

Common variants in *LSP1*, 2q35 and 8q24 and breast cancer risk for *BRCA1* and *BRCA2* mutation carriers

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Genome-wide association studies of breast cancer have identified multiple single nucleotide polymorphisms (SNPs) that are associated with increased breast cancer risks in the general population. In a previous study, we demonstrated that the minor alleles at three of these SNPs, in *FGFR2*, *TNRC9* and *MAP3K1*, also confer increased risks of breast cancer for *BRCA1* or *BRCA2* mutation carriers. Three additional SNPs rs3817198 at *LSP1*, rs13387042 at 2q35 and rs13281615 at 8q24 have since been reported to be associated with breast cancer in the general population, and in this study we evaluated their association with breast cancer risk in 9442 *BRCA1* and 5665 *BRCA2* mutation carriers from 33 study centres. The minor allele of rs3817198 was associated with increased breast cancer risk only for *BRCA2* mutation carriers [hazard ratio (HR) = 1.16, 95% CI: 1.07–1.25, *P*-trend = 2.8×10^{-4}]. The best fit for the association of SNP rs13387042 at 2q35 with breast cancer risk was a dominant model for both *BRCA1* and *BRCA2* mutation carriers (*BRCA1*: HR = 1.14, 95% CI: 1.04–1.25, *P* = 0.0047; *BRCA2*: HR = 1.18 95% CI: 1.04–1.33, *P* = 0.0079). SNP rs13281615 at 8q24 was not associated with breast cancer for either *BRCA1* or *BRCA2* mutation carriers, but the estimated association for *BRCA2* mutation carriers (per-allele HR = 1.06, 95% CI: 0.98–1.14) was consistent with odds ratio estimates derived from population-based case–control studies. The *LSP1* and 2q35 SNPs appear to interact multiplicatively on breast cancer risk for *BRCA2* mutation carriers. There was no evidence that the associations vary by mutation type depending on whether the mutated protein is predicted to be stable or not.

INTRODUCTION

Pathogenic mutations in *BRCA1* and *BRCA2* confer high risks of breast and ovarian cancer. The breast cancer risks for *BRCA1* and *BRCA2* mutation carriers have been estimated to be between 40 and 87% by age 70 (1–7), but there is substantial evidence that these risks are modified by other factors that also cluster in families. Segregation analysis models have quantified the extent of variability in the breast cancer risk for *BRCA1* and *BRCA2* mutation carriers by means of polygenic-modifying variances, and suggest that the relative genetic variation in disease incidence is similar to that for women from the general population (2). A plausible explanation for this finding is that genetic variants associated with breast cancer risk for women in the general population also modify the disease risk for mutation carriers.

To evaluate this hypothesis, the Consortium of Investigators of Modifiers of *BRCA1/2* (CIMBA) evaluated the associations between the breast cancer risk for mutation carriers and three common breast cancer susceptibility alleles identified through genome-wide association studies (GWAS) (8–11). The minor alleles of single nucleotide polymorphisms (SNPs) in *FGFR2*, *TNRC9* and *MAP3K1* were all found to be associated with breast cancer risk for *BRCA2* mutation carriers, but only the *TNRC9* SNP was associated with breast cancer risk for *BRCA1* mutation carriers. The *TNRC9* SNP was estimated to account for ~0.5% of the familial/genetic variance of breast cancer risk for *BRCA1* mutation carriers estimated by segregation analyses, and the three SNPs in *FGFR2*, *TNRC9* and *MAP3K1* for 2.8% of the estimated familial/genetic variance of breast cancer risk for *BRCA2* mutation carriers (8). Therefore, the large majority of the variability in breast cancer risks for *BRCA1* and *BRCA2* remains unexplained. The GWAS by Easton *et al.* (9) and Stacey *et al.* (11) identified three additional breast cancer susceptibility loci at 8q24, 2q35 and on 11p15 close to the *LSP1* gene. To evaluate whether these loci are also associated with breast cancer risk for *BRCA1* and/or *BRCA2* mutation carriers, we genotyped these disease-associated SNPs in a large series of female carriers assembled by the CIMBA consortium.

RESULTS

Mutation carriers were recruited by 33 study centres in 21 countries (Table 1). After quality control exclusions, we analysed data from 8136 carriers censored at a first breast cancer diagnosis, 1430 individuals censored at an ovarian cancer diagnosis, 414 censored at bilateral prophylactic mastectomy and 5127 carriers censored at the age at last follow-up (Table 2). The estimated hazard ratios (HRs) associated with each SNP are shown in Table 3.

The *LSP1* SNP rs3817198 was not associated with an increased risk of breast cancer for *BRCA1* mutation carriers (P -trend = 0.09), but there was significant evidence of association with breast cancer risk for *BRCA2* mutation carriers (P -trend = 0.00028). The estimated association in *BRCA2* mutation carriers was consistent with a multiplicative model in which each copy of the minor allele was estimated to confer a HR of 1.16 (95% CI: 1.07–1.25). There was some evidence of heterogeneity in the study HRs for both *BRCA1*

and *BRCA2* mutation carriers (P = 0.002 and 0.013, respectively, Fig. 1). To investigate whether the observed heterogeneity was due to the inclusion of several small studies and whether this influenced our results, we repeated the analysis by excluding any studies with fewer than 100 *BRCA1* or 100 *BRCA2* mutation carriers. The results were similar to those from the overall analysis. The estimated per allele HR for *BRCA1* mutation carriers was 1.05 (95% CI: 0.99–1.12, P -trend = 0.12) and for *BRCA2* it was 1.17 (95% CI: 1.07–1.27, P -trend = 0.00027). However, there was no longer evidence of heterogeneity in the HRs across studies for either *BRCA1* or *BRCA2* (P = 0.06 and 0.18, respectively). There was no significant evidence that the HRs varied by age for either *BRCA1* or *BRCA2* mutation carriers (P = 0.99 and 0.17, respectively).

