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 1630 TT 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.

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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 methylene-tetrahydrofolate reductase (MTHFR), both of which are important in cell cycle control, DNA synthesis and methylation reactions.
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 3′UTR (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 1298 A>C (rs1801131), both associated with reduced enzyme activity in vitro have been described. The MTHFR 677TT (homozgyote) 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 has been investigated and its 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 677CC>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 × 10⁻⁵), 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
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
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 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)  |          |                  |               |           |         |
| *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)  |          |                  |               |           |         |
| *BRCA1* | CC     | 2368 (68.9) | 2368 (68.9) | 1.00 |         |
|        | CT     | 972 (28.3) | 972 (28.3) | 0.93 | 0.83–1.06 |
|        | TT     | 97 (2.8) | 97 (2.8) | 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. *Diagnosed with breast cancer.* **Diagnosed with ovarian cancer.**

### 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

| Gene | Geonotype | Unaffected n (%) | Ovarian cancer n (%) | HR 95% CI | P-value |
|------|-----------|------------------|----------------------|-----------|---------|
| PHB 1630 C>T |          |                  |                      |           |         |
| *BRCA1* | CC     | 1155 (69.8) | 334 (69.2) | 1.00 |         |
|        | CT     | 465 (28.1) | 130 (26.9) | 0.99 | 0.78–1.25 |
|        | TT     | 34 (2.1) | 19 (3.9) | 2.16 | 1.24–3.76 |
|        | Per-Allele |          |               | 1.16 | 0.93–1.41 |
|         |         |               |               | 0.19 |         |
| *BRCA2* excluding IHCC | CC     | 933 (67.9) | 233 (65.8) | 1.00 |         |
|        | CT     | 412 (30.0) | 103 (29.1) | 0.98 | 0.75–1.29 |
|        | TT     | 29 (2.1) | 18 (5.1) | 2.32 | 1.34–4.05 |
|        | Per-Allele |          |               | 1.18 | 0.94–1.48 |
|         |         |               |               | 0.15 |         |
| *BRCA2* | CC     | 616 (67.1) | 83 (71.6) | 1.00 |         |
|        | CT     | 282 (30.7) | 28 (24.1) | 0.69 | 0.45–1.07 |
|        | TT     | 20 (2.2) | 5 (4.3) | 1.46 | 0.44–4.81 |
|        | Per-Allele |          |               | 0.86 | 0.53–1.39 |
|         |         |               |               | 0.54 |         |
| *MTHFR 677 C>T |          |                  |                      |           |         |
| *BRCA1* | CC     | 976 (43.3) | 349 (43.1) | 1.00 |         |
|        | CT     | 1000 (44.4) | 361 (44.6) | 0.95 | 0.79–1.13 |
|        | TT     | 279 (12.4) | 99 (12.2) | 0.93 | 0.72–1.21 |
|        | Per-Allele |          |               | 0.96 | 0.85–1.08 |
|         |         |               |               | 0.51 |         |
| *BRCA2* | CC     | 471 (43.0) | 74 (42.1) | 1.00 |         |
|        | CT     | 481 (43.9) | 85 (48.3) | 1.09 | 0.76–1.57 |
|        | TT     | 143 (13.1) | 17 (9.7) | 0.74 | 0.41–1.34 |
|        | Per-Allele |          |               | 0.93 | 0.72–1.20 |
|         |         |               |               | 0.57 |         |

Abbreviations: MTHFR, methylene-tetrahydrofolate reductase; PHB, prohibitin. Significant results are marked in bold.
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, 2007; Pepe 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 (Beest 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 12.3% with an equal distribution in breast cancer patients, ovarian cancer patients 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.

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REFERENCES

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Conflict of interest

The authors declare no conflict of interest.

REFERENCES

Antoniou AC, Sinilnikova OM, Simard J, Léoné M, Dumont M, Neuhausen SL, Streuwing JP, Stoppa-Lyonnet D, Barjhoux L, Hughes DJ, Coupier I, Belotti M, Lasset C, Bonadona V, Bignon YJ, Genetic Modifiers of Cancer Risk in BRCA1/2 Mutation Carriers Study (GEMO), Rebbeck TR, Wagner T, Lynch HT, Anton-Culver H, Warner E, Lubinski J, Gronwald J, Gorski B, Cybulski C, Spurdle AB, Holland H, kConFab, Goldgar DE, John EM, Hopper JL, Southey M, Buys SB, Daly MB, Terry MB, Schmutzler RK, Wappenschmidt B, Engel C, Meindl A, Arnold N, Niederacher D, Deissler PHB and MTHFR polymorphisms and cancer risk in BRCA1/2 carriers

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Genetics and Genomics
modifiers of disease risk in carriers of high-risk mutations. *Genet Epidemiol* 36: 274–291

Beestma S, Suthers G, Dhillon V, Salisbury C, Turner J, Altrey M, McKinnon R, Fench M (2008) Methionine-dependence phenotype in the de novo pathway in BRCA1 and BRCA2 mutation carriers with and without breast cancer. *Cancer Epidemiol Biomarkers Prev* 17: 2565–2571

Black DM, Nicola PJ, Hamon J, Solomon E (1993) A somatic cell hybrid map of the long arm of human chromosome 17, containing the familial breast cancer locus (BRCA1). *Am J Hum Genet* 52: 702–710

Boos DD (1992) On generalised score tests. *American Statistician* 46: 327–333

Chenevix-Trench G, Milne RL, Antoniou AC, Couch FJ, Easton DF, Goldgar DE (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

