

Common variants associated with breast cancer in genome-wide association studies are modifiers of breast cancer risk in BRCA1 and BRCA2 mutation carriers

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Received February 8, 2010; Revised and Accepted April 21, 2010

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Recent studies have identified single nucleotide polymorphisms (SNPs) that significantly modify breast cancer risk in *BRCA1* and *BRCA2* mutation carriers. Since these risk modifiers were originally identified as genetic risk factors for breast cancer in genome-wide association studies (GWASs), additional risk modifiers for *BRCA1* and *BRCA2* may be identified from promising signals discovered in breast cancer GWAS. A total of 350 SNPs identified as candidate breast cancer risk factors ($P < 1 \times 10^{-3}$) in two breast cancer GWAS studies were genotyped in 3451 *BRCA1* and 2006 *BRCA2* mutation carriers from nine centers. Associations with breast cancer risk were assessed using Cox models weighted for penetrance. Eight SNPs in *BRCA1* carriers and 12 SNPs in *BRCA2* carriers, representing an enrichment over the number expected, were significantly associated with breast cancer risk ($P_{\text{trend}} < 0.01$). The minor alleles of rs6138178 in *SNRPB* and rs6602595 in *CAMK1D* displayed the strongest associations in *BRCA1* carriers (HR = 0.78, 95% CI: 0.69–0.90, $P_{\text{trend}} = 3.6 \times 10^{-4}$ and HR = 1.25, 95% CI: 1.10–1.41, $P_{\text{trend}} = 4.2 \times 10^{-4}$), whereas rs9393597 in *LOC134997* and rs12652447 in *FBXL7* showed the strongest associations in *BRCA2* carriers (HR = 1.55, 95% CI: 1.25–1.92, $P_{\text{trend}} = 6 \times 10^{-5}$ and HR = 1.37, 95% CI: 1.16–1.62, $P_{\text{trend}} = 1.7 \times 10^{-4}$). The magnitude and direction of the associations were consistent with the original GWAS. In subsequent risk assessment studies, the loci appeared to interact multiplicatively for breast cancer risk in *BRCA1* and *BRCA2* carriers. Promising candidate SNPs from GWAS were identified as modifiers of breast cancer risk in *BRCA1* and *BRCA2* carriers. Upon further validation, these SNPs together with other genetic and environmental factors may improve breast cancer risk assessment in these populations.

INTRODUCTION

Deleterious germline mutations in the *BRCA1* and *BRCA2* genes confer high risks of breast and ovarian cancer. However, there is a substantial variation in breast cancer risks among carriers of *BRCA1* and *BRCA2* mutations, due to a combination of genetic and lifestyle factors, with penetrance estimated at between 40 and 87% by age 70 (1–7). In addition, there is significant variability in the age of onset of cancer and the site of cancer in these populations (1–3). Studies of high-risk families quantifying the extent of risk variation have suggested that other genetic factors may modify the risk of breast cancer associated with *BRCA1* and *BRCA2* mutations (4–6).

Initial efforts to identify genetic modifiers of breast cancer risk in *BRCA1* and *BRCA2* mutation carriers through candidate gene studies established that homozygosity for the *RAD51* 135G>C allele is associated with increased breast cancer risk in *BRCA2* mutation carriers, but not in *BRCA1* carriers (7–10). Subsequent efforts have focused on evaluation of common breast cancer susceptibility loci that harbor single nucleotide polymorphisms (SNPs) exhibiting genome-wide significant ($P < 1 \times 10^{-7}$) associations with breast cancer (11–17). Studies of six of these SNPs by the Consortium of Investigators of Modifiers of *BRCA1/2* (CIMBA) recently showed that SNPs in the *FGFR2*, *MAP3K1*, *TNRC9*, *LSP1* and 2q35 loci but not the 8q24 locus were significantly associated with breast cancer risk in *BRCA2* mutation carriers (18,19). In contrast, only SNPs in the *TNRC9* and 2q35 loci exhibited significant associations with risk in *BRCA1* carriers (18,19). Since ~70% of breast tumors in *BRCA1* carriers are estrogen receptor (ER) negative, this finding is consistent with the observation that these SNPs are more strongly associated with ER positive than ER negative breast cancer in the general population (20). Together, these variants account for 0.7 and 3.7% of the genetic modifying variance of breast cancer risk in *BRCA1* and *BRCA2* carriers, respectively (21).

Thus, a large proportion of the genetic variability underlying breast cancer in *BRCA1* and *BRCA2* carriers remains to be elucidated.

These findings suggest that selection of SNPs displaying highly significant associations with breast cancer in genome-wide association study (GWAS) of unselected breast cancers is an efficient method for identification of modifiers of breast cancer risk in *BRCA1* and *BRCA2* carriers. Under this model, which relies on selection of small numbers of high-quality candidate SNPs displaying highly significant associations with the phenotype of interest, it may be possible to avoid the need for additional GWAS and to reduce the number of samples required to identify variants significantly associated with the related disease phenotype. In order to test this model and to identify additional common genetic variants associated with breast cancer risk in *BRCA1* and *BRCA2* carriers, we selected 295 SNPs highly significantly associated with breast cancer risk ($P < 10^{-3}$) in breast cancer GWAS conducted in the UK (UK1) (11,15) and by the Cancer Genetic Markers of Susceptibility (CGEMS) consortium (12,17), along with 55 SNPs selected from candidate SNPs displaying association with breast cancer risk in a Mayo Clinic breast cancer case–control study. These variants were genotyped in *BRCA1* and *BRCA2* mutation carriers from nine studies involved in CIMBA. Here we demonstrate that a number of SNPs associated with breast cancer risk in *BRCA1* and *BRCA2* mutation carriers can be identified using this model and we evaluate the influence of the SNPs on age-related risk of breast cancer to show the potential utility of risk modifiers for cancer risk assessment in the *BRCA1* and *BRCA2* carrier populations.

RESULTS

Genotyping data for 333 SNPs on 3451 *BRCA1* and 2006 *BRCA2* carriers from nine centers were available for analysis after exclusion of 225 samples and 17 SNPs during quality

Table 1. Associations between GWAS SNPs and risk of breast cancer ($P_{\text{trend}} \leq 0.01$) in *BRCA1* and *BRCA2* mutation carriers

