S1 in Additional file 1) [18]. Thirty seven SNPs denoted by CGEMS as being tagged to these genes were initially identified as showing some association with breast cancer risk ($P<0.05$) (Supplementary table S2 in Additional file 2). Of these 37 SNPs, a panel of 32 variants were selected after successful assay design and genotyped on two platforms, using the Illumina GoldenGate assay (Illumina Inc.) and the Sequenom MassARRAY iPLEX platform (Sequenom, San Diego, CA, USA), as previously described [21, 22]. The genotyping method used for each participating study is detailed in Table 1. Five SNPs tagged to five candidate genes (*JUND, MAGED2, MLF2, MLH1, STIP1*) had call rates

less than 95% and were excluded from the analysis. The minor allele frequencies of three SNPs (rs2893535 - *ELMO1*, MAF=0.033; rs2304911 - *PER1* MAF=0.043, and rs3802957 – *MS4A1,* MAF=0.04) were considered too small for reliable analysis. Therefore, the number of genes assessed for their associations with breast cancer risk for *BRCA1* and *BRCA2* mutation carriers was eight and 10, respectively.

Statistical Methods

Relative Risks (RR) and 95% Confidence Intervals (CIs) were estimated using weighted Cox proportional hazards models. Each subject was followed from birth to the earliest of breast cancer, bilateral mastectomy, ovarian cancer, last follow-up, or age 80. The phenotype of interest was time to breast cancer. Mutation-specific weights were calculated using the age distribution of affected and unaffected individuals according to the methods previously outlined by Antoniou et al [23]. Analyses were stratified by year of birth, ethnicity, country of residence, study site, and mutation status. A robust variance estimate was used to account for relatedness amongst individuals. Primary SNP analyses assumed a log-additive relationship between the number of minor alleles carried by each individual and time to breast cancer. Wald p-values below 0.05 were declared of interest. Secondary analyses were carried out in which RR estimates were separately generated for those carrying one and two copies of the minor allele versus those with two copies of the major allele. Between-study heterogeneity was examined in each SNP by including an interaction term between the genotype and study centre.

Due to the highly-selected nature of subjects, a number of sensitivity analyses were examined. To limit the effect of potential survival bias, subjects diagnosed more than five years prior to study enrolment were excluded (N affected analyzed=1342 and 762 for BRCA1 and BRCA2 carriers, respectively). Other models were examined that excluded women with ovarian cancer (N excluded=491 and 151 BRCA1 and BRCA2 carriers, respectively). Finally, as risk of breast cancer

is reduced after bilateral oophorectomy [24, 25], analyses were carried out by treating oophorectomy as a time-dependent covariate in the Cox proportional hazards models. All p-values are two sided and analyses were carried out using R.

RESULTS AND DISCUSSION

A cohort of up to 4,724 *BRCA1* and 2,693 *BRCA2* female mutation carriers was used for this study. Of these, 4,035 mutation carriers were diagnosed with breast or ovarian cancer at the end of follow-up and 3,382 were censored as unaffected at a mean age of 44 years. The patient characteristics of *BRCA1* and *BRCA2* mutation carriers are shown in Table 2.

The RR estimates for the association between SNP genotypes and risk of breast cancer for *BRCA1* and *BRCA2* mutation carriers are shown in Table 3 and Table 4, respectively. Of the 24 SNPs that passed quality control, the minor alleles of two SNPs were found to be associated with increased risk for *BRCA1* mutation carriers (rs10242920 – *ELMO1*, P=0.043; rs480092 - *LSM2*, P=0.015) and the minor alleles of three SNPs with increased risk for *BRCA2* mutation carriers (rs1559949 - *HNRPDL*, P=0.021; rs3825977 – *SMAD3*, P=0.018; rs7166081 – *SMAD3*, P=0.004). The minor alleles of two SNPs, rs1559949 (*HNRPDL*) and rs3808814 (*ABL1*), were associated with decreased risk for *BRCA1* (P=0.022) and BRCA2 (P=0.030) mutation carriers, respectively.