There was significant evidence that rs13387042 at 2q35 was associated with breast cancer risk for both *BRCA1* and *BRCA2* mutation carriers (2df P = 0.003 and 0.015, respectively). However, for both *BRCA1* and *BRCA2* mutation carriers, the estimated risk for carriers homozygous for the risk allele was lower than the estimated risk for heterozygotes. Therefore, the effects were not consistent with a multiplicative model (P = 0.001 for *BRCA1*, 0.013 for *BRCA2*). The most parsimonious model for the association of the risk allele was a dominant model for both *BRCA1* and *BRCA2* (P = 0.07 and 0.27, respectively, compared with the general model). Under the dominant model, the HR for carriers who had a copy of the A allele at this SNP was estimated to be 1.14 (95% CI: 1.04–1.25, P = 0.0047) for *BRCA1* mutation carriers and 1.18 (95% CI: 1.04–1.33, P = 0.0079) for *BRCA2* mutation carriers. There was no evidence of heterogeneity in the HRs across studies for *BRCA1* mutation carriers (P = 0.13), but there was significant evidence of heterogeneity for *BRCA2* mutation carriers (P = 0.9×10^{-5} ; Fig. 2). The heterogeneity for *BRCA2* mutation carriers remained after the exclusion of studies with fewer than 100 *BRCA2* mutation carriers ($P_{\text{het}} = 7 \times 10^{-5}$); this finding was largely driven by the UCI study ($P_{\text{het}} = 0.44$ after excluding UCI). Exclusion of this study, however, made little difference to the overall HR estimates (P -dominant = 0.014, HR = 1.18, 95% CI: 1.03–1.34). There was no evidence that the HRs varied by age (P = 0.86 and 0.34 for *BRCA1* and *BRCA2*, respectively).

SNP rs13281615 at 8q24 was not associated with breast cancer risk for either *BRCA1* or *BRCA2* mutation carriers (P -trend = 0.88 and 0.15, respectively). The estimated per allele HR for *BRCA1* mutation carriers was 1.00 (95% CI: 0.94–1.05) and for *BRCA2* mutation carriers it was 1.06 (95% CI: 0.98–1.14). There was evidence of heterogeneity in the HRs across studies for *BRCA1*, but not for *BRCA2* mutation carriers (P -heterogeneity = 0.0002 and 0.59, respectively; Fig. 3). There was also evidence that the per-allele HR in *BRCA1* mutation carriers decreased with age (P -trend = 0.002). However, this was mainly driven by a significant association for women aged 60–69 years (HR = 0.68, 95% CI: 0.52–0.90; Supplementary Material, Table S1).

To investigate whether the inclusion of prevalent cancer cases influenced our HR estimates, we repeated the analysis after excluding carriers diagnosed with breast or ovarian cancer more than 5 years prior to recruitment, leaving 6507 *BRCA1* carriers (4086 unaffected, 2421 affected) and 3997

Table 1. Number of eligible *BRCA1* and *BRCA2* carriers by study group

Study	Country ^a	<i>BRCA1</i> , N	<i>BRCA2</i> , N	Genotyping platform
Medical University of Vienna (MUV)	Austria	284	122	iPLEX ^b
Breast Cancer Family Registry (BCFR)	USA, Canada, Australia	499	359	Taqman
Copenhagen Breast Cancer Study (CBCS)	Denmark	92	51	Taqman
Spanish National Cancer Centre (CNIO)	Spain, Greece	170	199	Taqman
Deutsches Krebsforschungszentrum (DKFZ)	Germany	68	27	Taqman
HEreditary Breast and Ovarian study Netherlands (HEBON)	The Netherlands	769	294	iPLEX ^b
EMBRACE	UK, Eire	807	634	iPLEX ^b
Fox Chase Cancer Centre (FCCC)	USA	81	54	iPLEX ^b
German Consortium of Hereditary Breast and Ovarian Cancer (GC-HBOC)	Germany	802	378	Taqman
Genetic Modifiers of cancer risk in <i>BRCA1/2</i> mutation carriers (GEMO)	France, USA	1123	567	Taqman
Gynecologic Oncology Group (GOG)	USA	398	282	Taqman
Hospital Clinico San Carlos (HCSC)	Spain	109	94	Taqman
Helsinki Breast Cancer Study (HEBCS)	Finland	102	104	iPLEX ^b
Iceland Landspítali—University Hospital (ILUH)	Iceland	0	87	Sequencing
Interdisciplinary Health Research International Team Breast Cancer Susceptibility (INHERIT BRCA)	Quebec, Canada	73	82	Taqman
kConFab	Australia	489	390	iPLEX ^b
University of California Irvine (UCI)	USA	168	121	Taqman
Mayo Clinic (MAYO)	USA	213	118	iPLEX ^b
Milan Breast Cancer Study Group (MBCSG)	Italy	346	218	Taqman
Memorial Sloane Kettering Cancer Center (MSKCC)	USA	258	155	Taqman
National Cancer Institute (NCI)	USA	156	73	Taqman
National Israeli Cancer Control Center (NICCC)	Israel	315	200	Taqman
Ontario Cancer Genetics Network (OCGN)	Canada	219	171	Taqman
Ohio State University Clinical Cancer Center (OSU CCG)	USA	60	31	Taqman
Odense University Hospital (OUH)	Denmark	216	132	Taqman
Pisa Breast Cancer Study (PBCS)	Italy	73	41	iPLEX ^b
Istituto Oncologico Veneto—Hereditary Breast Ovarian Cancer Study (IOVHBOCS)	Italy	95	88	Taqman
Sheba Medical Centre (SMC)—Tel Hashomer	Israel	400	190	Taqman
Swedish Breast Cancer Study (SWE-BRCA)	Sweden	413	121	iPLEX ^b
N.N. Petrov Institute of Oncology (NNPIO)	Russia	67	0	Taqman
Modifier Study of Quantitative Effects on Disease (ModSQuaD)	Czech Republic, Belgium	272	130	Taqman
University of Turin Breast Cancer Study (UTBCS)	Italy	60	43	Taqman
University of Pennsylvania (UPENN)	USA	245	109	iPLEX ^b
Total		9442	5665	

^aCountry of the clinic at which carriers are recruited.

