British Journal of Cancer (2012) 106, 2016–2024

www.bjcancer.com

Association of PHB 1630 C>T and MTHFR 677 C>T polymorphisms with breast and ovarian cancer risk in BRCA1/2 mutation carriers: results from a multicenter study

BACKGROUND: The variable penetrance of breast cancer in *BRCA1/2* mutation carriers suggests that other genetic or environmental factors modify breast cancer risk. Two genes of special interest are prohibitin (*PHB*) and methylene-tetrahydrofolate reductase (*MTHFR*), both of which are important either directly or indirectly in maintaining genomic integrity.

METHODS: To evaluate the potential role of genetic variants within *PHB* and *MTHFR* in breast and ovarian cancer risk, 4102 *BRCA1* and 2093 *BRCA2* mutation carriers, and 6211 *BRCA1* and 2902 *BRCA2* carriers from the Consortium of Investigators of Modifiers of *BRCA1* and *BRCA2* (CIMBA) were genotyped for the *PHB* 1630 C>T (rs6917) polymorphism and the *MTHFR* 677 C>T (rs1801133) polymorphism, respectively.

RESULTS: There was no evidence of association between the *PHB* 1630 C>T and *MTHFR* 677 C>T polymorphisms with either disease for *BRCA1* or *BRCA2* mutation carriers when breast and ovarian cancer associations were evaluated separately. Analysis that evaluated associations for breast and ovarian cancer simultaneously showed some evidence that *BRCA1* mutation carriers who had the rare homozygote genotype (TT) of the *PHB* 1630 C>T polymorphism were at increased risk of both breast and ovarian cancer (HR 1.50, 95%CI 1.10–2.04 and HR 2.16, 95%CI 1.24–3.76, respectively). However, there was no evidence of association under a multiplicative model for the effect of each minor allele.

CONCLUSION: The *PHB* 1630TT genotype may modify breast and ovarian cancer risks in *BRCA1* mutation carriers. This association need to be evaluated in larger series of *BRCA1* mutation carriers.

British Journal of Cancer (2012) **106,** 2016–2024. doi:10.1038/bjc.2012.160 www.bjcancer.com
Published online 15 May 2012

Keywords: *BRCA1/2* mutation carriers; *PHB* 1630 C>T polymorphism; MTHFR 677 C>T polymorphism; breast/ovarian cancer risk

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Received 14 November 2011; revised 18 March 2012; accepted 25 March 2012; published online 15 May 2012

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Breast and ovarian cancers are among the most common malignancies diagnosed in women. The major inherited susceptibilities to breast and/or ovarian cancer are germline mutations in either *BRCA1* or *BRCA2*. Even though both *BRCA1* and *BRCA2* confer a high risk of disease, it is not identical for all mutation carriers, which suggests there are other genetic and environmental factors that are capable of modifying disease penetrance. The identification of additional genetic factors that could modify disease expression in *BRCA1* or *BRCA2* mutation carriers is an important facet to improving risk assessment. Two genes of special interest are prohibitin (*PHB*) and methylenetetrahydrofolate reductase (*MTHFR*), both of which are important either directly or indirectly in maintaining genomic integrity, including cell cycle control, DNA synthesis and methylation (Roskams *et al*, 1993; Bagley and Selhub, 1998; Friso *et al*, 2002).

The *PHB* gene is located on human chromosome 17q21, a region that undergoes frequent loss of heterozygosity in familial and sporadic breast and ovarian cancers (White *et al*, 1991; Black *et al*, 1993; Nagai *et al*, 1994). The gene product is a 30-kD intracellular antiproliferative protein, which interacts with the retinoblastoma tumour suppressor protein to regulate E2F-mediated transcription (White *et al*, 1991; Wang *et al*, 1999). The 3′ untranslated region (3′UTR) of the *PHB* gene encodes a tumour suppressive *trans*-acting regulatory RNA molecule that arrests cell proliferation between the G1 and S phases of the cell cycle in normal epithelial cells and tumour breast cell lines (Jupe *et al*, 1996a; Manjeshwar *et al*, 2003). A single-nucleotide polymorphism (SNP), a C-to-T transition at position 1630 in the 3UTR (rs6917) creates a variant, which lacks antiproliferative activity (Jupe *et al*, 1996b) and significantly reduces cell motility (Manjeshwar *et al*, 2004). The presence of the T allele was shown to cause inactivation of the bioactive rRNA resulting in the loss of its proapoptotic function and a subsequent risk of malignant growth (Manjeshwar *et al*, 2003), and was reported to be associated with significantly increased risk of breast cancer in women aged less than 50 years who had a first-degree relative with breast cancer (Jupe *et al*, 2001).