rsID	Gene name	HR (95% CI) (per allele)	P_{trend}	FDR q_{value}	HR (95% CI)		$P_{2\text{df}}$
					One copy	Two copies	
BRCA1 carriers							
rs6138178	<i>SNRPB</i>	0.78 (0.69–0.90)	3.6×10^{-4}	0.07	0.75 (0.63–0.90)	0.66 (0.49–0.90)	0.0015
rs6602595	<i>CAMK1D</i>	1.25 (1.10–1.41)	4.2×10^{-4}	0.07	1.23 (1.02–1.48)	1.56 (1.21–2.01)	0.0019
rs6590224	<i>KIRREL3</i>	1.26 (1.10–1.45)	0.0013	0.13	1.25 (1.05–1.50)	1.61 (1.10–2.37)	0.0056
rs1048635	<i>TRIM45</i>	1.50 (1.17–1.94)	0.0015	0.13	1.59 (1.22–2.09)	1.15 (0.24–5.44)	0.0029
rs7629693	<i>HMG2P7</i>	0.83 (0.73–0.94)	0.0041	0.25	0.88 (0.74–1.05)	0.65 (0.49–0.86)	0.0104
rs6005863	<i>HSCB</i>	0.85 (0.76–0.95)	0.0049	0.25	1.01 (0.84–1.22)	0.66 (0.52–0.85)	0.0007
rs4415084	<i>MRPS30</i>	0.84 (0.75–0.95)	0.0052	0.25	0.80 (0.67–0.96)	0.73 (0.57–0.93)	0.015
rs2292326	<i>NECAB2</i>	1.27 (1.06–1.52)	0.0087	0.36	1.31 (1.07–1.61)	1.33 (0.68–2.57)	0.026
BRCA2 carriers							
rs9393597	<i>LOC134997</i>	1.55 (1.25–1.92)	6.0×10^{-5}	0.018	1.56 (1.18–2.08)	2.33 (1.23–4.39)	0.00030
rs12652447	<i>FBXL7</i>	1.37 (1.16–1.62)	1.7×10^{-4}	0.028	1.25 (0.96–1.62)	1.92 (1.39–2.67)	0.00041
rs6837016	<i>LOC729902</i>	0.76 (0.63–0.90)	0.0020	0.20	0.68 (0.53–0.88)	0.60 (0.42–0.85)	0.0046
rs909116	<i>TNNT3</i>	0.79 (0.68–0.92)	0.0025	0.20	0.88 (0.68–1.14)	0.61 (0.45–0.84)	0.0078
rs2067980	<i>MRPS30</i>	1.42 (1.12–1.80)	0.0036	0.22	1.31 (1.02–1.69)	3.86 (1.40–10.6)	0.0053
rs4660891	<i>MAST2</i>	1.32 (1.09–1.60)	0.0040	0.22	1.50 (1.18–1.92)	1.44 (0.88–2.35)	0.0040
rs10941679	<i>MRPS30</i>	1.31 (1.08–1.58)	0.0054	0.26	1.33 (1.06–1.68)	1.65 (1.00–2.72)	0.019
rs1533802	<i>LOC339778</i>	0.79 (0.67–0.94)	0.0072	0.28	0.89 (0.70–1.13)	0.50 (0.32–0.78)	0.0090
rs17827708	<i>DSEL</i>	0.74 (0.59–0.93)	0.0093	0.28	0.70 (0.54–0.90)	0.77 (0.37–1.62)	0.018
rs7185203	<i>CMIP</i>	0.79 (0.67–0.95)	0.010	0.28	0.84 (0.66–1.07)	0.59 (0.40–0.88)	0.032
rs6504950	<i>STXBP4</i>	1.27 (1.06–1.53)	0.010	0.028	1.31 (1.04–1.65)	1.53 (0.94–2.49)	0.034
rs7090828	<i>PCBD1</i>	0.81 (0.69–0.95)	0.010	0.28	0.93 (0.72–1.21)	0.62 (0.45–0.87)	0.012

FDR, false discovery rate; 2df, two degree of freedom model.

assessment. A total of 3030 carriers from the nine study groups were diagnosed with breast cancer, while 2427 additional carriers were unaffected at the date of last observation ($n = 1807$), were diagnosed with ovarian cancer ($n = 412$) or had undergone a bilateral prophylactic mastectomy ($n = 208$). From the overall analysis of individual SNPs, a total of 8 displayed significant associations ($P < 0.01$) with breast cancer risk in *BRCA1* mutation carriers and 12 exhibited associations ($P < 0.01$) with breast cancer in *BRCA2* mutation carriers. In contrast, only three were expected to be associated with breast cancer in *BRCA1* and *BRCA2* carriers by chance. The estimated HRs and P -values of the SNPs exhibiting significant associations ($P < 0.01$) are shown in Table 1.

Several SNPs recently identified and validated as genetic risk factors for breast cancer in the general population through breast cancer GWAS were included in this study and were evaluated as modifiers of breast cancer risk in *BRCA1* and *BRCA2* carriers. rs4415084 from the *MRPS30* locus on chromosome 5p12 (14) ($P = 0.005$) and rs2107425 from *H19* (11) ($P = 0.015$) were significantly associated with breast cancer in *BRCA1* carriers, whereas the *MRPS30* SNPs, rs10941679 ($P = 0.0054$) and rs2067980 ($P = 0.0036$), but not rs4415084, were associated with breast cancer in *BRCA2* carriers (Table 1; Supplementary Material, Table S1). In addition, rs6504950 from the *STXBP4/COX11* locus at 17q23.2 (15), rs16886165 from the *MAP3K1* locus (11), rs6735174 from the *TNPI* locus at 2q35 in high LD ($r^2 = 0.80$) with rs13387042 (17) and rs2107425 from the *H19* locus (11) displayed marginally significant associations ($P < 0.05$) in *BRCA2* carriers, but not in *BRCA1* carriers (Table 1; Supplementary Material, Table S5). In contrast, rs1357245 from *NEK10* at 3p24 (15), rs4973768 from *SLC4A7* at 3p24 (15) and rs999737 from *RAD51L1* at 1p11.2 (17) did not display significant associations with

breast cancer risk in either *BRCA1* or *BRCA2* carriers (Table 1; Supplementary Material, Table S1 and S5).

The SNPs showing the strongest associations with breast cancer in *BRCA1* carriers in this study were rs6138178 in *SNRPB* and rs6602595 in *CAMK1D* (HR = 0.78; 95% CI: 0.69–0.90, $P_{\text{trend}} = 0.00036$, and HR = 1.25; 95% CI: 1.10–1.41, $P_{\text{trend}} = 0.00042$, respectively). When applying false discovery rate (FDR) to account for multiple testing, these SNPs showed probabilities of false positive associations of less than 7% ($q = 0.07$) in *BRCA1* carriers (Table 1). For both SNPs, the estimated risk for heterozygotes and homozygotes were consistent with a multiplicative model (rs6138178 HR(het) = 0.75; 95% CI: 0.63–0.90 and HR(hom) = 0.66; 95% CI: 0.49–0.90) (rs6602595 HR(het) = 1.23; 95% CI: 1.02–1.48 and HR(hom) = 1.56; 95% CI: 1.21–2.01). No apparent heterogeneity in the study-specific HR was observed for rs6138178 ($P_{\text{het}} = 0.82$) or rs6602595 ($P_{\text{het}} = 0.27$) for *BRCA1* mutation carriers (Fig. 1).

To investigate whether the inclusion of ovarian cancer cases influenced associations with breast cancer risk in *BRCA1* mutation carriers, analyses were performed after excluding all 315 individuals diagnosed with ovarian cancer only or ovarian cancer prior to breast cancer. A total of 32 SNPs displayed significant associations with breast cancer including rs6138178 in *SNRPB* and rs6602595 in *CAMK1D* ($P_{\text{trend}} = 0.03$ and 0.004, respectively). Interestingly, rs6590224 from the *KIRREL3* locus was also highly significantly associated with risk (HR = 1.45; 95% CI: 1.23–1.71, $P_{\text{trend}} = 1.0 \times 10^{-5}$) in the absence of the ovarian cases (Supplementary Material, Table S2). Next, the influence of inclusion of prevalent cases was assessed by exclusion of all cases diagnosed with breast cancer more than 5 years before recruitment. A total of 21 SNPs including rs6138178 in *SNRPB* ($P_{\text{trend}} = 0.006$) and rs6602595 in *CAMK1D* ($P_{\text{trend}} = 0.017$) displayed