^bIndicates centralized genotyping (Queensland Institute of Medical Research).

BRCA2 carriers (2386 unaffected, 1611 affected). The HR estimates were similar to those from the analysis which included all cancer cases (Supplementary Material, Table S2).

Approximately 72% of the *BRCA1* mutation carriers harboured class 1 mutations and 22% class 2 mutations, and the remainder of mutations were unclassifiable. To investigate whether there were differences in the strength of the associations by mutation type, we carried out separate analyses for *BRCA1* class 1 and class 2 mutations (Table 4). rs13281615 (8q24) and rs3817198 (*LSP1*) were not associated with breast cancer risk for either class 1 or class 2 mutation carriers, consistent with the analysis in which all *BRCA1* mutation carriers were combined. The 2q35 SNP (rs13387042) appeared to be more strongly associated with breast cancer risk for *BRCA1* class 2 mutation carriers (under dominant model: HR = 1.30, 95% CI: 1.06–1.60, $P = 0.013$) than class 1 mutation carriers (HR = 1.10, 95% CI: 0.99–1.23, $P = 0.069$), but the difference in HR was not statistically significant ($P = 0.17$). The number of *BRCA2* mutation carriers harbouring class 2 mutations was too small to warrant separate analyses.

We also conducted analyses restricted to carriers of the most frequent mutations, 185delAG and 5382insC in *BRCA1* (2532 carriers) and 6174delT in *BRCA2* (834 carriers). The estimated HRs did not differ from those for the overall study (results not shown).

To evaluate the combined associations of rs3817198 in *LSP1* and rs13387042 at 2q35 on breast cancer risk for *BRCA2* mutation carriers, we fitted a multiplicative model that included a HR parameter for the effect of each SNP and compared this against a fully saturated model which included a separate HR parameter for each combined genotype (Table 5). The genotype-specific HR ratio estimates for the combined associations of these SNPs were similar with the multiplicative and saturated models, and there was no evidence that the fully saturated model gave a better fit than the multiplicative model ($P = 0.16$, 6df).

DISCUSSION

Three GWAS in 2007 identified six loci with common SNPs that are associated with increased breast cancer risk (9–11).

Table 2. Summary characteristics for the 15 107 eligible *BRCA1* and *BRCA2* carriers used in the analysis

Characteristic	<i>BRCA1</i>		<i>BRCA2</i>	
	Unaffected	Breast cancer	Unaffected	Breast cancer
Number	4462	4980	2509	3156
Person-years follow-up	190 973	203 416	111 555	139 078
Median age at censure (IQR ^a)	41 (34–50)	40 (34–46)	43 (35–53)	43 (37–50)
Age at censure, <i>N</i> (%)				
<30	630 (14.1)	444 (8.9)	322 (12.8)	149 (4.7)
30–39	1284 (28.8)	2027 (40.7)	677 (27.0)	991 (31.4)
40–49	1338 (30.0)	1702 (34.2)	694 (27.7)	1184 (37.5)
50–59	776 (17.4)	603 (12.1)	467 (18.6)	570 (18.1)
60–69	287 (6.4)	155 (3.1)	225 (9.0)	206 (6.5)
70+	147 (3.3)	49 (1.0)	124 (4.9)	56 (1.8)
Year of birth, <i>N</i> (%)				
<1920	32 (0.7)	43 (0.9)	20 (0.8)	37 (1.2)
1920–1929	124 (2.8)	193 (3.9)	89 (3.6)	157 (5.0)
1930–1939	326 (7.3)	459 (9.2)	216 (8.6)	396 (12.6)
1940–1949	685 (15.4)	1163 (23.4)	356 (14.2)	776 (24.6)
1950–1959	1062 (23.8)	1568 (31.5)	594 (23.7)	984 (31.2)
1960+	2233 (50.0)	1554 (31.2)	1234 (49.2)	806 (25.5)
Mutation class, <i>N</i> (%)				
Class 1 ^b	3388 (75.9)	3387 (68.0)	2367 (94.3)	2926 (92.7)
Class 2 ^b	838 (18.8)	1222 (24.5)	52 (2.1)	80 (2.5)
Other	236 (5.3)	371 (7.5)	90 (3.6)	150 (4.8)
Mutation description, <i>N</i> (%)				
Ashkenazi Jewish ^c	1210 (27.1)	1322 (26.6)	440 (17.5)	394 (12.5)
Other	3252 (72.9)	3658 (73.5)	2069 (82.5)	2762 (87.5)

^aIQR, interquartile range.^bSee methods for definitions.^cAshkenazi Jewish includes 185delAG and 5382insC for *BRCA1* and 6174 delT for *BRCA2*.