All SNPs selected for this study (Supplementary table S2 in Additional file 2) had previously been reported to be 1) at least marginally associated ($p<0.05$) with breast cancer risk through the CGEMS Phase 1 Breast Cancer Whole Genome Association Scan [18], and 2) tagged to a gene whose expression level was associated with *BRCA1* and/or *BRCA2* mutation status in irradiated LCLs [18]. The minor allele of four out of six SNPs shown here to be associated with risk in *BRCA1* (rs1559949, *HNRPDL*; rs480092, *LSM2*) or *BRCA2* (rs3825977 and rs7166081, *SMAD3*) mutation carriers had risk estimates for the homozygous genotype that were concordant with the odds-ratio reported by the CGEMS study (Table 3, Table 4 and Supplementary table S2 in Additional file 2). Furthermore, the expression of *HNRPDL* and *LSM2* was associated with *BRCA1* mutation status and the expression of *SMAD3* was associated with BRCA2 mutation status [18]. The risk estimate of rs10242920 (*ELMO1*) was also concordant with the odds-ratio determined by

the CGEMS study, and although the expression of *ELMO1* was not associated with *BRCA1* mutation status at P<0.001 there was an association with gene expression at P<0.005 [18]. In contrast, the risk estimates of rs3808814 (*ABL1*) and rs1559949 (*HNRPDL*) in *BRCA2* mutation carriers are not concordant with the odds-ratio determined by the CGEMS study. Forest plots of study groups with 70 or more carriers and tests of heterogeneity are shown for two of the most significant SNPs (rs3825977, P-het= 0.619 and rs7166081, P-het=0.218 at the *SMAD3* locus) stratified by study site (Figure 1). The minor alleles of rs3825977 and rs7166081 are in high linkage disequilibrium (r^2=0.78), which would be expected if their association with increased breast cancer risk is bona fide.

Although further study is required to confirm whether genetic variation in *SMAD3* plays a role in modifying risk of breast cancer, SMAD3 has been shown to interact with the BRCA2 protein suggesting a possible mechanism through which SMAD3 may modify BRCA2 function [26]. Furthermore, SMAD3 is a critical regulatory factor of the transforming growth factor beta (TGF-beta) pathway, which is known to play a key role in the development of breast cancer as well as other many other cancers [27, 28]. In addition, a recent study comparing dense breast tissue (a known breast cancer risk factor) with non-dense tissue identified reduced expression of SMAD3 to be associated with dense tissue, indirectly supporting a role of SMAD3 expression with breast cancer risk [28].

Choosing candidate *BRCA1* and *BRCA2* modifier genes from a novel combinatorial approach [18], we show that four SNPs tagged to three of the 14 candidate genes show an association with breast cancer risk for *BRCA1* or *BRCA2* mutation carriers. However, we initiated this study with the expectation that SNPs in eight of the 14 genes may be associated with altered expression in *BRCA1* mutation carriers, and 10 of the 14 genes with altered expression in *BRCA2* carriers. Thus it can be argued that 3 of 18 (17%) of "valid" comparisons showed an association with risk. For either

interpretation, the rate of observed association is greater than the one in 20 (5%) expected by chance. In addition, post-hoc mining of the expression dataset showed that another SNP (rs10242920 - *ELMO1*) association that was consistent with the effect reported in the CGEMS dataset was also actually associated with altered expression in carriers, albeit with less significance (P=0.005) than originally used for gene and SNP selection. These findings suggest that the combinatorial approach may be a useful method to prioritize candidate modifier genes for polymorphism association studies. It is notable that CIMBA GWA studies of *BRCA1* and *BRCA2* mutation carriers are currently underway [29]. One might therefore anticipate that the combinatorial approach would provide even greater enrichment for prioritizing SNPs from GWA studies that directly relate to the disease state under study. Further studies with larger cohort size are therefore warranted to assess benefit of carrying such an approach.

CONCLUSIONS

We have explored the value of using biological information embedded in gene expression data to prioritise candidate modifier genes for SNP association studies. Using this combinatorial approach we were able to demonstrate a three-fold enrichment of genes that contain SNPs associated with breast cancer risk for *BRCA1* or *BRCA2* mutation carriers. Most notable was the evidence that the *SMAD3* gene, which encodes a key regulatory protein in the TGF-beta signalling pathway, may contribute to increased risk of breast cancer in *BRCA2* mutation carriers. These results suggest that the combinatorial approach may be a useful method to prioritise candidate modifier genes for polymorphism association studies.