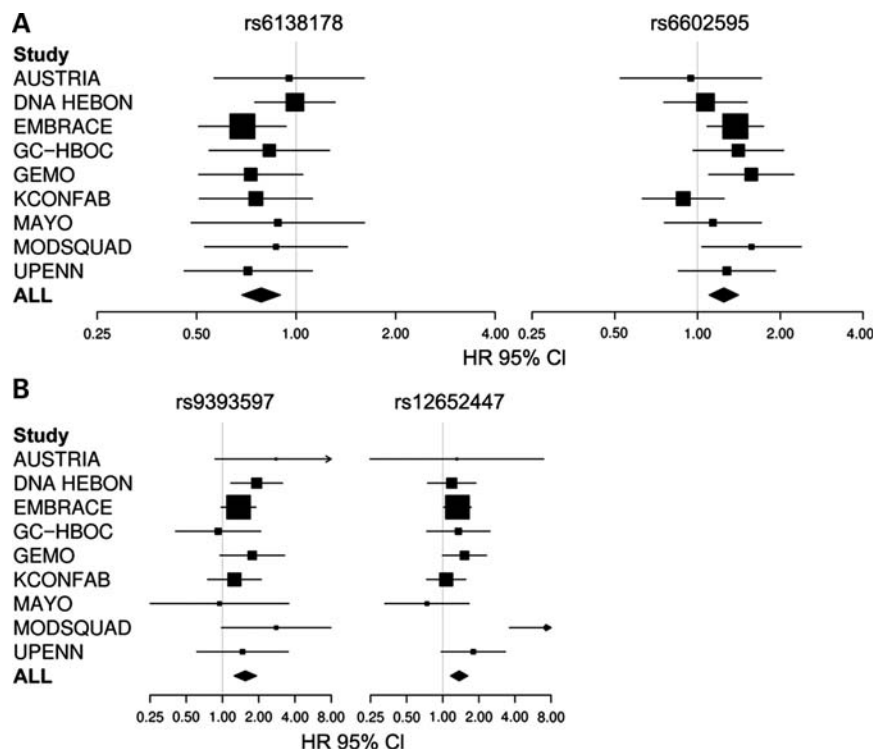


Figure 1. Study-specific per allele hazards ratio estimates for selected SNPs in *BRCA1* and *BRCA2* mutation carriers. (A) HR estimates for rs6138178 from the *SNRPB* locus and rs6602595 from the *CAMK1D* locus. (B) HR estimates for rs12652447 from the *FBXL7* locus and rs9393597 from the *LOC134997* locus. The area of each square is proportional to the sample size of each study. Horizontal lines represent the 95% CI for each study.

significance in the absence of the prevalent cases (Supplementary Material, Table S3). In addition, because risk-reducing oophorectomy (RRO) reduces the risk of breast cancer in *BRCA1* carriers by up to 50% (21), we included RRO as a time-dependent covariate in the analysis. Little influence on the associations with breast cancer risk was observed (Supplementary Material, Table S4).

In *BRCA2* carriers, rs9393597 in *LOC134997* (HR = 1.57; 95% CI: 1.25–1.92, $P_{\text{trend}} = 6 \times 10^{-5}$) and rs12652447 in *FBXL7* (HR = 1.37; 95% CI: 1.16–1.62, $P_{\text{trend}} = 1.7 \times 10^{-4}$) displayed the strongest associations with breast cancer risk (Table 1). When applying FDR, rs9393597 in *LOC134997* and rs12652447 in *FBXL7* showed probabilities of false positive associations of less than 5% ($q < 0.05$) (Table 1). For both SNPs, the estimated risk in homozygotes was higher than in heterozygotes. While there was no evidence of heterogeneity in the per-allele HRs for rs9393597 ($P_{\text{het}} = 0.48$) among *BRCA2* mutation carriers from the nine centers, some evidence of heterogeneity for rs12652447 ($P_{\text{het}} = 0.002$) was observed (Fig. 1). When repeating the analysis after exclusion of the Austria, Mayo and ModSquad studies that contributed less than 100 *BRCA2* carriers (Table 2), there was no evidence of heterogeneity ($P_{\text{het}} = 0.66$) and little change in the per-allele HR (1.37–1.33).

The sensitivity of the SNP associations with breast cancer to the presence of ovarian cancer cases and prevalent breast cancer cases in *BRCA2* carriers was also assessed. After exclusion of all ovarian cancer cases, 30 SNPs displayed significance ($P < 0.05$) including rs9393597 in *LOC134997* ($P_{\text{trend}} = 0.008$) and rs12652447 in *FBXL7* ($P_{\text{trend}} = 0.0006$)

(Supplementary Material, Table S6). Similarly, exclusion of prevalent cases yielded 29 SNPs displaying significant associations ($P < 0.05$) with breast cancer, including rs9393597 in *LOC134997* ($P_{\text{trend}} = 0.0002$) and rs12652447 in *FBXL7* ($P_{\text{trend}} = 0.005$) (Supplementary Material, Table S7). Likewise, little effect on the associations with breast cancer was observed after multivariate analysis including RRO as a time-dependent covariate (Supplementary Material, Table S8).

It has previously been postulated that genetic modifiers of breast cancer risk may prove useful for refined cancer risk assessment for *BRCA1* and *BRCA2* carriers (18). Thus, in subsequent risk assessment studies, we investigated the possible application of SNPs with strongest associations to cancer risk assessment in *BRCA1* and *BRCA2* carriers. Initially, the proportion of risk-modifying variance in *BRCA1* mutation carriers explained by *SNRPB* rs6138178 and *CAMK1D* rs6602595 was estimated at 1.6 and 2.0%, respectively. Subsequently, assessment of associations between combinations of these SNPs and breast cancer risk showed that a model containing only the two ordinal SNP effects performed as well as more complex models (Table 3). Under this simple model, the (TT,GG) genotype had the highest HR of 1.81, whereas the (TG,AG) and (GG,GG) genotypes had the same average risk as observed in all *BRCA1* carriers (Table 3). Figure 2 shows the estimated magnitude of the effects of the individual SNPs and the combined SNPs under the simple ordinal model on the cumulative incidence of breast cancer.

Similarly, the proportion of risk-modifying variance in *BRCA2* mutation carriers predicted to be explained by *LOC134997* rs9393597 and *FBXL7* rs12652447 was 3.9 and

Table 2. Age distribution and breast cancer (affected and unaffected) status of 5457 eligible *BRCA1* and *BRCA2* mutation carriers from CIMBA nine study centers

	BRCA1		BRCA2	
	n	Age ^a (SD)	n	Age ^a (SD)
Austria				
Affected	92	39.6 (8.4)	30	40.7 (7.3)
Unaffected	93	38.9 (10.3)	15	43.0 (9.6)
DNA Hebon				
Affected	285	39.6 (9.0)	103	43.5 (9.4)
Unaffected	290	43.7 (14.0)	168	43.8 (13.5)
EMBRACE				
Affected	421	40.5 (8.5)	318	44.2 (8.6)
Unaffected	394	41.4 (11.8)	326	44.2 (11.9)
GC-HBOC				
Affected	261	39.4 (9.9)	121	46.7 (10.0)
Unaffected	137	41.2 (13.0)	42	45.7 (12.7)
GEMO				
Affected	230	40.9 (9.5)	151	43.5 (10.2)
Unaffected	178	42.9 (12.2)	75	44.3 (11.7)
kConFab				
Affected	250	41.3 (9.3)	203	44.2 (9.9)
Unaffected	199	42.9 (11.9)	150	44.9 (13.0)
Mayo				
Affected	108	44.0 (10.0)	58	45.2 (9.9)
Unaffected	103	46.7 (12.0)	38	47.8 (12.6)
MODSQUAD				
Affected	110	40.8 (9.6)	60	44.9 (10.3)
Unaffected	48	42.4 (12.0)	31	42.0 (13.1)
UPENN				
Affected	154	39.4 (8.9)	75	42.3 (10.1)
Unaffected	98	43.5 (11.9)	42	43.1 (10.8)
All Subjects				
Affected ^b	1911	40.5 (9.2)	1119	44.2 (9.6)
Unaffected ^c	1540	42.6 (12.4)	887	44.3 (12.5)
Total	3451	41.4 (10.6)	2006	44.2 (10.9)

^aAges are summarized as mean age; SD: standard deviation.

^bAffected refers to carriers diagnosed with breast cancer.

^cUnaffected (earliest of age at last follow up, age at ovarian cancer diagnosis, age at prophylactic mastectomy; age 80).

2.9%, respectively. Here a model including the two ordinal SNP effects together with a multiplicative interaction fit the observed data better than the simple model that included only the two ordinal SNP effects ($P = 0.032$). Figure 2 shows the estimated magnitude of the effects of the individual and the combined SNPs under this model on the cumulative incidence of breast cancer. *BRCA2* mutation carriers homozygous for the minor risk alleles at both loci (GG,AA) and with (AG,AA), (GG,GA), (AG,GA) and (AA,AA) genotypes had higher age-related cumulative breast cancer risk than the average incidence in the *BRCA2* carriers, consistent with multiplicative interactions between disease alleles (Table 3 and Fig. 2). Approximately 31% of *BRCA2* mutation carriers were predicted to have HRs in excess of 1.5 (Table 3 and Fig. 2).