We previously evaluated SNPs at three of these loci (*FGFR2*, *TNRC9*, *MAP3K1*) in *BRCA1* and *BRCA2* carriers (8). In the present analysis we evaluated the SNPs in the three additional loci for association with breast cancer risk for carriers. Our results provide evidence that rs3817198 in *LSP1* is associated with breast cancer risk for *BRCA2* mutation carriers, but we found no evidence of an association for *BRCA1* mutation carriers. The 95% CI for the HR estimates, however, included the estimated odds ratio (OR) from population-based studies (1.07 (9)). For the SNP at 2q35, we found evidence of association for both *BRCA1* and *BRCA2* mutation carriers. The estimated HR for homozygotes was lower than that for heterozygotes, such that a log-additive model was rejected. This was in contrast to the population-based studies which clearly showed a higher risk for homozygotes (11,12). The effect did not appear to be driven by any particular study: the heterozygote HR estimate was greater than, or equal to, the HR in the homozygotes for 19 of the studies, including 4 of the largest studies (BCFR, EMBRACE, GEMO and GC-HBOC). This effect was also observed in analyses subdivided by mutation class, and when prevalent cases were excluded. It is possible that this departure from log-additivity is due to chance, although it might also reflect some distinct mechanism that operates only in mutation carriers. It may be possible to clarify this once the causal variant(s) are identified, and/or by studying larger numbers of carriers. The two SNPs in *LSP1* and 2q35 appeared to interact multiplicatively on the breast cancer risk for *BRCA2* mutation carriers. Finally, the 8q24 SNP was not significantly associated with risk for either *BRCA1*

or *BRCA2* carriers. Although the CI for *BRCA1* carriers excludes the estimated OR from population-based studies (1.08), the 95% CI HR estimate for *BRCA2* carriers (0.98–1.14) includes this estimate.

There was significant evidence of heterogeneity in the HR estimates across studies for the associations of rs3817198 (*LSP1*) and rs13387042 (2q35) for *BRCA2* mutation carriers. This heterogeneity is unlikely to be due to genotyping quality issues as all genotyping centres were required to adhere to strict genotyping quality control criteria, and studies which failed these criteria were excluded from the analyses. Our analyses indicate that much of the heterogeneity was driven by studies with small numbers of mutation carriers (Table 1). When studies with fewer than 100 mutation carriers were excluded from the analysis there was no longer evidence of heterogeneity in the HRs across studies for the *LSP1* SNP, and yet the results remained virtually unchanged. The heterogeneity in the HR estimates for rs13387042 (2q35) and breast cancer risk for *BRCA2* mutation carriers was largely due to one study (UCI) with 121 *BRCA2* carriers; removal of this study, however, did not materially affect the overall association. We conclude that the associations we observed are likely to apply broadly to mutation carriers of European origin.

In our analysis of the *FGFR2*, *TNRC9* and *MAP3K1* loci, we noted that the differences in the associations between *BRCA1* and *BRCA2* mutation carriers may reflect the differences in the distribution of tumour subtype. Approximately 90% of *BRCA1* breast cancer tumours have been reported to be estrogen receptor (ER)-negative whereas tumours in

Table 3. Genotype frequencies by disease status and HR estimates

Mutation/gene	Genotype	Unaffected (%)	Affected (%)	HR	95% CI	P-value
<i>LSP1</i> rs3817198 <i>BRCA1</i>	TT	1940 (46.2)	2114 (44.2)	1.00		
	TC	1810 (43.0)	2112 (44.2)	1.05	0.96–1.13	
	CC	453 (10.8)	555 (11.6)	1.11	0.97–1.27	
	2df test					0.24
<i>BRCA2</i>	Per allele			1.05	0.99–1.11	0.090
	TT	1090 (45.3)	1283 (42.3)	1.00		
	TC	1057 (44.0)	1375 (45.4)	1.11	1.00–1.24	
	CC	257 (10.7)	372 (12.3)	1.39	1.16–1.67	
2q35 rs13387042 <i>BRCA1</i>	2df test					0.00098
	Per allele			1.16	1.07–1.25	0.00028
	GG	1029 (24.1)	1007 (21.1)	1.00		
	GA	2034 (47.7)	2423 (50.9)	1.18	1.07–1.30	
<i>BRCA2</i>	AA	1205 (28.2)	1333 (28.0)	1.08	0.97–1.21	
	2df test					0.003
	Per allele			1.03	0.98–1.09	0.24
	Dominant			1.14	1.04–1.25	0.0047
<i>BRCA2</i>	GG	604 (25.1)	672 (22.1)	1.00		
	GA	1143 (47.5)	1526 (50.2)	1.21	1.06–1.37	
	AA	660 (27.4)	844 (27.7)	1.12	0.97–1.31	
	2df test					0.015
8q24 rs13281615 <i>BRCA1</i>	Per allele			1.06	0.98–1.14	0.14
	Dominant			1.18	1.04–1.33	0.0079
	AA	1366 (32.1)	1581 (33.2)	1.00		
	AG	2135 (50.2)	2325 (48.8)	0.96	0.88–1.04	
<i>BRCA2</i>	GG	753 (17.7)	856 (18.0)	1.01	0.90–1.13	
	2df test					0.54
	Per allele			1.00	0.94–1.05	0.88
	AA	821 (34.1)	938 (31.0)	1.00		
<i>BRCA2</i>	AG	1182 (49.1)	1547 (51.1)	1.07	0.95–1.20	
	GG	405 (16.8)	540 (17.9)	1.11	0.96–1.29	
	2df test					0.35
	Per allele			1.06	0.98–1.14	0.15