ABBREVIATIONS

CI, Confidence Intervals; CGEMS, Cancer Genetic Markers of Susceptibility; CIMBA, Consortium of Investigators of Modifiers of *BRCA1* and *BRCA2*; GWA, Genome wide association; HR, Hazard ratio; LCL, Lymphoblastoid cell line; RR, Relative risk; SNP, Single nucleotide polymorphism; TGF-beta, transforming growth factor beta.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

LCW, ABS and FJC conceived and designed the study. LCW, GCT, ABS and FJC coordinated the study and LCW drafted the manuscript. ABS and FJC supervised the analysis and participated in manuscript writing. ZSF and VSP carried out the statistical analysis and ZSF contributed to the manuscript writing. XW, RT, NML, JB, and XC processed samples and acquired data. DS-L, CT, SG, SM, DM, J-PF, CD, RKS, BW, CE, IS, HD, AM, FBH, MV, MJH, AMWvdO, MRN, MGEMA, CMA, CJvA, PD, MMG, QW, CIS, DFE, SP, MC, CTO, DF, PH, DGE, FL, RE, LI, CC, RD, DE, K-RO, JC, TR, KLN, SMD, CFS, DG-K, A-CD, GP, AKG, TH, HN,BAA, MAC, HO,

UK, AL BA, PK, BM, OMS, LM, ACA, GCT and FJC provided samples and information on the *BRCA1* and *BRCA2* mutation carriers included in this study. SH and OMS provided assistance with mutation nomenclature and classifications. LM and ACA maintained the database of *BRCA1* and *BRCA2* mutation carriers. All authors have read and approved the manuscript.

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Table 1. Distribution of *BRCA1* and *BRCA2* mutation carriers by study site

Study	Country[a]	*BRCA1*	*BRCA2*	Genotyping Platform
HEBON	Netherlands	807	308	iPLEX[b]; Golden Gate[c]
EMBRACE	UK	841	656	iPLEX[b]; Golden Gate[c]
FCCC	USA	82	53	iPLEX[b]; Golden Gate[c]
GC-HBOC	Germany	398	163	Golden Gate[c]
GEMO	France/USA	408	226	Golden Gate[c]
GEORGETOWN	USA	27	14	iPLEX[b]; Golden Gate[c]
HEBCS	Finland	103	104	iPLEX[b]; Golden Gate[c]
ILUH	Iceland	6	87	iPLEX[b]; Golden Gate[c]
KCONFAB	Australia/New Zealand	531	427	iPLEX[b]; Golden Gate[c]
MAYO	USA	227	123	iPLEX[b]; Golden Gate[c]
MOD SQUAD	USA	158	91	Golden Gate[c]
MUV	Austria	298	126	iPLEX[b]; Golden Gate[c]
PBCS	Italy	76	43	iPLEX[b]
SWE-BRCA	Sweden	489	141	iPLEX[b]; Golden Gate[c]
UPENN	USA	273	131	iPLEX[b]; Golden Gate[c]

[a] Coordinating centre.
[b] Samples were genotyped at the Queensland Institute of Medical Research.
[c] Samples were genotyped at the Mayo Clinic.

Table 2. Patient characteristics

Characteristic	*BRCA1* mutation carriers		*BRCA2* mutation carriers	
	Unaffected	**Breast cancer**	**Unaffected**	**Breast cancer**
Number of Carriers	2193	2531	1189	1504
Length of follow-up (person-years)	93521	102870	53147	66764
Mean (SD) age at censure	43 (12.6)	41 (9.4)	45 (13.2)	44 (9.7)
Age at censure:				
<30	344 (16%)	252 (10%)	144 (12%)	52 (3%)
30 – 39	658 (30%)	1060 (42%)	343 (29%)	474 (32%)
40 – 49	608 (28%)	809 (32%)	331 (28%)	587 (39%)
50 – 59	374 (17%)	310 (12%)	215 (18%)	273 (18%)
60 – 69	143 (6%)	87 (3%)	101 (8%)	93 (6%)
70+	66 (3%)	13 (1%)	55 (5%)	25 (2%)
Year of birth				
Before 1949	523 (24%)	840 (33%)	281 (24%)	602 (40%)
1949 – 1959	508 (23%)	816 (32%)	307 (26%)	518 (35%)
1960 – 1968	594 (27%)	602 (24%)	302 (25%)	303 (20%)
After 1968	568 (26%)	273 (11%)	299 (25%)	81 (5%)
Oophorectomy	260 (12%)	77 (3%)	126 (11%)	47 (3%)
Ethnicity				
Caucasian	2127 (97%)	2446 (97%)	1159 (97%)	1464 (97%)
Ashkenazi Jewish	66 (3%)	85 (3%)	30 (3%)	40 (3%)

Table 3. Genotype distributions of 24 candidate modifier SNPs and HR estimates for *BRCA1* mutation carriers