DISCUSSION

By evaluating candidate genetic risk factors for breast cancer risk from two recent breast cancer GWAS, we have identified several potential modifiers of breast cancer risk in *BRCA1* and

Table 3. Association of combinations of rs6138178 and rs6602595 among *BRCA1* carriers and combinations of rs9393597 and rs12652447 among *BRCA2* carriers and breast cancer risk

SNP1	SNP2	Frequency (%)	HR	95% CI	P-value
BRCA1					
rs6138178	rs6602595				
TT	AA	18.1	1.00	(Reference)	
TT	AG	25.0	1.34	1.04–1.73	0.0251
TT	GG	8.6	1.81	1.29–2.54	0.0006
TG	AA	15.1	0.84	0.63–1.12	0.2376
TG	AG	18.9	0.98	0.74–1.29	0.8650
TG	GG	7.2	1.19	0.81–1.76	0.3767
GG	AA	2.3	0.79	0.44–1.44	0.4461
GG	AG	3.5	0.80	0.50–1.27	0.3371
GG	GG	1.3	0.96	0.48–1.92	0.9069
BRCA2					
rs9393597	rs12652447				
AA	GG	26.0	1.00	(Reference)	
AA	GA	34.9	1.13	0.83–1.53	0.4437
AA	AA	12.3	1.52	1.03–2.22	0.0331
AG	GG	8.0	1.06	0.63–1.78	0.8235
AG	GA	11.4	1.82	1.22–2.73	0.0036
AG	AA	4.7	4.23	2.25–7.94	<0.0001
GG	GG	0.8	2.65	0.91–7.69	0.0738
GG	GA	1.4	2.14	0.85–5.40	0.1065

BRCA2 mutation carriers. In particular, rs6138178 in *SNRPB* and rs6602595 in *CAMK1D* were significantly associated with breast cancer in *BRCA1* carriers, while rs9393597 in *LOC134997* and rs12652447 in *FBXL7* were significantly associated with breast cancer in *BRCA2* carriers, each reaching $P < 0.001$. Of these, the rare allele of rs6138178 in *SNRPB* was associated with a reduced risk of breast cancer, whereas the rare alleles of rs6602595 in *CAMK1D*, rs9393597 in *LOC134997* and rs12652447 in *FBXL7* were all associated with increased risk. Importantly, the HR estimates for these four SNPs were quite consistent across the nine study groups (Fig. 1). These observations and the finding that the SNPs remained significantly associated with breast cancer risk after exclusion of ovarian cases, after exclusion of prevalent cases, and after evaluation of the influence of RRO, support a role for these variants as risk modifiers.

Although none of the associations for SNPs evaluated in this study reached 'genome-wide' levels of significance, there was an excess of association significant at the $P < 0.01$ level (8 in *BRCA1* carriers, 12 in *BRCA2* carriers, compared with 3 expected by chance). Also only 1 of the 8 SNPs in *BRCA1* carriers and 2 of the 12 SNPs in *BRCA2* carriers were previously established in breast cancer loci (all in 5p12). Since previous CIMBA studies have shown that the majority of established breast cancer SNPs are associated with breast cancer risk in *BRCA2* carriers and, in some cases, *BRCA1* carriers, the suggestion is that some of the other significant SNPs reported here may reflect true associations with breast cancer in *BRCA1* and *BRCA2* mutation carriers. The results also suggest that SNPs in GWAS that do not reach genome levels of significance ($P < 1 \times 10^{-7}$) for association with breast cancer may still account for effects in a *BRCA1* or *BRCA2* mutant background. Thus, evaluation of larger numbers of SNPs displaying moderate associations in GWAS, such as $P < 1 \times 10^{-3}$, may be an efficient strategy

for identifying further genetic modifiers in *BRCA1* and *BRCA2* carriers. This approach may also be a useful strategy for identifying genetic modifiers for familial forms of other diseases, such as hereditary nonpolyposis colorectal cancer. Further studies with larger numbers of *BRCA1* and *BRCA2* carriers will be needed to establish which particular SNP associations can be replicated, and to estimate precisely the risks associated with each locus. In addition, the results raise the possibility that certain SNPs that show modest associations in case-control studies may show stronger effects in *BRCA1* or *BRCA2* carriers because breast cancer in *BRCA1* or *BRCA2* carriers may have less heterogeneity. Thus, additional studies using unselected breast cancer cases, perhaps in subgroups of cases defined by pathology, should be undertaken to determine if these SNPs are genetic risk factors for breast cancer in non-carriers.

None of the four most strongly associated SNPs are putative functional variants. The rs6138178 variant is located in the first intron of *SNRPB* on chromosome 20p13 and encodes a nuclear component of small ribonucleoprotein particles (snRNPs) which are essential for pre-mRNA splicing. The SNP is in high LD with rs3746687 ($r^2 > 0.8$), a variant in the 3'-UTR of *ZNF434*, encoding a zinc finger protein of unknown function. Interestingly, rs6138178 was selected for this study based on an association with breast cancer in premenopausal women ($P_{2df} = 7 \times 10^{-4}$) in a Mayo Clinic breast cancer case-control study, which is consistent with the association with risk in *BRCA1* carriers who develop breast cancer at a younger age. SNP rs6602595, identified through the UK1 GWAS, is located in the first intron of *CAMKID*, which encodes a calcium-/calmodulin-dependent serine/threonine protein kinase. *CAMKID* is frequently amplified and overexpressed in basal-like breast tumors, and has been shown to promote an epithelial-to-mesenchymal transition of breast epithelial cells, consistent with the basal-like tumor phenotype (22). Since *BRCA1* mutant breast tumors are predominantly basal-like (23), it is possible that common genetic variation in *CAMKID* may contribute to this tumor phenotype. The rs9393597 SNP associated with risk in *BRCA2* carriers was also initially associated with breast cancer in the UK1 GWAS. The closest gene to rs9393597 is *FAM65B*, encoding a potential myogenic factor in cell differentiation, cytoskeletal rearrangements and filopodia formation. The rs12652447 SNP associated with risk in *BRCA2* carriers was selected following near-genome wide significant validation by CGEMS of a UK1 GWAS candidate SNP. This variant is located within an intron of *FBXL7*, which encodes an F-box containing protein that constitutes ubiquitin-dependent ligase complexes (SKP1-Cullin-F-box) essential in phosphorylation-dependent ubiquitination and proteasome-dependent degradation.

As noted earlier, none of the 8 SNPs in *BRCA1* carriers and 12 SNPs in *BRCA2* carriers displayed significant associations in both *BRCA1* and *BRCA2* carriers. The absence of SNPs displaying significance in both populations (Supplementary Material, Tables S1 and S2) is consistent with evidence that *BRCA1* and *BRCA2* carriers represent pathologically distinct diseases (23). Analysis of genetic risk factors for breast cancer identified through GWAS suggests that more of these loci are significantly associated with breast cancer risk in *BRCA2* carriers than *BRCA1* carriers (18,19), which is

consistent with the outcome of our own study. The ER-negative nature of *BRCA1* breast cancer also raises the possibility of the existence of a distinct set of SNPs that modify risk in *BRCA1* carriers but may not necessarily display strong associations in the general population.