The *MTHFR* gene produces a key enzyme in folate metabolism that catalyses the irreversible conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which is the primary circulating form of folate. This reaction is essential for both purine nucleotide biosynthesis and remethylation of homocysteine to methionine used in DNA methylation (Kim, 1999; Choi and Mason, 2002). Two functional SNPs in the *MTHFR* gene, 677 C>T (rs1801133) and 1298A>C (rs1801131), both associated with reduced enzyme activity *in vitro* have been described. The MTHFR 677TT (homozygote) genotype results in 30% enzyme activity *in vitro* compared with the CC wild-type, whereas the MTHFR 1298 CC genotype has been found to result in 60% enzyme activity *in vitro* compared with the AA wild-type (Frosst *et al*, 1995; Weisberg *et al*, 1998; Weisberg *et al*, 2001). Reduction of the MTHFR enzyme activity may result in cancer risk increase through impaired DNA repair synthesis and disruption of DNA methylation. In addition, it has been suggested that breast carcinogenesis could be associated with alteration of oestrogen receptor gene methylation patterns (Nass *et al*, 2000) and global DNA methylation (Soares *et al*, 1999). The association of MTHFR 677 C>T and 1298 A>C polymorphisms with breast cancer risk have been investigated and results of meta-analyses have shown a statistically significant association of the MTHFR 677 C>T polymorphism with breast cancer risk (Macis *et al*, 2007; Zhang *et al*, 2010; Qi *et al*, 2010).

Recently, the *PHB* 1630 C>T SNP was shown to be associated with a twofold increased breast cancer risk in Polish *BRCA1* mutation carriers of the CT, TT and combined CT + TT genotypes (Jakubowska *et al*, 2007a). Similarly the *MTHFR* 677C>T SNP was associated with a two to threefold increased risk of breast and ovarian cancer in the same population (Jakubowska *et al*, 2007b).

In the current study we have evaluated both associations in a large series of *BRCA1* and *BRCA2* mutation carriers from the Consortium of Investigators of Modifiers of *BRCA1* and *BRCA2* (CIMBA) (Chenevix-Trench *et al*, 2007).

MATERIALS AND METHODS

Patients

Eligible study subjects were women who carried a deleterious germ line mutation in BRCA1 or BRCA2 and were 18 years old or older. Information on study subjects was submitted by centres participating in CIMBA. Details of the CIMBA initiative, information about the participating centres and detailed inclusion criteria for subjects can be found elsewhere (Chenevix-Trench *et al*, 2007). Briefly, collected data included year of birth, mutation description, family membership, ethnicity, country of residence, age at last follow-up, ages at breast and ovarian cancer diagnosis, and information on bilateral prophylactic mastectomy and prophylactic oophorectomy. Related individuals were identified through a unique family identifier. Only carriers of pathogenic mutations were included in the study. These were mutations generating a premature termination codon (frameshifts, small deletions and insertions, nonsense mutations, splice site mutations and large genomic rearrangements), large in-frame deletions that span one or more exons, deletions of transcription regulatory regions (promoter and/or first exons) expected to cause lack of expression of mutant allele and missense variants classified as pathogenic by Breast Cancer Information Core (BIC) or using the algorithms of Goldgar *et al* (2004) and Chenevix-Trench *et al* (2007). Truncating variants in exon 27 of *BRCA2* were excluded.

All analyses were restricted to mutation carriers of self-reported white European ancestry. A total of 4108 *BRCA1* mutation carriers, 2093 *BRCA2* mutation carriers derived from 13 centres participating in CIMBA were included in the analysis of rs6917 in *PHB* gene, and 7056 *BRCA1* mutation carriers and 3341 *BRCA2* mutation carriers from 23 centres in that of rs1801133 in *MTHFR* gene. The analysis included both related and unrelated mutation carriers in order to maximise the number of samples in the analysis.

All carriers participated in clinical or research studies at the host institutions under ethically approved protocols and data were analysed anonymously.

Genotyping

Genotypes for the two polymorphisms rs6917 in *PHB* and rs1801133 in *MTHFR* were determined for each sample using PCR-RFLP (Jakubowska *et al*, 2007a, b), Taqman or iPLEX analyses (Table 1). The CIMBA genotyping quality control criteria, described in detail in http://www.srl.cam.ac.uk/consortia/cimba/eligibility/eligibility.html, were applied. Based on these criteria one study (169 carriers) was excluded due to low concordance rate for rs1801133, and 6 *BRCA1* carriers for rs6917 were excluded because of low number.

As an additional genotyping quality control assessment Hardy–Weinberg equilibrium (HWE) was evaluated in unrelated subjects for each polymorphism. There was no significant evidence of deviation from HWE except for one study (1115 carriers) for rs1801133 (HWE P-value $= 8 \times 10^{-6}$), so this was also excluded from the analysis. After all exclusions the rs6917 in *PHB* gene was analysed in 4102 *BRCA1* and 2093 *BRCA2* mutation carriers, and the rs1801133 in *MTHFR* gene in 6211 *BRCA1* and 2902 *BRCA2* mutation carriers (Table 1).