SNP	Gene	Minor allele	MAF	Heterozygous		Homozygous		per-allele HR	95% CI	P_{trend}
				HR	95% CI	HR	95% CI			
rs7026988	*ABL1*	A	0.12	1.08	0.88-1.34	1.61	0.82-3.15	1.13	0.93-1.36	0.212
rs3808814	*ABL1*	A	0.09	0.90	0.72-1.14	0.65	0.22-1.91	0.89	0.72-1.10	0.284
rs1889532	*ARHGEF2*	G	0.25	0.99	0.83-1.18	1.12	0.80-1.56	1.03	0.90-1.18	0.708
rs10242920	*ELMO1*	A	0.24	1.06	0.89-1.27	1.61	1.12-2.32	1.16	1.00-1.33	*0.043*
rs6964474	*ELMO1*	C	0.22	1.08	0.90-1.29	0.71	0.49-1.02	0.96	0.84-1.10	0.568
rs2541095	*ELMO1*	G	0.12	1.03	0.84-1.27	1.12	0.48-2.63	1.04	0.86-1.26	0.683
rs6956864	*ELMO1*	G	0.41	1.04	0.76-1.42	1.35	0.18-10.03	1.05	0.78-1.42	0.755
rs1559949	*HNRPDL*	G	0.14	0.78	0.65-0.94	0.91	0.51-1.64	0.82	0.70-0.97	*0.022*
rs4285076	*HNRPDL*	A	0.29	0.95	0.80-1.12	1.00	0.73-1.37	0.98	0.86-1.11	0.746
rs4787956	*IL4R*	G	0.34	0.99	0.83-1.18	1.11	0.84-1.46	1.03	0.91-1.17	0.611
rs16976728	*IL4R*	A	0.38	0.92	0.77-1.10	1.07	0.82-1.39	1.00	0.88-1.13	0.978
rs480092	*LSM2*	G	0.16	1.25	1.04-1.51	1.30	0.81-2.08	1.21	1.04-1.42	*0.015*
rs2253820	*PER1*	A	0.17	1.02	0.9-1.16	0.72	0.52-1.00	0.96	0.87-1.06	0.412
rs4888201	*PLCG2*	A	0.16	1.10	0.91-1.34	1.39	0.77-2.51	1.13	0.95-1.33	0.168
rs10514519	*PLCG2*	A	0.18	1.06	0.88-1.28	1.59	0.92-2.75	1.11	0.95-1.31	0.195
rs4997772	*PLCG2*	A	0.39	1.13	0.95-1.35	1.07	0.84-1.37	1.05	0.94-1.18	0.377
rs3936112	*PLCG2*	A	0.39	0.98	0.87-1.10	0.97	0.82-1.16	0.98	0.91-1.07	0.700
rs4254419	*PLD3*	A	0.15	0.98	0.81-1.18	0.87	0.50-1.52	0.96	0.82-1.13	0.648
rs10758	*SLC20A1*	G	0.26	0.98	0.82-1.17	0.95	0.68-1.33	0.98	0.85-1.12	0.729
rs3825977	*SMAD3*	A	0.20	0.98	0.88-1.11	0.94	0.71-1.24	0.98	0.89-1.07	0.638
rs7166081	*SMAD3*	G	0.24	0.98	0.87-1.11	1.03	0.80-1.33	1.00	0.91-1.10	0.995
rs3777663	*THEM2*	G	0.24	1.02	0.86-1.22	1.17	0.84-1.63	1.05	0.92-1.20	0.453
rs2075642	*TOMM40*	A	0.20	0.97	0.81-1.16	1.06	0.69-1.63	0.99	0.86-1.15	0.931
rs12211125	*VNN2 / VNN3*	G	0.09	0.96	0.83-1.11	1.19	0.73-1.94	0.99	0.87-1.12	0.882

Abbreviation: SNP, single nucleotide polymorphism; MAF, Minor allele frequency; HR, hazard ratio; CI, confidence interval.