In an effort to demonstrate the potential utility for breast cancer risk modifiers in cancer risk assessment for *BRCA1* and *BRCA2* carriers, we conducted preliminary analyses in which the effects of the SNPs with strongest associations and combinations of the SNPs on the age-related cumulative risk of breast cancer in carriers were estimated (Fig. 2). The average cumulative risk at the age of 50 years was estimated at 43% among *BRCA1* carriers and almost 20% among *BRCA2* carriers, which is consistent with previous assessments (1). In *BRCA1* carriers, subjects with the combination of the minor alleles of rs6602595 and the major alleles of rs6138178 showed an increase in risk at age 50 from 43 to 58%, whereas individuals with the opposite alleles showed a decrease in risk at age 50–27%. Overall, significantly more carriers were predicted to have elevated breast cancer risk than reduced risk (40.8 versus 20.9%). In *BRCA2* carriers, the minor alleles of the SNPs were associated with an increase in risk from an average of 20 up to 50% by age 50. These observations serve to demonstrate the potential utility of SNPs for cancer risk assessment in *BRCA1* and *BRCA2* carriers. The results suggest that combinations of several modifier loci, when identified, may allow identification of subsets of carriers at high risk of cancer at young age who can benefit from early intervention, and carriers at low or even population-based levels of risk who may delay or avoid current intervention methods such as prophylactic oophorectomy and mastectomy. However, in the context of the current study, it should be recognized that the influence of the SNPs on age-related risk of breast cancer may well be attenuated upon further validation with larger numbers of *BRCA1* and *BRCA2* carriers. Similarly, the incorporation of family history and environmental risk factors that also influence the lifetime risk of breast cancer may substantially modify the age-related risk models shown here. Despite the need for more refined estimation of risk in the future, our results support the notion that the addition of fully characterized modifier loci to risk models will lead to improved risk assessment for patients carrying deleterious mutations in *BRCA1* and *BRCA2*. Thus, in time modifier loci may significantly influence the approach to genetic counseling, prevention and routine care of these high-risk patients.

MATERIALS AND METHODS

BRCA1 and *BRCA2* mutation carriers

Nine large studies from eight countries (Australia, Austria, Britain, Czech Republic, France, Germany, the Netherlands and the USA) provided DNA samples, clinical and risk factor data from *BRCA1* and *BRCA2* mutation carriers for this study (Table 2). The majority of carriers were recruited through cancer genetics clinics offering genetic testing, and were enrolled into site-specific studies (Mayo and UPENN) or national studies. Eligibility was restricted to individuals with pathogenic *BRCA1* or *BRCA2* mutations (10) over 18

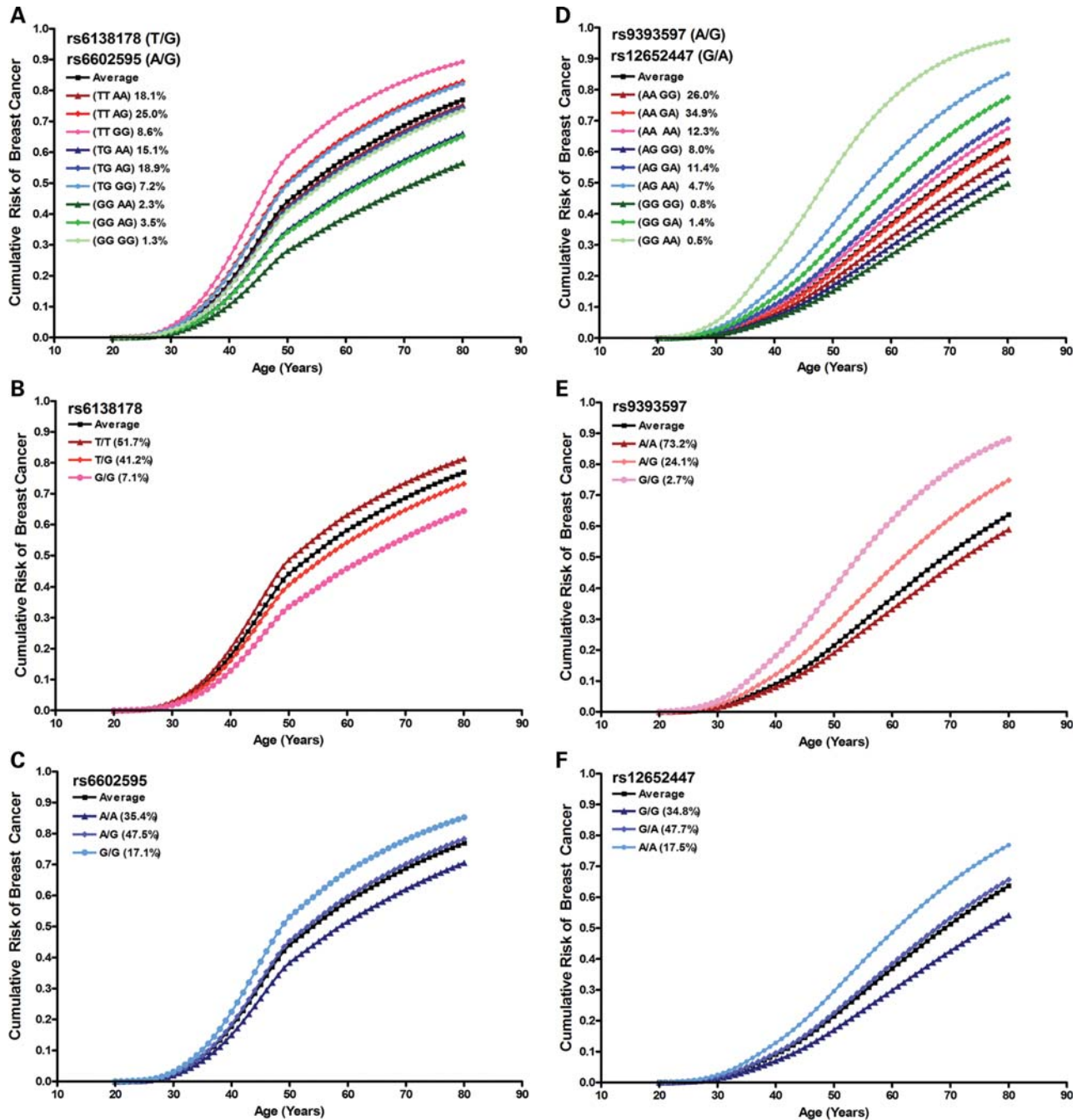


Figure 2. Age-related modifying effects of breast cancer risk by selected SNPs in *BRCA1* and *BRCA2* mutation carriers. Cumulative risk modifying effects of rs6138178 and rs6602595 combined (A), rs6138178 (B) and rs6602595 (C) genotypes in *BRCA1* mutation carriers. Cumulative risk modifying effects of rs9393597 and rs12652447 combined (D), rs9393597 (E) and rs12652447 (F) genotypes in *BRCA2* mutation carriers. All possible genotypes of the loci and their frequencies in *BRCA1* or *BRCA2* mutation carriers are shown. 'Average' represents the cumulative breast cancer risk over all possible modifying effects among *BRCA1* or *BRCA2* mutation carriers.

years of age, with available genomic DNA from blood samples, and phenotypic information including country of residence, year of birth, mutation description, age at last follow-up, diagnosis date of invasive breast and/or ovarian cancer, age of bilateral mastectomy or oophorectomy, and family identifiers (Table 4). All carriers were recruited to studies at the host institutions under IRB-approved protocols. Only self-reporting Caucasian

women were included in the analysis. Carriers with both *BRCA1* and *BRCA2* mutations were excluded. Duplicate samples were identified based on the year of birth, exact mutation description, age at onset of disease and SNP genotyping data from the present and past CIMBA studies and were included only once in the analysis. The date of blood draw was identified as the date of ascertainment for each carrier.