Statistical analysis

The aim of the analysis was to evaluate the associations between the two polymorphisms and the risk of breast or ovarian cancer for *BRCA1* and *BRCA2* mutation carriers. For this purpose women were classified according to their age of cancer diagnosis or their age at last observation. Data were analysed within a retrospective likelihood framework by modelling the likelihood of the observed genotypes conditional on the disease phenotypes. This approach, described in detail elsewhere (Antoniou *et al*, 2007), adjusts for the fact that *BRCA1* and *BRCA2* mutation carriers were not randomly sampled with respect to their phenotype. Two types of analyses were carried out for each polymorphism. For the primary analysis, the associations with breast and ovarian cancer were evaluated separately for each disease. For the breast cancer risk association analysis, mutation carriers were censored at the age of the first breast cancer diagnosis, ovarian cancer diagnosis, bilateral

Table 1 Number of *BRCA1* and *BRCA2* carriers by study group and genotyping platforms

Study	Country[a]	*BRCA1*, N	*BRCA2*, N	Genotyping Platform
***PHB* 1630 C>T**				
Epidemiological study of BRCA1 & BRCA2 mutation carriers (EMBRACE)	UK and Eire	823	651	iPLEX
Fox Chase Cancer Centre (FCCC)	USA	78	51	iPLEX
Georgetown University	USA	32	15	
Hereditary Breast and Ovarian study Netherlands (HEBON)	The Netherlands	760	285	iPLEX
Helsinki Breast Cancer Study (HEBCS)	Finland	103	104	iPLEX
International Hereditary Cancer Centre (IHCC)	Poland	696	0	PCR-RFLP
Iceland Landspitali - University Hospital (ILUH)	Iceland	0	86	iPLEX
Interdisciplinary Health Research International Team Breast Cancer Susceptibility (INHERIT BRCAs)	Quebec -Canada	73	82	Taqman
kConFab	Australia	519	415	iPLEX
Mayo Clinic (MAYO)	USA	214	115	iPLEX
Pisa Breast Cancer Study (PBCS)	Italy	76	41	iPLEX
Swedish Breast Cancer Study (SWE-BRCA)	Sweden	468	127	iPLEX
University of Pennsylvania (UPENN)	USA	260	121	iPLEX
Total		4102	2093	
***MTHFR*677 C>T**				
Spanish National Cancer Centre (CNIO)	Spain, Greece	168	202	Taqman
Deutsches Krebsforschungszentrum (DKFZ)	Germany	68	29	Taqman
Epidemiological study of BRCA1 & BRCA2 mutation carriers (EMBRACE)	UK and Eire	642	469	iPlex
Fox Chase Cancer Centre (FCCC)	USA	80	54	iPlex
German Consortium of Hereditary Breast and Ovarian Cancer (GC-HBOC)	Germany	559	283	Taqman
Genetic modifiers of cancer risk in *BRCA1*/2 mutation carriers (GEMO)	France, USA	1075	536	iPlex
Hereditary Breast and Ovarian study Netherlands (HEBON)	The Netherlands	480	0	iPlex
Helsinki Breast Cancer Study (HEBCS)	Finland	101	103	iPlex
International Hereditary Cancer Centre (IHCC)	Poland	686	0	PCR-RFLP
Interdisciplinary Health Research International Team Breast Cancer Susceptibility (INHERIT BRCAs)	Quebec -Canada	72	82	Taqman
kConFab	Australia	409	322	iPlex
Mayo Clinic (MAYO)	USA	106	56	iPlex
Milan Breast Cancer Study Group (MBCSG)	Italy	246	132	Taqman
Modifier Study of Quantitative Effects on Disease (ModSQuaD)	Czech Republic, Belgium	132	35	Taqman
National Cancer Institute (NCI)	USA	139	57	Taqman
National Israeli Cancer Control Center (NICCC)	Israel	221	129	Taqman
Ontario Cancer Genetics Network (OCGN)	Canada	180	130	Taqman
Odense University Hospital (OUH)	Denmark	109	0	Taqman
Pisa Breast Cancer Study (PBCS)	Italy	56	32	Taqman
Swedish Breast Cancer Study (SWE-BRCA)	Sweden	427	130	Taqman
University of Pennsylvania (UPENN)	USA	255	121	Taqman
Total		6211	2902	