Choi SW, Mason JB (2002) Folate status: effects on pathways of colorectal carcinogenesis. *J Nutr* 132: 2415S–2418S

Friso S, Choi SW, Girelli D, Mason JB, Dolnikowski GG, Bagley PJ, Olivieri O, Jacques PF, Rosenberg JH, Corrocher R, Selhub J (2002) A common mutation in the 5,10-methylenetetrahydrofolate reductase gene affects genomic DNA methylation through an interaction with folate status. *Proc Natl Acad Sci USA* 99: 5606–5611

Frost P, Blom JH, Milos R, Goyette P, Matthews RG, Boers CJ, den Heijer M, Klijtmans LA, van den Heuvel LP (1995) A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. *Nat Genet* 10: 111–113

Gershoni-Baruch R, Dagan I, Israel O, Kinsztal L, Kadouri E, Friedman E (2000) Association of the C677T polymorphism in the MTHFR gene with breast and/or ovarian cancer risk in Jewish women. *Eur J Cancer* 36: 2316–2316

Goldgar DE, Easton DF, Deffenbaugh AM, Monteiro AN, Tartgivan SV, Couch FJ (2004) Integrated evaluation of DNA sequence variants of untranslated region RNA in human cancers. *Genet Epidemiol* 35: 639–646

Jakubowska A, Gronwald J, Górska B, Huzarski T, Byrski T, Gronwald J, Górska B, Cybulski C, Debnik T, Ossorio A, Durán M, Tejada MJ, Benitez J, Hamann U, Rookus MA, Verhoef S, Timmins-Linthurst MA, Vreeswijk MP, Bodmer D, Ausems MG, van Os TA, Asperen CJ, Blod MJ, Meijers-Heijboer HE, HEBON, EMBRACE, Peock S, Cook M, Oliver C, Drost F, Dunning AM, Evans DG, Eeles R, Pichert G, Cole T, Hodgson S, Brewer C, Morrison PJ, Porteous M, Kennedy MJ, Rogers MT, Side LE, Donaldson A, Gregory H, Godwin A, Stoppa-Lyonnet D, Moncoutier V, Castera L, Mazoyer S, Barjhoux L, Bonadona V, Leroux D, Fairev L, Lidereau R, Nogues B, Cignon BY, Prieur F, Collange-Rame MA, Venat-Bouvet L, Fert-Ferrer S, GEMO Study Collaborators, Miron A, Buys SS, Hopper JL, Daly MB, John EM, Terry MB, Goldgar D, BCFR, Hanssen TN, Jonson L, Eijfertsen B, Agnarsson BA, Offit K, Kirchhoff T, Vijai J, Dutra-Claro AL, Przybylo JA, Casella C, Imyanitov EN, Janavicius R, Blanco I, Lázaro C, Mioszyk KB, Karlan BY, Gross J, Beattie MS, Schmutzler R, Wappenschmidt B, Meindl A, Ruehl I, Fibig B, Sutter C, Arnold N, Deissler H, Varon-Mateeva R, Kast K, Niederacher D, Gadzicki D, Caldes T, de la Hoya M, Nevanlinna H, Altumlki K, Simard J, Soucy P, kConFab Investigators, Spurdle AB, Holland H, Chenevix-Trench G, Easton DF, Antoniou AC (2011) Genetic variation at 9p22.2 and ovarian cancer risk for BRCA1 and BRCA2 mutation carriers. *J Natl Cancer Inst* 103: 105–116

Roksmans AJ, Friedman V, Wood CM, Walker L, Owens GA, Stewart DA, Altus MS, Danner DB, Liu XT, McClung JK (1993) Cell cycle activity and expression of prohibitin mRNA. *J Cell Physiol* 157: 289–295

Soares J, Pinto AE, Cunha CV, Andre S, Barlo I, Sousa JM, Carvo M (1999) Global DNA hypomethylation in breast carcinoma: correlation with prognostic factors and tumor progression. *Cancer* 85: 112–118

Wang S, Nath N, Adlam M, Chellappan S (1999) Prohibitin, a potential tumor suppressor, interacts with RB and regulates E2F function. *Oncogene* 18: 3501–3510

Weisberg I, Tran P, Christiansen B, Sibani S, Rozan R (1998) A second genetic polymorphism in methylenetetrahydrofolate reductase (MTHFR) associated with decreased enzyme activity. *Mol Genet Metab* 64: 169–172

Weisberg IS, Jacobs PF, Selhub J, Bostom AG, Chen Z, Curtis ER, Eckfeldt JH, Rozen R (2001) The 1298A→C polymorphism in methylenetetrahydrofolate reductase (MTHFR); *in vitro* expression and association with homocysteine. *Atherosclerosis* 156: 409–415

White JJ, Ledbetter DH, Eddy Jr RL, Shows TB, Stewart DA, Nuell MJ, Friedman V, Wood CM, Owens GA, McClung JK, Danner DB, Morton CC (1991) Assignment of the human prohibitin (sic) gene (PHB) to chromosome 17 and identification of a DNA polymorphism. *Genomics* 11: 226–230

Zhang J, Qiu LX, Wang ZH, Wu XH, Liu XJ, Wang BY, Xu XC (2010) MTHFR C677T polymorphism associated with breast cancer susceptibility: a meta-analysis involving 15 260 cases and 20 411 controls. *Breast Cancer Res Treat* 123: 549–555