Table 4. Summary characteristics for the 5457 eligible *BRCA1* and *BRCA2* carriers used in the analysis

Characteristic	BRCA1		BRCA2	
	Unaffected ^a	Affected ^b	Unaffected ^a	Affected ^b
Number	1540	1911	887	1119
Person-years follow-up	65 612	77 316	39 322	49 428
Median age (IQR)	41 (34, 50)	39 (34, 46)	43 (35, 52)	43 (37, 50)
Age at censure, <i>n</i> (%)				
<30	232 (15.1)	186 (9.7)	101 (11.4)	42 (3.8)
30–39	473 (30.7)	823 (43.1)	267 (30.1)	356 (31.8)
40–49	445 (28.9)	605 (31.2)	253 (28.5)	441 (39.4)
50–59	251 (16.3)	223 (11.7)	164 (18.5)	198 (17.7)
60–69	93 (6.0)	67 (3.5)	71 (8.0)	67 (6.0)
70+	46 (3.0)	7 (0.4)	31 (3.5)	15 (1.3)
Year of birth, <i>n</i> (%)				
<1920	7 (0.5)	15 (0.8)	6 (0.7)	5 (0.5)
1920–29	34 (2.2)	74 (3.9)	23 (2.6)	47 (4.2)
1930–39	115 (7.5)	180 (9.4)	65 (7.3)	146 (13.1)
1940–49	221 (14.4)	427 (22.3)	130 (14.7)	292 (26.1)
1950–59	371 (24.1)	606 (31.7)	225 (25.4)	366 (32.7)
1960+	792 (51.4)	609 (31.9)	438 (49.4)	263 (23.5)
Mutation description, <i>n</i> (%)				
Ashkenazi Jewish ^c	130 (8.5)	144 (7.6)	41 (4.6)	49 (4.4)
Other	1410 (91.5)	1767 (92.4)	846 (95.4)	1070 (95.6)
After ovarian case exclusion				
Number	1226	1911	784	1119
Person-years follow-up	50 240	77 316	33 678	49 428
Median age (IQR)	39 (32, 48)	39 (34, 46)	41 (34, 50)	43 (37, 50)
After prevalent cases exclusion				
Number	1540	894	887	544
Person-years follow-up	65 580	36 730	39 331	24 491
Median age (IQR)	41 (34, 50)	40 (34, 46)	43 (35, 52)	44 (38, 50)

IQR, interquartile range.

^aUnaffected (earliest of age at last follow up, age at ovarian cancer diagnosis, age at prophylactic mastectomy; age 80).

^bAffected refers to carriers diagnosed with breast cancer.

^cAshkenazi Jewish includes 185delAG and 5382insC for *BRCA1* and 6174delT for *BRCA2*.

SNP selection and genotyping

The exact concentration of DNA in each sample was measured using the Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen). The quality of the DNA was assessed by electrophoresis on E-Gel-96 1% agarose gels (Invitrogen). Samples with severely degraded DNA were excluded from the study. A total of 5685 samples from mutation carriers and 110 duplicates (2%) at a concentration of 50 ng/μl (250 ng total per sample) were selected for genotyping. A single Illumina custom-designed 384-plex OPA containing 350 non-overlapping SNPs from two breast cancer GWAS and a Mayo Clinic candidate SNP study were generated. This included 142 SNPs displaying the most significant associations ($P < 1 \times 10^{-3}$) with breast cancer in stage 2 of the CGEMS breast cancer GWAS (17), 54 SNPs from the UK1 GWAS (11) displaying the most significant associations ($P < 1 \times 10^{-3}$) with breast cancer when combining data from SEARCH and CGEMS, 99 SNPs displaying the most significant associations ($P < 1 \times 10^{-3}$) with breast cancer in the combined SEARCH stage 1 and 2 data and 55 SNPs displaying the most significant associations ($P < 1 \times 10^{-2}$) with breast cancer in associations studies using the Mayo Clinic Breast Cancer Study (MCBCS) (24–26). DNA samples were arranged as a random mixture of breast cancer affected and unaffected carriers in 63 × 96 well plates with four DNA controls consisting of a trio of

CEPH controls plus a random genomic control per plate. Following genotyping, SNPs with a minor allele frequency (MAF) < 0.05 or with a call rate $< 95\%$ were excluded from the analysis ($n = 13$). An additional four SNPs which deviated significantly from Hardy–Weinberg equilibrium (HWE) ($P < 1 \times 10^{-7}$) were excluded. A total of 154 samples were excluded due to missing phenotypic data, and 74 samples were excluded due to SNP call rates $< 95\%$. Using the genotypes available, three duplicate individuals from different study centers were identified and removed. The concordance rate between duplicates was 100%. After all quality control was completed, a total of 5457 unique mutation carriers (3451 *BRCA1* and 2006 *BRCA2*) with genotyping data for 333 SNPs were analyzed (Table 2).

Statistical analyses

Associations between SNPs and breast cancer risk in *BRCA1* and *BRCA2* mutation carriers were evaluated using a Cox proportional hazards model. Each subject was followed from birth to breast cancer, or when unaffected by breast cancer to the earliest of bilateral mastectomy, ovarian cancer, last follow-up or age 80. To adjust for the non-random sampling of mutation carriers, analyses were carried out within a weighted cohort framework (27). Individuals were assigned weights based upon mutation status, breast cancer affected/unaffected status

and age at event or age at censoring, such that the observed breast cancer incidence rates in the study sample were consistent with established breast cancer risk estimates for *BRCA1* and *BRCA2* mutation carriers (27). Analyses were stratified by year of birth (based on quartiles of unaffected), ethnicity, country of residence, study site and mutation status. A robust variance approach was used to estimate the standard errors of the parameters to allow for the dependence of individuals from the same family. Each of the 333 SNPs that passed quality control was formally evaluated for associations with risk under a log-additive model. In addition, estimates were separately generated for one and two copies of the minor allele versus two copies of the major allele. Between-study heterogeneity for each SNP was assessed by fitting a model that estimated study-specific log-hazard ratios (HR), and comparing this against the model that estimated a single-pooled log-hazard ratio via a likelihood ratio test. Forest plots were used to assess consistency of study specific effects for rs6138178 (*SNRPB*), rs6602595 (*CAMK1D*), rs9393597 (*LOC134997*) and rs12652447 (*FBXL7*).

To determine the degree to which the inclusion of ovarian cancer cases may have influenced the risk association, analyses were performed after excluding all individuals diagnosed with ovarian cancer. To further explore the influence of inclusion of prevalent cases in the study, analyses were performed after exclusion of subjects who were diagnosed with breast cancer more than 5 years prior to age of mutation diagnosis. Because of the good 5-year survival rate for breast cancer, this avoids substantial survival bias in the collection of *BRCA1* carriers. In addition, a further analysis treating prophylactic oophorectomy as a time-dependent covariate was conducted.

Cumulative risk assessment in *BRCA1* and *BRCA2* mutation carriers

The proportion of genetic modifying variance explained by each of the SNPs was estimated by $\ln(c)/\sigma^2$, as previously described (18,28,29), where c was the estimated coefficient of variation in incidence rates due to each SNP, obtained from the SNP genotype frequencies and the ordinal hazard ratio estimates, and σ^2 was the total polygenic variance component for breast cancer risk, estimated at 1.32 for *BRCA1* and 1.73 for *BRCA2* mutation carriers (5). In addition, the HR estimates of candidate SNPs were integrated with estimates of either *BRCA1* or *BRCA2* penetrance (1) to further estimate the influence of candidate SNPs on the absolute risk of breast cancer. This was achieved by constraining breast cancer incidence to match estimates of *BRCA1* or *BRCA2* penetrance by year of age and by estimating the cumulative incidence of breast cancer that corresponded to each genotype, or genotype combination, by applying the estimated HRs such that the appropriate baseline breast cancer incidence was retained while reflecting the relative risk differences among the genotypes, or genotype combinations. Plots of the resulting breast cancer cumulative incidence estimates corresponding to each genotype of each candidate SNP were generated.

A series of three models were fit to the data for the top two SNPs, separately for *BRCA1* and *BRCA2* carriers, in order to

assess the combined effects of two candidate *BRCA1* modifiers and also two candidate *BRCA2* modifiers on breast cancer risk. The first model estimated a separate HR for each combination of genotypes, with those simultaneously homozygous for the major alleles serving as the reference group. The second model estimated an ordinal (log-additive) trend for each SNP, together with a multiplicative interaction between the ordinal effects for the two SNPs. The third model simply estimated an ordinal effect for each of the two SNPs. Score tests that accounted for familial clustering were used to test if increasing model complexity significantly improved model fit. Estimates from the models that most parsimoniously fit the data from the two SNPs were integrated with estimates of *BRCA1* and *BRCA2* penetrance (1) to calculate the cumulative risk of breast cancer for each combination of SNP genotypes in *BRCA1* and *BRCA2* carriers separately.

SUPPLEMENTARY MATERIAL

Supplementary Material is available at *HMG* online.

ACKNOWLEDGEMENTS

We thank the CGEMS and UK1 GWAS for providing candidate SNPs.