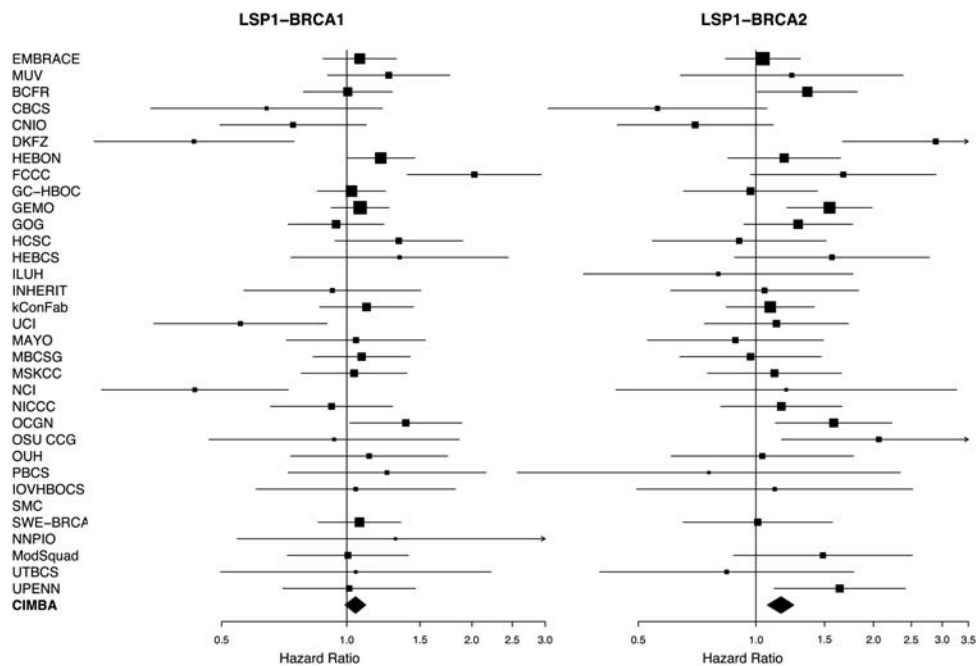


Figure 1. Study-specific per-allele HR estimates for SNP rs3817198 in *LSP1*. The area of the square is proportional to the inverse of the variance of the estimate. Horizontal lines represent the 95% confidence intervals.

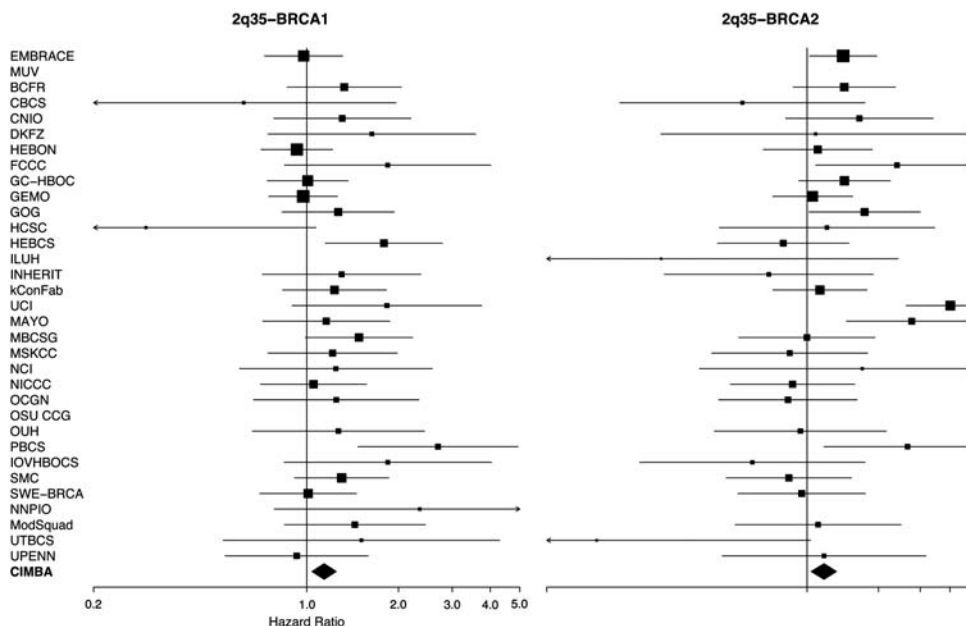


Figure 2. Study-specific HR estimates for SNP rs13387042 at 2q35 under the dominant model. The area of the square is proportional to the inverse of the variance of the estimate. Horizontal lines represent the 95% confidence intervals.

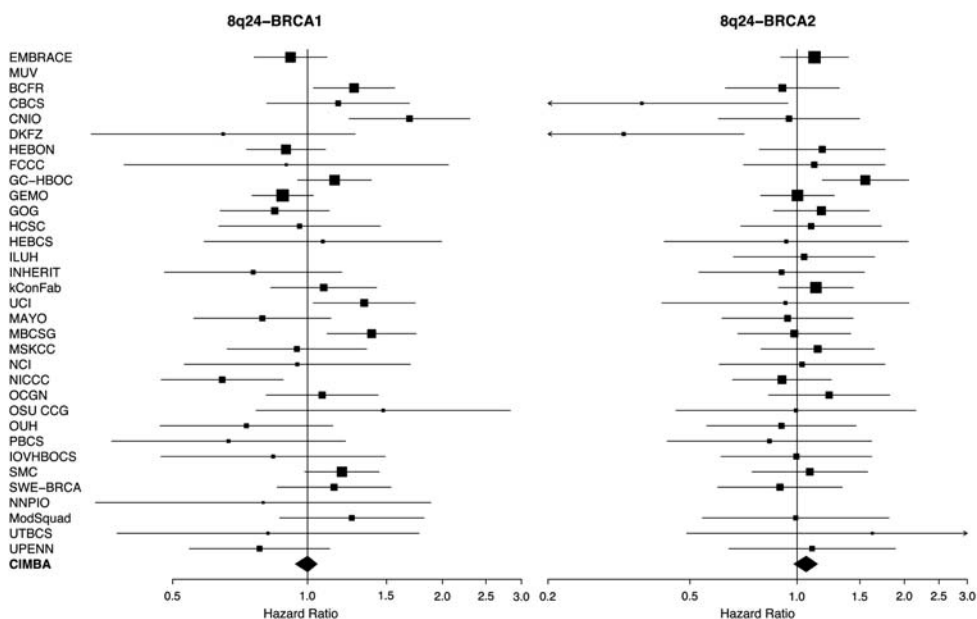


Figure 3. Study-specific per-allele HR estimates for SNP rs13281615 at 8q24. The area of the square is proportional to the inverse of the variance of the estimate. Horizontal lines represent the 95% confidence intervals.