Abbreviations: MTHFR, methylene-tetrahydrofolate reductase; PHB, prohibitin; RFLP, restriction fragment length polymorphism. [a]Country of the clinic at which carriers are recruited

prophylactic mastectomy or the age at last observation. For this analysis, only mutation carriers censored at breast cancer were considered as affected. To evaluate the associations with ovarian cancer risk, carriers were censored at the age of ovarian cancer diagnosis, bilateral prophylactic oophorectomy or age at last observation, whichever occurred first. Only women censored at ovarian cancer diagnosis were considered as affected in this analysis. To allow for the fact that mutation carriers are at risk of developing both breast and ovarian cancer, in a second analysis we evaluated the associations between the SNPs with both breast and ovarian cancer simultaneously using a competing risk analysis, by estimating simultaneously HRs for both breast and ovarian cancers. Details of this method have been described elsewhere (Antoniou *et al*, 2010; Ramus *et al*, 2011; Barnes *et al*, 2012). A different censoring process was used in this case, whereby individuals were followed up to the age of the first breast or ovarian cancer diagnosis, and were considered to have developed the corresponding disease. No follow-up was considered after the first cancer diagnosis. Individuals were censored for breast cancer at the age of bilateral prophylactic mastectomy and for ovarian cancer at the age of bilateral oophorectomy, and were assumed to be unaffected for the corresponding disease. The remaining individuals were censored at the age at last observation and were assumed to be unaffected for both diseases.

All analyses were stratified by study group and country of residence, and used calendar-year and cohort-specific cancer incidences for *BRCA1* and *BRCA2* (Antoniou *et al*, 2008). A robust variance-estimation approach was used to allow for the non-independence among related carriers (Boos, 1992).

RESULTS AND DISCUSSION

In this study, a total of 6195 individuals including 4102 *BRCA1* and 2093 *BRCA2* mutation carriers from 11 countries were eligible for inclusion in the analysis of the *PHB* 1630 C>T (rs6917) polymorphism (Table 1). The main analysis included all available mutation carriers, including the Polish *BRCA1* mutation carriers used in the previous reports (Jakubowska *et al*, 2007a; Jakubowska *et al*, 2007b). There was no evidence of an association of rs6917 with breast or ovarian cancer risk for mutation carriers when the risks were evaluated separately (Table 2). However, the competing risk analysis, where associations were evaluated simultaneously for breast and ovarian cancer provided some evidence of association between the rare homozygote TT genotype with both breast cancer risk (HR 1.50, 95%CI 1.10–2.04) and ovarian cancer risk (HR 2.16, 95%CI 1.24–3.76) for *BRCA1* mutation carriers (Table 3). The breast and ovarian cancer HRs for the TT genotype in the competing risk analysis were in the same direction as the

Table 2 *PHB* 1630 C>T genotype frequencies by (a) disease status and breast cancer hazard ratio estimates; (b) disease status and ovarian cancer hazard ratio estimates

Gene	Genotype	Unaffected *n* (%)	Affected *n* (%)	HR	95% CI	*P*-value
(a)[a]						
BRCA1	CC	1443 (69.7)	1388 (68.3)	1.00		
	CT	575 (27.8)	574 (28.3)	1.04	0.92–1.18	
	TT	52 (2.5)	70 (3.4)	1.35	0.99–1.84	
	2df test					0.17
	Per-Allele			1.08	0.97–1.21	0.15
BRCA2	CC	672 (67.9)	714 (64.7)	1.00		
	CT	293 (29.9)	354 (32.1)	1.15	0.96–1.37	
	TT	25 (2.5)	35 (3.2)	1.13	0.70–1.82	
	2df test					0.29
	Per-Allele			1.12	0.96–1.30	0.14
(b)[b]						
BRCA1	CC	2368 (68.9)	463 (69.6)	1.00		
	CT	972 (28.3)	177 (26.6)	0.93	0.83–1.06	
	TT	97 (2.8)	25 (3.8)	1.49	0.91–2.45	
	2df test					0.18
	Per-Allele			1.03	0.87–1.23	0.73
BRCA2	CC	1274 (66.0)	112 (68.3)	1.00		
	CT	603 (31.3)	44 (26.8)	0.80	0.55–1.15	
	TT	52 (2.7)	8 (4.9)	1.63	0.67–3.99	
	2df test					0.21
	Per-Allele			0.96	0.67–1.38	0.84

Abbreviation: PHB, prohibitin. [a]Diagnosed with breast cancer. [b]Diagnosed with ovarian cancer.

corresponding breast and ovarian cancer HR estimates in the analysis in which the breast and ovarian cancer associations were assessed separately (Table 2). The analyses that investigated the breast and ovarian cancer risk associations separately yielded no evidence of association with the TT genotype. When evaluating the associations with a single disease (breast or ovarian) in the primary analysis, individuals who developed the other disease were assumed to be unaffected in the analysis (i.e. treated as 'controls'). Under this analysis, a potential bias could arise if *PHB* 1630 C>T is associated with both breast and ovarian cancer: if the magnitude of the true breast and ovarian cancer relative risks conferred by *PHB* 1630 C>T are in the same direction, then such an analysis could lead to an attenuation of the estimated associations (Barnes *et al*, 2012). Therefore, a plausible explanation for the apparent discrepancy between the two analyses could be due to this source of bias. However, the number of *BRCA1* mutation carriers with the *PHB* 1630 TT genotype is limited and larger studies will be required to clarify this. The association with the TT genotype remained significant after excluding the Polish samples from the previously published study (Table 3).