Table 4. Genotype distributions of 24 candidate modifier SNPs and HR estimates for *BRCA2* mutation carriers

SNP	Gene	Minor allele	MAF	Heterozygous		Homozygous		per-allele HR	95% CI	P_{trend}
				HR	95% CI	HR	95% CI			
rs7026988	*ABL1*	A	0.12	0.96	0.73-1.26	0.93	0.41-2.10	0.96	0.76-1.20	0.713
rs3808814	*ABL1*	A	0.09	0.72	0.51-1.02	0.50	0.17-1.42	0.71	0.53-0.97	*0.030*
rs1889532	*ARHGEF2*	G	0.25	1.32	1.05-1.67	0.99	0.64-1.54	1.13	0.95-1.33	0.172
rs10242920	*ELMO1*	A	0.24	1.01	0.79-1.30	0.86	0.52-1.43	0.97	0.80-1.17	0.747
rs6964474	*ELMO1*	C	0.22	1.09	0.86-1.40	1.47	0.89-2.45	1.15	0.95-1.39	0.153
rs2541095	*ELMO1*	G	0.12	1.04	0.8-1.35	0.72	0.27-1.94	1.00	0.78-1.26	0.971
rs6956864	*ELMO1*	G	0.41	0.76	0.48-1.18	2.20	0.16-29.7	0.78	0.50-1.22	0.279
rs1559949	*HNRPDL*	G	0.14	1.29	0.96-1.72	2.06	0.99-4.28	1.33	1.04-1.70	*0.021*
rs4285076	*HNRPDL*	A	0.29	0.86	0.67-1.09	1.47	0.95-2.26	1.03	0.85-1.25	0.737
rs4787956	*IL4R*	G	0.34	1.10	0.87-1.41	1.31	0.90-1.91	1.13	0.95-1.35	0.167
rs16976728	*IL4R*	A	0.38	1.16	0.91-1.48	1.38	0.95-1.99	1.17	0.98-1.39	0.075
rs480092	*LSM2*	G	0.16	0.92	0.72-1.18	1.09	0.55-2.16	0.96	0.78-1.19	0.735
rs2253820	*PER1*	A	0.17	0.85	0.70-1.02	1.13	0.68-1.87	0.90	0.77-1.06	0.209
rs4888201	*PLCG2*	A	0.16	0.98	0.75-1.27	1.16	0.54-2.52	1.01	0.80-1.27	0.964
rs10514519	*PLCG2*	A	0.18	0.85	0.66-1.09	1.92	0.95-3.88	0.99	0.79-1.24	0.933
rs4997772	*PLCG2*	A	0.39	1.21	0.95-1.55	1.26	0.89-1.78	1.14	0.97-1.34	0.107
rs3936112	*PLCG2*	A	0.39	0.91	0.76-1.09	0.94	0.73-1.22	0.96	0.85-1.08	0.483
rs4254419	*PLD3*	A	0.15	0.95	0.73-1.22	0.74	0.36-1.51	0.92	0.74-1.14	0.448
rs10758	*SLC20A1*	G	0.26	1.12	0.88-1.42	1.11	0.67-1.84	1.08	0.90-1.30	0.388
rs3825977	*SMAD3*	A	0.20	1.10	0.91-1.33	1.83	1.23-2.73	1.20	1.03-1.40	*0.018*
rs7166081	*SMAD3*	G	0.24	1.17	0.97-1.42	1.74	1.21-2.49	1.25	1.07-1.45	*0.004*
rs3777663	*THEM2*	G	0.24	0.95	0.75-1.21	1.11	0.69-1.79	0.99	0.82-1.20	0.945
rs2075642	*TOMM40*	A	0.20	1.14	0.89-1.46	1.19	0.68-2.09	1.12	0.92-1.37	0.267
rs12211125	*VNN2 / VNN3*	G	0.09	1.01	0.81-1.26	1.31	0.49-3.56	1.02	0.83-1.26	0.818

Abbreviation: SNP, single nucleotide polymorphism; MAF, Minor allele frequency; HR, hazard ratio; CI, confidence interval.

FIGURE LEGENDS

Figure 1. *BRCA2* plots of Study Group-Specific RR for rs3825977 and rs7166081 at the *SMAD3* locus. Study groups with 70 or more carriers and tests of heterogeneity are shown for A) rs3825977 [Overall RR (95% CI) = 1.20 (1.03, 1.40) P−trend = 0.018], and B) rs7166081 [Overall RR (95% CI) = 1.25 (1.07, 1.45) P−trend = 0.004].

Additional files

Additional file 1: Supplementary table S1. Genes predicted to modify risk in *BRCA1* and/or *BRCA2* mutation carriers by Walker et al [18].

Additional file 2: Supplementary table S2. List of 37 candidate *BRCA1/2* risk modifier SNPs. Each SNP listed was tagged to a gene and shown to be associated with breast cancer risk from the CGEMS Study version 1.