BRCA2 mutation carriers have an ER distribution similar to those arising in women from the general population, the majority of which are ER-positive (13). All three SNPs were associated with risk in *BRCA2* carriers, whereas only the *TNRC9* SNP was associated with risk in *BRCA1* carriers, consistent with the differences in association between ER-positive and ER-negative disease reported by the Breast Cancer Association Consortium (BCAC) (8,14). BCAC has also investigated the associations of the *LSP1* and 8q24 SNPs by the ER status of cancer cases (14). For the 8q24 SNP, the

association was stronger for ER-positive disease, with no evidence of an association for ER-negative disease. Although we did not detect a significant difference in the HR estimates between *BRCA1* and *BRCA2* carriers, the absence of an association for *BRCA1* carriers (HR 1.0) and the proximity of the HR estimate derived from 5665 *BRCA2* carriers (1.06) to that reported from the population-based studies (1.08) is consistent with the differential effect of this SNP between ER-positive and ER-negative disease. BCAC did not observe a significant difference between ER-positive and ER-negative disease for

Table 4. *BRCA1* analysis by mutation class

Mutation/gene	Genotype	Unaffected (%)	Affected (%)	HR	95% CI	P-value
<i>LSP1</i> rs3817198 <i>BRCA1</i> —Class 1	TT	1476 (46.4)	1421 (43.7)	1.00		
	TC	1353 (42.5)	1445 (44.5)	1.05	0.96–1.16	
	CC	353 (11.1)	382 (11.8)	1.12	0.96–1.29	
	2df test					0.27
	Per allele			1.06	0.99–1.13	0.11
<i>BRCA1</i> —Class 2	TT	347 (44.1)	531 (45.7)	1.00		
	TC	362 (46.1)	504 (43.3)	0.95	0.79–1.15	
	CC	77 (9.8)	128 (11.0)	1.11	0.81–1.53	
	2df test					0.63
	Per allele			1.02	0.88–1.17	0.82
2q35 rs13387042 <i>BRCA1</i> —Class 1	GG	791 (24.4)	717 (22.1)	1.00		
	GA	1550 (47.8)	1620 (50.0)	1.13	1.01–1.26	
	AA	899 (27.8)	904 (27.9)	1.07	0.94–1.21	
	2df test					0.11
	Per Allele			1.03	0.97–1.10	0.37
	Dominant			1.10	0.99–1.23	0.069
<i>BRCA1</i> —Class 2	GG	186 (23.3)	218 (18.8)	1.00		
	GA	379 (47.4)	608 (52.3)	1.35	1.09–1.67	
	AA	234 (29.3)	336 (28.9)	1.21	0.95–1.56	
	2df test					0.021
	Per allele			1.08	0.95–1.22	0.22
	Dominant			1.30	1.06–1.60	0.013
8q24 rs13281615 <i>BRCA1</i> —Class 1	AA	1044 (32.2)	1083 (33.4)	1.00		
	AG	1635 (50.4)	1582 (48.7)	0.95	0.87–1.05	
	GG	564 (17.4)	582 (17.9)	1.01	0.88–1.15	
	2df test					0.54
	Per allele			1.00	0.93–1.06	0.91
<i>BRCA1</i> —Class 2	AA	246 (31.2)	376 (32.4)	1.00		
	AG	397 (50.3)	566 (48.8)	0.98	0.82–1.17	
	GG	146 (18.5)	218 (18.8)	1.01	0.79–1.30	
	2df test					0.95
	Per allele			1.00	0.89–1.13	0.98

Genotype frequencies and HR estimates.

Table 5. HR estimates for the combined effects of *LSP1* and 2q35 on breast cancer risk for *BRCA2* mutation carriers

<i>LSP1</i> /2q35 genotype	Hazard ratio	
	Multiplicative model ^a	Saturated model
TT/GG	1.00	1.00
TT/GA	1.14	1.09
TT/AA	1.14	1.24
TC/GG	1.16	1.20
TC/GA	1.32	1.35
TC/AA	1.32	1.18
CC/GG	1.34	1.14
CC/GA	1.53	1.64
CC/AA	1.53	1.60

^aHR estimates under multiplicative mode; *LSP1*: per-allele HR = 1.16 (1.07–1.26); 2q35 dominant HR = 1.14 (1.02–1.27).

the effect of the *LSP1* SNP rs3817198 (14), although the estimated OR was higher for ER-positive than ER-negative disease. Therefore, our observation that this SNP is only associated with breast cancer risk for *BRCA2* mutation carriers is also consistent with the BCAC results. Stacey *et al.* (11)

reported that SNP rs13387042 at 2q35 was associated only with risk of ER-positive breast cancer. Recent results from the much larger BCAC study, however, indicated that this SNP is associated with the risk of both ER-positive and ER-negative disease (12), a result that is more consistent with our observation of an association with disease for both *BRCA1* and *BRCA2* carriers. Overall, the results for these three SNPs are consistent with the hypothesis that the breast cancer relative risks conferred by these SNPs are broadly similar for *BRCA1* and *BRCA2* carriers to those conferred on women from the general population, considering ER-positive and ER-negative disease separately.