A total of 6211 *BRCA1* and 2902 *BRCA2* participants from 16 countries were assessed for the associations between the common polymorphism 677C>T in *MTHFR* (rs1801133) and breast or ovarian cancer risk for women who harboured a germline mutation in either *BRCA1* or *BRCA2* (Table 1). When breast and ovarian cancer associations were evaluated separately (Table 4) or simultaneously (competing risk analysis) (Table 3), there was no evidence of association between the polymorphism with either disease for *BRCA1* or *BRCA2* mutation carriers. This observation is in contrast to previous findings in smaller studies of *BRCA1* mutation carriers (Gershoni-Baruch *et al*, 2000; Pepe *et al*, 2007).

Table 3: *PHB* 1630 C>T and *MTHFR* 677 C>T genotype frequencies by disease status, *BRCA1/2* mutation and, breast and ovarian cancer hazard ratio estimates in competing risk analysis

	Unaffected *n* (%)	Ovarian cancer *n* (%)	HR	95% CI	*P*-value	Breast cancer *n* (%)	HR	95% CI	*P*-value
PHB 1630 C>T									
BRCA1									
CC	1155 (69.8)	334 (69.2)	1.00			1342 (68.3)	1.00		
CT	465 (28.1)	130 (26.9)	0.99	0.78-1.25		554 (28.2)	1.04	0.92-1.19	
TT	34 (2.1)	19 (3.9)	**2.16**	**1.24–3.76**		69 (3.5)	**1.50**	**1.10–2.04**	
Per-allele			1.16	0.93–1.41	0.19		1.11	0.99–1.23	0.06
BRCA1 excluding IHCC									
CC	933 (67.9)	233 (65.8)	1.00			1136 (67.7)	1.00		
CT	412 (30.0)	103 (29.1)	0.98	0.75–1.29		478 (28.5)	0.99	0.86–1.14	
TT	29 (2.1)	18 (5.1)	**2.32**	**1.34–4.05**		64 (3.8)	**1.43**	**1.05–1.98**	
Per-allele			1.18	0.94–1.48	0.15		1.07	0.95–1.20	0.27
BRCA2									
CC	616 (67.1)	83 (71.6)	1.00			687 (64.9)	1.00		
CT	282 (30.7)	28 (24.1)	0.69	0.45–1.07		337 (31.8)	1.08	0.90–1.30	
TT	20 (2.2)	5 (4.3)	1.46	0.44–4.81		35 (3.3)	1.24	0.81–1.89	
Per-Allele			0.86	0.53–1.39	0.54		1.09	0.94–1.27	0.23
MTHFR 677 C>T									
BRCA1									
CC	976 (43.3)	349 (43.1)	1.00			1344 (42.7)	1.00		
CT	1000 (44.4)	361 (44.6)	0.95	0.79–1.13		1425 (45.3)	0.99	0.89–1.09	
TT	279 (12.4)	99 (12.2)	0.93	0.72–1.21		378 (12.0)	0.96	0.82–1.13	
Per-allele			0.96	0.85–1.08	0.51		0.98	0.99–1.06	0.63
BRCA2									
CC	471 (43.0)	74 (42.1)	1.00			688 (42.2)	1.00		
CT	481 (43.9)	85 (48.3)	1.09	0.76–1.57		741 (45.4)	1.05	0.90–1.23	
TT	143 (13.1)	17 (9.7)	0.74	0.41–1.34		202 (12.4)	0.95	0.76–1.20	
Per-allele			0.93	0.72–1.20	0.57		1.00	0.90–1.11	0.98

Abbreviations: MTHFR, methylene-tetrahydrofolate reductase; PHB, prohibitin. Significant results are marked in bold.

Table 4 *MTHFR* 677 C>T genotype frequencies by (a) disease status and breast cancer hazard ratio estimates; (b) disease status and ovarian cancer hazard ratio estimates