(a) **rs3825977**

Study	# Affected	# Unaffected
EMBRACE	324	327
GC−HBOC	121	42
GEMO	151	75
HEBCS	59	45
HEBON	111	190
KCONFAB	218	175
MAYO	74	48
MODSQUAD	60	31
SWE−BRCA	62	59
UPENN	80	50
ALL	**1260**	**1042**

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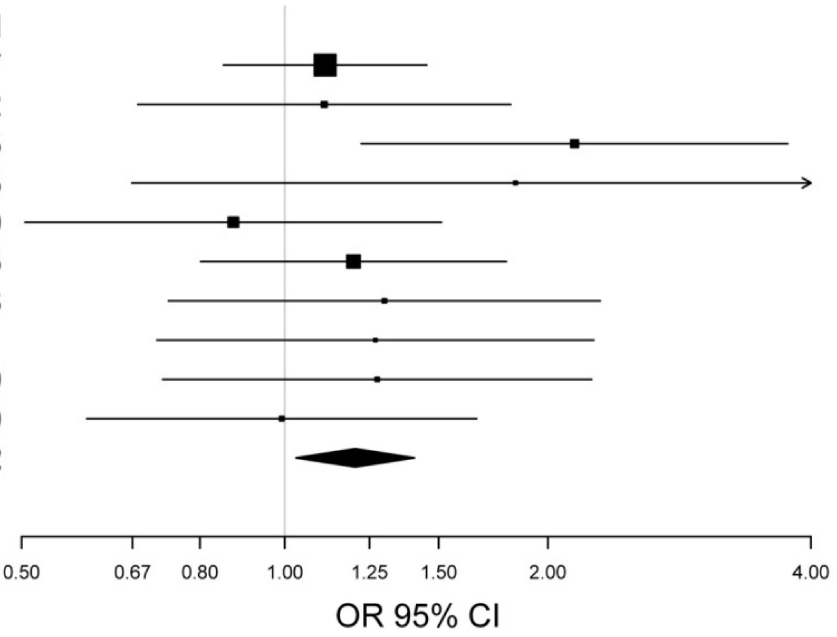

(b) **rs7166081**

Study	# Affected	# Unaffected
EMBRACE	324	329
GC−HBOC	121	42
GEMO	151	75
HEBCS	59	45
HEBON	113	191
KCONFAB	217	176
MAYO	74	48
MODSQUAD	60	31
UPENN	85	51
ALL	**1204**	**988**

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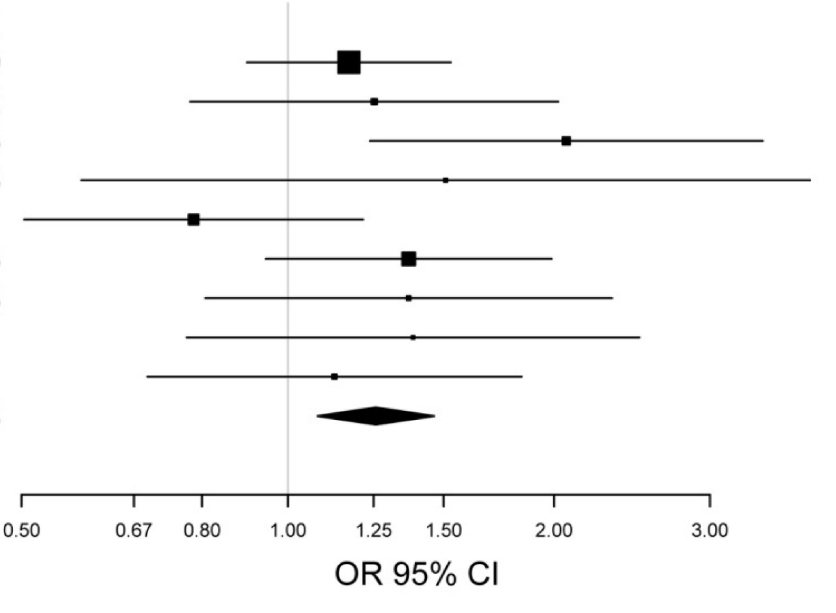

Figure 1

Additional files provided with this submission:

Additional file 1: SMAD3 modifier manuscript_Suppl1.docx, 35K
http://breast-cancer-research.com/imedia/1564691076441624/supp1.docx
Additional file 2: Additional data 2.docx, 38K
http://breast-cancer-research.com/imedia/3400841624897908/supp2.docx