Conflict of Interest statement. None declared.

FUNDING

The work was supported by R01 grants (CA122340, CA128978) and a Breast Cancer Specialized Program of Research Excellence grant [P50 CA116201 (SPORE)] from the National Cancer Institute at the National Institutes of Health, an award from the Breast Cancer Research Foundation and an award from the Komen Race for the Cure Foundation to F.J.C. The UK1 GWAS was funded by a Cancer Research UK award to D.F.E. The CGEMS GWAS was funded by intramural funds from the National Institutes of Health.

APPENDIX

EPIDEMIOLOGICAL STUDY OF *BRCA1* & *BRCA2* MUTATION CARRIERS (EMBRACE)

EMBRACE Collaborating Centers are: Coordinating Centre, Cambridge: Susan Peock, Margaret Cook, Clare Oliver, Debra Frost. North of Scotland Regional Genetics Service, Aberdeen: Helen Gregory, Zosia Miedzybrodzka. Northern Ireland Regional Genetics Service, Belfast: Patrick Morrison. West Midlands Regional Clinical Genetics Service, Birmingham: Trevor Cole, Carole McKeown, Amy Taylor. South West Regional Genetics Service, Bristol: Alan Donaldson. East Anglian Regional Genetics Service, Cambridge: Joan Paterson. Medical Genetics Services for Wales, Cardiff: Alexandra Murray, Mark Rogers, Emma McCann. St James's Hospital, Dublin, and National Centre for Medical Genetics, Dublin: John Kennedy, David Barton. South East of Scotland Regional Genetics Service, Edinburgh: Mary Porteous.

Peninsula Clinical Genetics Service, Exeter: Carole Brewer, Emma Kivuva, Anne Searle, Selina Goodman. West of Scotland Regional Genetics Service, Glasgow: Rosemarie Davidson, Victoria Murday, Nicola Bradshaw, Lesley Snadden, Mark Longmuir, Catherine Watt. South East Thames Regional Genetics Service, Guys Hospital London: Louise Izatt, Gabriella Pichert, Caroline Langman. North West Thames Regional Genetics Service, Harrow: Huw Dorkins. Leicestershire Clinical Genetics Service, Leicester: Julian Barwell. Yorkshire Regional Genetics Service, Leeds: Carol Chu, Tim Bishop, Julie Miller. Merseyside and Cheshire Clinical Genetics Service, Liverpool: Ian Ellis. Manchester Regional Genetics Service, Manchester: D. Gareth Evans, Fiona Laloo, Felicity Holt. North East Thames Regional Genetics Service, NE Thames: Alison Male, Anne Robinson. Nottingham Centre for Medical Genetics, Nottingham: Carol Gardiner. Northern Clinical Genetics Service, Newcastle: Fiona Douglas, Oonagh Claber. Oxford Regional Genetics Service, Oxford: Lisa Walker, Diane McLeod. The Institute of Cancer Research and Royal Marsden NHS Foundation Trust: Ros Eeles, Susan Shanley, Nazneen Rahman, Richard Houlston, Elizabeth Bancroft, Lucia D'Mello, Elizabeth Page, Audrey Ardern-Jones, Anita Mitra. North Trent Clinical Genetics Service, Sheffield: Jackie Cook, Oliver Quarrell, Cathryn Bardsley. South West Thames Regional Genetics Service, London: Shirley Hodgson, Sheila Goff, Glen Brice, Lizzie Winchester. Wessex Clinical Genetics Service, Princess Anne Hospital, Southampton: Diana Eccles, Anneke Lucassen, Gillian Crawford, Emma Tyler, Donna McBride. The EMBRACE study was funded by Cancer Research UK. S.P. is funded by Cancer Research-UK Grants C1287/A10118 and C1287/A8874. D.G.E. and F.L. are supported by an NIHR grant to the Biomedical Research Centre, Manchester. The Investigators at The Institute of Cancer Research and The Royal Marsden NHS Foundation Trust are supported by an NIHR grant to the Biomedical Research Centre at The Institute of Cancer Research and The Royal Marsden NHS Foundation Trust. R.E./E.B./L.D'M. are also supported by Cancer Research UK Grant C5047/A8385. A.C.A. is a Cancer Research UK Senior Cancer Research Fellow. D.F.E. is a Principal Research Fellow of Cancer Research UK.

GERMAN CONSORTIUM OF HEREDITARY BREAST AND OVARIAN CANCER (GC-HBOC)

We thank all families for providing samples for this study. This work was supported by grants from the German Cancer Aid (Grant numbers 107054 and 107353).

THE GEMO STUDY (GENETIC MODIFIERS OF CANCER RISK IN BRCA1/2 MUTATION CARRIERS: CANCER GENETICS NETWORK 'GROUPE GÉNÉTIQUE ET CANCER', FÉDÉRATION NATIONALE DES CENTRES DE LUTTE CONTRE LE CANCER, FRANCE)

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KCONFAB (KATHLEEN CUNNINGHAM FOUNDATION CONSORTIUM FOR RESEARCH INTO FAMILIAL BREAST CANCER)

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. A.B.S. and G.C.T. are Senior and Senior Principal Research Fellows, respectively, of the NHMRC.

MODIFIER STUDY OF QUANTITATIVE EFFECTS ON DISEASE (MODSQUAD)