Gene	Genotype	Unaffected *n* (%)	Affected *n* (%)	HR	95% CI	*P*-value
(a)[a]						
BRCA1	CC	1281 (43.3)	1388 (42.7)	1.00		
	CT	1313 (44.3)	1473 (45.3)	1.00	0.90–1.10	
	TT	367 (12.4)	389 (12.0)	0.96	0.82–1.12	
	2 df test					0.84
	Per-Allele			0.98	0.92–1.05	0.64
BRCA2	CC	530 (43.3)	703 (41.9)	1.00		
	CT	542 (44.3)	765 (45.6)	1.05	0.91–1.22	
	TT	152 (12.4)	210 (12.5)	1.02	0.82–1.27	
	2 df test					0.78
	Per-Allele			1.02	0.93–1.13	0.66
(b)[b]						
BRCA1	CC	2190 (43.3)	479 (42.6)	1.00		
	CT	2259 (44.7)	527 (45.8)	1.01	0.88–1.16	
	TT	610 (12.1)	146 (12.7)	1.04	0.84–1.28	
	2 df test					0.88
	Per-Allele			1.02	0.92–1.12	0.73
BRCA2	CC	1132 (42.7)	101 (40.7)	1.00		
	CT	1193 (44.9)	114 (46.0)	1.05	0.75–1.41	
	TT	329 (12.4)	33 (13.3)	1.14	0.74–1.76	
	2 df test					0.84
	Per-Allele			1.06	0.87–1.30	0.57

Abbreviation: MTHFR, methylene-tetrahydrofolate reductase. [a]Diagnosed with breast cancer. [b]Diagnosed with ovarian cancer.

In this multicentre study we were unable to confirm the modifying effect of the *MTHFR* 677C>T polymorphism on breast cancer risk for *BRCA1* carriers. We also did not detect an association of the above polymorphism with breast and/or ovarian cancer risk for *BRCA2* mutation carriers. Previous studies were restricted to specific populations, and may potentially represent population specific effects (Gershoni-Baruch *et al*, 2000; Jakubowska *et al*, 2007b; Pepe *et al*, 2007; Beetstra *et al*, 2008). This explanation is confirmed by the fact that the frequency of 677TT genotype was substantially different between studies: 8.5% (52 in 609 carriers) in the Polish study (Jakubowska *et al*, 2007b), 13.5% (5 in 37 carriers) in a small study from Australia (Beetstra *et al*, 2008), 17% (82 in 484 carriers) in an Italian study (Pepe *et al*, 2007) and 21% (43 in 205 carriers) among Jewish carriers (Gershoni-Baruch *et al*, 2000). It is also noticeable that in Polish and Jewish carriers the modifying effect of *MTHFR* 677C>T polymorphism was observed for 677T homozygotes, whereas in the Italian study an increased risk of breast cancer was detected in carriers of the 677T allele. The genotype frequency of *MTHFR* 677CTT in this multi-population study was 12.2% with an equal distribution in breast cancer patients, ovarian cancer patients and unaffected individuals (12.1, 12.8 and 12.2, respectively). However, the most likely explanation could be the fact that all previous studies were based on much smaller data sets and associations based on those studies could represent false-positive findings. Although the present analysis has been performed in a much larger sample set of mutation carriers and therefore has a greater power to detect an association compared with any previously published studies it remains underpowered to identify weaker effects.

In conclusion, these findings show that in general the *MTHFR* 677C>T polymorphism is not likely to have an important role as modifier of breast and/or ovarian cancer risks in *BRCA1/2* mutation carriers. There was some evidence that the *PHB* 1630 C>T polymorphism is associated with breast and ovarian cancer risks in *BRCA1* mutation carriers in the competing risk analysis, but this would need to be evaluated in additional analyses with larger number of mutation carriers. Future analyses should also aim to assess the associations with other clinical and tumour characteristics.

ACKNOWLEDGEMENTS

The CIMBA data management and analysis are supported by Cancer Research – UK. Antonis C Antoniou is a Cancer Research – UK Senior Cancer Research Fellow.

Spanish National Cancer Centre (CNIO)

The research leading to these results has been partially funded by Mutua Madrileña Foundation, 'Red de Investigación en Cáncer RD06/0020/1160' and Spanish Ministry of Science and Innovation (FIS PI08 1120 and SAF2010-20493).

Deutsches Krebsforschungszentrum (DKFZ)

The DKFZ study was supported by the DKFZ

Epidemiological study of BRCA1 and BRCA2 mutation carriers (EMBRACE)

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EMBRACE is supported by Cancer Research UK grants C1287/A10118 and C1287/A11990. D Gareth Evans and Fiona Lalloo 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. Ros Eeles, Elizabeth Bancroft and Lucia D'Mello are also supported by Cancer Research UK Grant C5047/A8385.

Fox Chase Cancer Center (FCCC)

Andrew Godwin was funded by U01CA69631, 5U01CA113916, the Ovarian Cancer Research Fund, the Eileen Stein Jacoby Fund. The author acknowledges the support from The University of Kansas Cancer Center and the Kansas Bioscience Authority Eminent Scholar Program. AKG is the Chancellors Distinguished Chair in Biomedical Sciences endowed Professor.

The German Consortium of Hereditary Breast and Ovarian Cancer (GC-HBOC)

GC-HBOC is supported by a grant of the German Cancer Aid (grant 109076) and by the Centre of Molecular Medicine Cologne (CMMC).