In summary, of the common breast cancer susceptibility variants identified by GWAS evaluated to date, the present and previous (8) results indicate that SNPs in *FGFR2*, *TNRC9*, *MAP3K1*, *LSP1* and 2q35 are all associated with breast cancer risk for *BRCA2* mutation carriers, but only the *TNRC9* and 2q35 SNPs show evidence of association with breast cancer risk for *BRCA1* mutation carriers. Based on the estimated HRs, and estimates of the genetic variance of breast cancer risk for *BRCA1* and *BRCA2* mutation carriers, it is predicted that the five SNPs would account for 3.7% of the *BRCA2* genetic-modifying variance. The *TNRC9* and

2q35 SNPs are estimated to account for 0.7% of the *BRCA1* genetic modifying variance. These findings suggest that the majority of the genetic variability in breast cancer risk for *BRCA1* and *BRCA2* mutation carriers still remains unexplained and future studies to identify further genetic modifiers of risk will be worthwhile.

MATERIALS AND METHODS

Subjects

Subjects were mutation carriers recruited by 33 study centres in 21 countries through the CIMBA initiative (Table 1). The large majority of carriers were recruited through cancer genetics clinics offering genetic testing, and enrolled into national (Australia, Austria, France, Netherlands, UK/Eire) or regional studies. Some carriers were identified by population-based sampling of cases, and some by community recruitment (e.g. in Ashkenazi Jewish populations). Eligibility to participate in CIMBA was restricted to carriers of pathogenic *BRCA1* or *BRCA2* mutations who were 18 years old or older at recruitment. Information collected included the year of birth; mutation description, including nucleotide position and base change; age at last follow-up; ages at breast and ovarian cancer diagnosis; and age or date at bilateral prophylactic mastectomy. Information was also available on the country of residence, which was defined to be the country of the clinic at which the carrier family was recruited to the study. Related individuals were identified through a unique family identifier. Women were included in the analysis if they carried mutations that were pathogenic according to generally recognized criteria (15) (Breast Cancer Information Core). All carriers participated in clinical or research studies at the host institutions under ethically approved protocols. Further details of the CIMBA initiative can be found elsewhere (16).

Women who self-reported as 'non-white' and those who carried pathogenic mutations in both *BRCA1* and *BRCA2* were excluded from the current analysis. We investigated possible overlap between studies by comparing the year of birth, exact mutation description and the reported ages, to identify potential duplicate individuals. Where possible, we also used SNP genotype data available within the CIMBA database. When a potential duplicate was identified, we contacted the relevant centres for further information about these individuals, in a manner that protected the identity of the individuals in question, in order to determine precisely the extent of true overlap in subjects and families appearing more than once in the data set. Duplicated mutation carriers were included only once in the analysis. To avoid inclusion of families extending over several studies, we included the individual in the study with the most complete version of the family. 106 mutation carriers were excluded for this reason.

Genotyping

The genotyping platforms used by each study are shown in Table 1. The DNA samples from 10 studies were genotyped using the iPLEX Mass Array platform at a single genotyping centre. One study genotyped by direct sequencing. All remain-

ing studies used the 5' endonuclease assay (Taqman), with reagents supplied by Applied Biosystems and tested centrally. All centres included at least 2% of the samples in duplicate, no template controls in every plate and a random mixture of affected and unaffected carriers. Samples that failed for two or more of the SNPs genotyped (or $\geq 20\%$ of the SNPs typed if more than three SNPs were analysed using multi-PLEX genotyping platforms) were excluded from the analysis. A study was included in the analysis only if the call rate was over 95% after samples that failed at multiple SNPs had been excluded. The concordance between duplicates had to be at least 98%. To assess the accuracy of genotyping across genotyping centres, all centres genotyped 95 DNA samples from a standard test plate (Coriell Institute) for all three SNPs. If the genotyping was inconsistent for more than one sample in the test plate, the study was excluded from the analysis of that SNP. Three studies failed these criteria for one or more of the SNPs. One study was excluded from all three SNP analyses, another from the analysis of rs13281615 and rs13387042 and one from the analysis of rs13387042. As an additional genotyping quality-control check, we also evaluated the deviation from Hardy-Weinberg equilibrium (HWE) for unrelated subjects separately for each SNP and study. For one study, the HWE *P*-value for rs3817198 was $P = 2 \times 10^{-13}$ and this study excluded from the analysis of that SNP. If HWE *P*-values were in the range 0.01–0.05 (six studies for the 8q24 SNP and four studies for the *LSP1* SNP) we examined the cluster plots; none revealed any unusual patterns and these studies were included in all the analyses. After the above exclusions a total of 15 107 unique mutation carriers (9442 *BRCA1* and 5665 *BRCA2*) from 33 studies had an observed genotype for at least of one of the SNPs and were therefore included in the analysis (Table 1).

Statistical analysis

The aim of the analysis was to evaluate the association between each genotype and breast cancer risk. The phenotype of each individual was therefore defined by their age at diagnosis of breast cancer or their age at last follow-up. For this purpose, individuals were censored at the age of the first breast cancer diagnosis, ovarian cancer diagnosis, or bilateral prophylactic mastectomy or the age at last observation. Mutation carriers censored at ovarian cancer diagnosis were considered unaffected. Since mutation carriers were not sampled randomly with respect to their disease status, standard methods of survival analysis (such as Cox regression) may lead to biased estimates of the HRs (17). This can be illustrated by considering an individual affected at age *t*. In a standard analysis of a cohort study, the SNP genotype for the individual will be compared with those of all individuals at risk at age *t*. This analysis leads to consistent estimates of the HR. However, in the present design, mutation carriers are already selected on the basis of disease status (where affected individuals are over-sampled). If standard cohort analysis were applied to these data, it would lead to affected individuals at age *t* being compared with unaffected carriers selected on the basis of their future disease status. If the genotype is associated with the disease, the risk estimate will be biased to zero because too many affected individuals (in