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REFERENCES

- Antoniou, A., Pharoah, P.D., Narod, S., Risch, H.A., Eyfjord, J.E., Hopper, J.L., Loman, N., Olsson, H., Johannsson, O., Borg, A. *et al.* (2003) Average risks of breast and ovarian cancer associated with BRCA1 or BRCA2 mutations detected in case series unselected for family history: a combined analysis of 22 studies. *Am. J. Hum. Genet.*, **72**, 1117–1130.
- Simchoni, S., Friedman, E., Kaufman, B., Gershoni-Baruch, R., Orr-Urtreger, A., Kedar-Barnes, I., Shiri-Sverdlow, R., Dagan, E., Tsabari, S., Shohat, M. *et al.* (2006) Familial clustering of site-specific cancer risks associated with BRCA1 and BRCA2 mutations in the Ashkenazi Jewish population. *Proc. Natl Acad. Sci. USA*, **103**, 3770–3774.
- Rebbeck, T.R. (2002) Inherited predisposition and breast cancer: modifiers of BRCA1/2-associated breast cancer risk. *Environ. Mol. Mutagen.*, **39**, 228–234.
- Antoniou, A.C., Pharoah, P.D., McMullan, G., Day, N.E., Stratton, M.R., Peto, J., Ponder, B.J. and Easton, D.F. (2002) A comprehensive model for familial breast cancer incorporating BRCA1, BRCA2 and other genes. *Br. J. Cancer*, **86**, 76–83.
- Antoniou, A.C., Cunningham, A.P., Peto, J., Evans, D.G., Lalloo, F., Narod, S.A., Risch, H.A., Eyfjord, J.E., Hopper, J.L., Southey, M.C. *et al.* (2008) The BOADICEA model of genetic susceptibility to breast and ovarian cancers: updates and extensions. *Br. J. Cancer*, **98**, 1457–1466.
- Begg, C.B., Haile, R.W., Borg, A., Malone, K.E., Concannon, P., Thomas, D.C., Langholz, B., Bernstein, L., Olsen, J.H., Lynch, C.F. *et al.* (2008) Variation of breast cancer risk among BRCA1/2 carriers. *J. Am. Med. Assoc.*, **299**, 194–201.
- Chenevix-Trench, G., Milne, R.L., Antoniou, A.C., Couch, F.J., Easton, D.F. and Goldgar, D.E. (2007) An international initiative to identify genetic modifiers of cancer risk in BRCA1 and BRCA2 mutation carriers: the Consortium of Investigators of Modifiers of BRCA1 and BRCA2 (CIMBA). *Breast Cancer Res.*, **9**, 104.
- Couch, F.J., Sinilnikova, O., Vierkant, R.A., Pankratz, V.S., Fredericksen, Z.S., Stoppa-Lyonnet, D., Coupier, I., Hughes, D., Hardouin, A., Berthet, P. *et al.* (2007) AURKA F31I polymorphism and breast cancer risk in BRCA1 and BRCA2 mutation carriers: a consortium of investigators of modifiers of BRCA1/2 study. *Cancer Epidemiol. Biomarkers Prev.*, **16**, 1416–1421.
- Gao, B., Xie, X.J., Huang, C., Shames, D.S., Chen, T.T., Lewis, C.M., Bian, A., Zhang, B., Olopade, O.I., Garber, J.E. *et al.* (2008) RASSF1A polymorphism A133S is associated with early onset breast cancer in BRCA1/2 mutation carriers. *Cancer Res.*, **68**, 22–25.
- Antoniou, A.C., Sinilnikova, O.M., Simard, J., Leone, M., Dumont, M., Neuhausen, S.L., Struwing, J.P., Stoppa-Lyonnet, D., Barjhoux, L., Hughes, D.J. *et al.* (2007) RAD51 135G > C modifies breast cancer risk among BRCA2 mutation carriers: results from a combined analysis of 19 studies. *Am. J. Hum. Genet.*, **81**, 1186–1200.
- Easton, D.F., Pooley, K.A., Dunning, A.M., Pharoah, P.D., Thompson, D., Ballinger, D.G., Struwing, J.P., Morrison, J., Field, H., Luben, R. *et al.* (2007) Genome-wide association study identifies novel breast cancer susceptibility loci. *Nature*, **447**, 1087–1093.
- Hunter, D.J., Kraft, P., Jacobs, K.B., Cox, D.G., Yeager, M., Hankinson, S.E., Wacholder, S., Wang, Z., Welch, R., Hutchinson, A. *et al.* (2007) A genome-wide association study identifies alleles in FGFR2 associated with risk of sporadic postmenopausal breast cancer. *Nat. Genet.*, **39**, 870–874.
- Stacey, S.N., Manolescu, A., Sulem, P., Rafnar, T., Gudmundsson, J., Gudjonsson, S.A., Masson, G., Jakobsdottir, M., Thorlacius, S., Helgason, A. *et al.* (2007) Common variants on chromosomes 2q35 and 16q12 confer susceptibility to estrogen receptor-positive breast cancer. *Nat. Genet.*, **39**, 865–869.
- Stacey, S.N., Manolescu, A., Sulem, P., Thorlacius, S., Gudjonsson, S.A., Jonsson, G.F., Jakobsdottir, M., Bergthorsson, J.T., Gudmundsson, J., Aben, K.K. *et al.* (2008) Common variants on chromosome 5p12 confer susceptibility to estrogen receptor-positive breast cancer. *Nat. Genet.*, **40**, 703–706.
- Ahmed, S., Thomas, G., Ghoussaini, M., Healey, C.S., Humphreys, M.K., Platte, R., Morrison, J., Maranian, M., Pooley, K.A., Luben, R. *et al.* (2009) Newly discovered breast cancer susceptibility loci on 3p24 and 17q23.2. *Nat. Genet.*, **41**, 585–590.
- Zheng, W., Long, J., Gao, Y.T., Li, C., Zheng, Y., Xiang, Y.B., Wen, W., Levy, S., Deming, S.L., Haines, J.L. *et al.* (2009) Genome-wide association study identifies a new breast cancer susceptibility locus at 6q25.1. *Nat. Genet.*, **41**, 324–328.
- Thomas, G., Jacobs, K.B., Kraft, P., Yeager, M., Wacholder, S., Cox, D.G., Hankinson, S.E., Hutchinson, A., Wang, Z., Yu, K. *et al.* (2009) A multistage genome-wide association study in breast cancer identifies two new risk alleles at 1p11.2 and 14q24.1 (RAD51L1). *Nat. Genet.*, **41**, 579–584.
- Antoniou, A.C., Spurdle, A.B., Sinilnikova, O.M., Healey, S., Pooley, K.A., Schmutzler, R.K., Vermold, B., Engel, C., Meindl, A., Arnold, N. *et al.* (2008) Common breast cancer-predisposition alleles are associated with breast cancer risk in BRCA1 and BRCA2 mutation carriers. *Am. J. Hum. Genet.*, **82**, 937–948.
- Antoniou, A.C., Sinilnikova, O.M., McGuffog, L., Healey, S., Nevanlinna, H., Heikkinen, T., Simard, J., Spurdle, A.B., Beesley, J., Chen, X. *et al.* (2009) Common variants in LSP1, 2q35 and 8q24 and breast cancer risk for BRCA1 and BRCA2 mutation carriers. *Hum. Mol. Genet.*, **18**, 4442–4456.
- Garcia-Closas, M., Hall, P., Nevanlinna, H., Pooley, K., Morrison, J., Richesson, D.A., Bojesen, S.E., Nordestgaard, B.G., Axelsson, C.K., Arias, J.I. *et al.* (2008) Heterogeneity of breast cancer associations with five susceptibility loci by clinical and pathological characteristics. *PLoS Genet.*, **4**, e1000054.
- Rebbeck, T.R., Lynch, H.T., Neuhausen, S.L., Narod, S.A., Van't Veer, L., Garber, J.E., Evans, G., Isaacs, C., Daly, M.B., Matloff, E. *et al.* (2002)

- Prophylactic oophorectomy in carriers of BRCA1 or BRCA2 mutations. *N. Engl. J. Med.*, **346**, 1616–1622.
22. Bergamaschi, A., Kim, Y.H., Kwei, K.A., La Choi, Y., Bocanegra, M., Langerod, A., Han, W., Noh, D.Y., Huntsman, D.G., Jeffrey, S.S. *et al.* (2008) CAMK1D amplification implicated in epithelial-mesenchymal transition in basal-like breast cancer. *Mol. Oncol.*, **2**, 327–339.
 23. Lakhani, S.R., Reis-Filho, J.S., Fulford, L., Penault-Llorca, F., van der Vijver, M., Parry, S., Bishop, T., Benitez, J., Rivas, C., Bignon, Y.J. *et al.* (2005) Prediction of BRCA1 status in patients with breast cancer using estrogen receptor and basal phenotype. *Clin. Cancer Res.*, **11**, 5175–5180.
 24. Wang, X., Goode, E.L., Fredericksen, Z.S., Vierkant, R.A., Pankratz, V.S., Liu-Mares, W., Rider, D.N., Vachon, C.M., Cerhan, J.R., Olson, J.E. *et al.* (2008) Association of genetic variation in genes implicated in the beta-catenin destruction complex with risk of breast cancer. *Cancer Epidemiol. Biomarkers Prev.*, **17**, 2101–2108.
 25. Kelemen, L.E., Couch, F.J., Ahmed, S., Dunning, A.M., Pharoah, P.D., Easton, D.F., Fredericksen, Z.S., Vierkant, R.A., Pankratz, V.S., Goode, E.L. *et al.* (2008) Genetic variation in stromal proteins decorin and lumican with breast cancer: investigations in two case-control studies. *Breast Cancer Res.*, **10**, R98.
 26. Olson, J.E., Wang, X., Goode, E.L., Pankratz, V.S., Fredericksen, Z.S., Vierkant, R.A., Pharoah, P.D., Cerhan, J.R. and Couch, F.J. (2010) Variation in genes required for normal mitosis and risk of breast cancer. *Breast Cancer Res. Treat.*, **119**, 453–462.
 27. Antoniou, A.C., Goldgar, D.E., Andrieu, N., Chang-Claude, J., Brohet, R., Rookus, M.A. and Easton, D.F. (2005) A weighted cohort approach for analysing factors modifying disease risks in carriers of high-risk susceptibility genes. *Genet. Epidemiol.*, **29**, 1–11.
 28. Risch, N. (1990) Linkage strategies for genetically complex traits. I. Multilocus models. *Am. J. Hum. Genet.*, **46**, 222–228.
 29. Antoniou, A.C. and Easton, D.F. (2003) Polygenic inheritance of breast cancer: implications for design of association studies. *Genet. Epidemiol.*, **25**, 190–202.