Genetic Modifiers of cancer risk in BRCA1/2 mutation carriers (GEMO)

Cancer Genetics Network 'Groupe Génétique et Cancer', Fédération Nationale des Centres de Lutte Contre le Cancer, France. We wish to thank all the GEMO collaborating groups for their contribution to this study. GEMO Collaborating Centers are: Coordinating Centres, Unité Mixte de Génétique Constitutionnelle des Cancers Fréquents, Centre Hospitalier Universitaire de Lyon/ Centre Léon Bérard, and Equipe «Génétique du cancer du sein», Centre de Recherche en Cancérologie de Lyon: Olga Sinilnikova, Sylvie Mazoyer, Laure Barjhoux, Carole Verny-Pierre, Sophie Giraud, Mélanie Léone; and Service de Génétique Oncologique, Institut Curie, Paris: Dominique Stoppa-Lyonnet, Marion Gauthier-Villars, Bruno Buecher, Claude Houdayer, Virginie Moncoutier, Muriel Belotti, Carole Tirapo, Antoine de Pauw. Institut Gustave Roussy, Villejuif: Brigitte Bressac-de-Paillerets, Véronique Byrde, Olivier Caron, Gilbert Lenoir. Centre Jean Perrin, Clermont–Ferrand: Yves-Jean Bignon, Nancy Uhrhammer. Centre Léon Bérard, Lyon: Christine Lasset, Valérie Bonadona. Centre François Baclesse, Caen: Agnès Hardouin, Pascaline Berthet. Institut Paoli Calmettes, Marseille: Hagay Sobol, Violaine Bourdon, Tetsuro Noguchi, Audrey Remenieras, François Eisinger. Groupe Hospitalier Pitié-Salpétrière, Paris: Florence Coulet, Chrystelle Colas, Florent Soubrier. CHU de Arnaud-de-Villeneuve, Montpellier: Isabelle Coupier, Pascal Pujol. Centre Oscar Lambret, Lille: Jean-Philippe Peyrat, Joëlle Fournier, Françoise Révillion, Philippe Vennin, Claude Adenis. Hôpital René Huguenin/Institut Curie, St Cloud: Etienne Rouleau, Rosette Lidereau, Liliane Demange, Catherine Nogues. Centre Paul Strauss, strasbourg: Danièle Muller, Jean-Pierre Fricker. Institut Bergonié, Bordeaux: Emmanuelle Barouk-Simonet, Françoise Bonnet, Virginie Bubien, Nicolas Sevenet, Michel Longy. Institut Claudius Regaud, toulouse: Christine Toulas, Rosine Guimbaud, Laurence Gladieff, Viviane Feillel. CHU de Grenoble: Dominique Leroux, Hélène Dreyfus, Christine Rebischung, Magalie Peysselon. CHU de Dijon: Fanny Coron, Laurence Faivre. CHU de St-Etienne: Fabienne Prieur, Marine Lebrun, Caroline Kientz. Hôtel Dieu Centre Hospitalier, Chambéry: Sandra Fert Ferrer. Centre Antoine Lacassagne, Nice: Marc Frénay. CHU de Limoges: Laurence Vénat-Bouvet. CHU de Nantes: Capucine Delnatte. CHU Bretonneau, Tours: Isabelle Mortemousque. Creighton University, Omaha, USA: Henry T Lynch, Carrie L Snyder.

The study was supported by the Ligue National Contre le Cancer; Association for International Cancer Research Grant (AICR-07-0454); and the Association 'Le cancer du sein, parlons-en!' Award.

Georgetown

Claudine Isaacs received support from the Familial Cancer Registry and the Tissue Culture Shared Resource at Georgetown University (NIH/NCI grant P30-CA051008), the Cancer Genetics Network (HHSN261200744000C), and Swing Fore the Cure.

The Hereditary Breast and Ovarian Cancer Research Group, Netherlands (HEBON)

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The HEBON study is supported by the Dutch Cancer Society grants NKI1998-1854, NKI2004-3088, NKI2007-3756 and the ZonMW grant 91109024.

International Hereditary Cancer Centre (IHCC)

Iceland, Landspitali - University Hospital (ILUH)

The ILUH was supported by Landspitali University Hospital Research Fund, Walking Together Research Fund.

Interdisciplinary Health Research International Team Breast Cancer Susceptibility (INHERIT BRCAs)

We would like to thank Dr Martine Dumont for sample management and Martine Tranchant for skillful technical assistance. JS is Chairholder of the Canada Research Chair in Oncogenetics.

This work was supported by the Canadian Institutes of Health Research for the 'CIHR Team in Familial Risks of Breast Cancer' program and by the Canadian Breast Cancer Research Alliance-grant #019511.