whom the at-risk genotype is overrepresented) are included in the comparison group. Simulation studies have shown that this effect can be quite marked (17). We therefore conducted the analysis by modelling the retrospective likelihood of the observed genotypes conditional on the disease phenotypes. The derivation of the retrospective likelihood is described in detail elsewhere (15). In this model, the breast cancer incidence was assumed to depend on the underlying SNP genotype through a Cox proportional hazards model: $\lambda_i(t) = \lambda_0(t) \exp(\beta_i)$, where $\exp(\beta_i)$ is the HR for genotype i and $\lambda_0(t)$ is the breast cancer incidence rate in the baseline category. Under this approach, the baseline age-specific incidence rates in the Cox proportional-hazards model were chosen such that the overall breast cancer incidence rates, averaged over all genotypic categories, agree with external estimates of incidence for *BRCA1* and *BRCA2* mutation carriers (2). The effect of each SNP was modelled either as a per-allele HR (multiplicative model) or as separate HRs for heterozygotes and homozygotes, and these were estimated on the log scale (i.e. β_i). Where there was evidence of deviation from the multiplicative model, dominant and recessive models were also fitted. The HRs were assumed to be independent of age (i.e. we used a Cox proportional-hazards model). The assumption of proportional hazards was tested by adding a 'genotype x age' interaction term to the model in order to fit models in which the HR changed with age. Analyses were carried out with the pedigree-analysis software MENDEL (18). We examined between-study heterogeneity by comparing the models that allowed for study-specific log-HRs against models in which the same log-HR was assumed to apply to all studies. All analyses were stratified by study group and country of residence and used calendar-year- and cohort-specific breast cancer incidence rates for *BRCA1* and *BRCA2* (2). We used a robust variance-estimation approach to allow for the non-independence among related carriers (19). The median family size in our sample was 1 (inter-quartile range: 1–2). To evaluate the combined effects of the significant SNPs on breast cancer risk, we fitted a multiplicative (log-additive) model that included a parameter for the log-HR for each of the SNPs (depending on the locus specific genetic model) and compared this with a fully saturated model in which a separate parameter was fitted for each multi-locus genotype.

To investigate whether our results were influenced by any of our assumptions we performed additional sensitivity analyses. If any of the SNPs were associated with disease survival, the inclusion of prevalent cases may influence the HR estimates. We therefore repeated our analysis by excluding mutation carriers diagnosed more than 5 years prior to the age at recruitment into the study. To examine whether SNP associations differed by type of mutations, we classified mutations according to their potential functional effect. Class 1 mutations comprised loss-of-function mutations, expected to result in a reduced transcript or protein level due to mRNA nonsense-mediated decay (NMD) and/or degradation or instability of truncated proteins (20–23), translation re-initiation but no production of stable protein (24), or the absence of expression because of the deletion of transcription regulatory regions. Class 2 mutations were those likely to generate potentially stable mutant proteins that might have dominant negative

action, partially preserved normal function, or loss of function. Class 2 mutations include missense substitutions, in-frame deletions and insertion, as well as truncating mutations with premature stop codons occurring in the last exon (22). Mutations, whose consequences at transcript or protein level could not be inferred, were not considered for this classification. These were mainly mutations located in splice sites but not characterised for their effect at transcript level, or large deletions or insertions with undetermined boundaries. Additional analyses were performed by restricting to carriers of the mutations 185delAG and 5382insC in *BRCA1* and 6174delT in *BRCA2*, which were the most frequent mutations observed.

The proportions of the modifying variance explained by the set of associated SNPs were estimated by $\ln(c)/\sigma^2$, where c is the estimated coefficient of variation in incidences associated with SNP (25,26) and σ^2 is the estimated modifying variance [1.32 and 1.73 for *BRCA1* and *BRCA2* mutation carriers, respectively (2)]. We estimated the total proportion of the modifying variance due to all SNPs by adding the individual proportions, i.e. by assuming that the loci combined multiplicatively.

WEB RESOURCES

Breast Cancer Information Core, <http://research.nhgri.nih.gov/projects/bic/>.

SUPPLEMENTARY MATERIAL

Supplementary Material is available at *HMG* online.

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Conflict of Interest statement: The authors declare no conflicts of interest.

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APPENDIX

CIMBA COLLABORATING CENTRES

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Epidemiological study of BRCA1 & BRCA2 mutation carriers (EMBRACE)

DFE is the PI of the study. EMBRACE Collaborating Centers are: Coordinating Centre, Cambridge: S.P., M.C., C.O., D.F. North of Scotland Regional Genetics Service, Aberdeen: Zosia Miedzobrodzka, Helen Gregory. Northern Ireland Regional Genetics Service, Belfast: Patrick Morrison. West Midlands Regional Clinical Genetics Service, Birmingham: T.C., Carole McKeown, Lucy Burgess. 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 & 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: R.D., Victoria Murday, Nicola Bradshaw, Lesley Snadden, Mark Longmuir, Catherine Watt. South East Thames Regional Genetics Service, Guys Hospital London: L.I., 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: C.C., Tim Bishop, Julie Miller. Merseyside & Cheshire Clinical Genetics Service, Liverpool: Ian Ellis. Manchester Regional Genetics Service, Manchester: D.G.E., F.L., Felicity Holt. D.G.E. and F.L. are supported the NIHR Biomedical research centre, Manchester. 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. Oxford Regional Genetics Service, Oxford: Lisa Walker, Sarah Durell. 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 Arden-Jones, Anita Mitra. North Trent Clinical Genetics Service, Sheffield: Jackie Cook, Oliver Quarrell, Cathryn Bardsley. South West Thames Regional Genetics Service, London: S.H., Sheila Goff, Glen Brice, Lizzie Winchester. Wessex Clinical Genetics Service, Princess Anne Hospital, Southampton: Gillian Crawford, Emma Tyler, Donna McBride, Anneke Lucassen. S.P., M.C., D.F., C.O. and R.P. are funded by Cancer Research-UK Grants C1287/A10118 and C1287/A8874. 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

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