The Kathleen Cuningham Foundation Consortium for Research into Familial Breast Cancer (kConFab)

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 2001-2009 by NHMRC and currently by the National Breast Cancer Foundation and Cancer Australia #628333) for their contributions to this resource, and the many families who contributed 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.

Modifiers and Genetics in Cancer (MAGIC)

NIH grants R01-CA083855 and R01-CA102776.

Mayo Clinic (MAYO)

MAYO was supported by a National Institutes of Health Specialized Program of Research Excellence (SPORE) in Breast Cancer (CA116201) to the Mayo Clinic and R01 CA128978, and grants from the Komen Foundation for the Cure and the Breast Cancer Research Foundation.

Milan Breast Cancer Study Group (MBCSG)

MBCSG thanks Paolo Radice, Bernard Peissel, Daniela Zaffaroni and Marco A: Pierotti of the Fondazione IRCCS Istituto Nazionale Tumori and Monica Barile of the Istituto Europeo di Oncologia, Milano, Italy.

Paolo Peterlongo is supported by funds from Italian citizens who allocated the 5×1000 share of their tax payment to the Fondazione IRCCS Istituto Nazionale Tumori, according to Italian laws (INT-Institutional strategic projects '5×1000').

Modifier Study of Quantitative Effects on Disease (ModSQuad)

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Faculty of Medicine, Charles University, Prague, Czech, Republic); Lenka Foretova, Machackova Eva, and Lukesova Miroslava (Masaryk Memorial, Cancer Institute, Brno, Czech Republic); Kathleen Claes, Kim De Leeneer, Bruce Poppe, Anne De Paepe (Ghent University, Ghent, Belgium).

CI Szabo is supported by Susan G Komen Foundation Basic, Clinical and Translational

Research grant (BCTR0402923) and the Mayo Rochester Early Career Development Award for Non-Clinician Scientists; We acknowledge the contributions of Petr Pohlreich and Zdenek Kleibl (Department of Biochemistry and Experimental Oncology, First Faculty of Medicine, Charles University, Prague, Czech Republic) and the support of the Grant Agency of the Czech republic grant No. 301/08/P103 (to MZ). Lenka Foretova, Machackova Eva and Lukesova Miroslava (Masaryk Memorial Cancer Institute, Brno, Czech Republic) are supported through the Ministry of Health of the CR grant -MZ0 MOU 2005. We acknowledge the contribution of Kim De Leeneer, Kathleen Claes and Anne De Paepe. This research was supported by grant 1.5.150.07 from the Fund for Scientific Research Flanders (FWO) to Kathleen Claes and by grant 12051203 from the Ghent university to Anne De Paepe. Bruce Poppe is Senior Clinical Investigator of the Fund for Scientific Research of Flanders (FWO – Vlaanderen).

National Cancer Institute (NCI)

National Israeli Cancer Control Center (NICCC)

The NICCC cohort is supported by the Breast Cancer Research Foundation (BCRF).

Ontario Cancer Genetics Network (OCGN)

We wish to thank Mona Gill, Lucine Collins, Nalan Gokgoz, Teresa Selander, Nayana Weerasooriya and members of the Ontario Cancer Genetics Network for their contributions to the study.

This work was supported by Cancer Care Ontario, Canada (ILA); and the National Cancer Institute, National Institutes of Health under RFA-CA-06-503 and through cooperative agreements with members of the Breast Cancer Family Registry and P.Is. The content of this manuscript does not necessarily reflect the views or policies of the National Cancer Institute or any of the collaborating centres in the CFR, nor does mention the trade names, commercial products, or organisations imply endorsement by the US Government or the CFR.

Odense University Hospital (OUH)

Pisa Breast Cancer Study (PBCS)

This research was supported by Istituto Toscano Tumori.

The Swedish BRCA1 and BRCA2 Study (SWE-BRCA)

SWE-BRCA collaborators: Per Karlsson, Margareta Nordling, Annika Bergman and Zakaria Einbeigi, Gothenburg, Sahlgrenska University Hospital; Marie Stenmark-Askmalm and Sigrun Liedgren, Linköping University Hospital; Åke Borg, Niklas Loman, Håkan Olsson, Maria Soller, Helena Jernström, Katja Harbst and Karin Henriksson, Lund University Hospital; Annika Lindblom, Brita Arver, Anna von Wachenfeldt, Annelie Liljegren, Gisela Barbany-Bustinza and Johanna Rantala, Stockholm, Karolinska University Hospital; Beatrice Melin, Henrik Grönberg, Eva-Lena Stattin and Monica Emanuelsson, Umeå University Hospital; Hans Ehrencrona, Richard Rosenquist and Niklas Dahl, Uppsala University Hospital.

University of Pennsylvania (UPENN)

We acknowledge the support by the Breast Cancer Research Foundation to KLN and the Susan G Komen Foundation to SMD.

Conflict of interest

The authors declare no conflict of interest.

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