

# 3' untranslated region 1630 C>T polymorphism of *prohibitin* increases risk of breast cancer

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**Background:** *Prohibitin* 3' untranslated region 1630 C>T (rs6917) polymorphism creates a variant T allele that lacks the antiproliferative activity of the more common functional C allele. Previous studies indicate that women carrying the *prohibitin* T allele have an increased susceptibility to breast cancer. However, the role of 1630 C>T polymorphism in mRNA expression of *prohibitin* and its contribution to carcinogenesis in the breast remains controversial.

**Methods:** Using mRNA expression data from the HapMap online database, we sought an association between *prohibitin* 1630 C>T polymorphism and its mRNA expression, then conducted a meta-analysis of *prohibitin* 1630 C>T polymorphism and risk of breast cancer.

**Results:** Although no significant association was found between *prohibitin* 1630 C>T polymorphism and mRNA expression in lymphoblastoid cell lines from the HapMap database ( $P_{\text{trend}} = 0.543$ ), the present meta-analysis involving 5072 cases and 4796 controls demonstrated that *prohibitin* 1630 C>T polymorphism was significantly correlated with breast cancer risk in allele contrast model T versus C (odds ratio [OR] 1.09, 95% confidence interval [CI] 1.01–1.18), the homozygote codominant model TT versus CC (OR 1.47, 95% CI 1.12–1.92), and the recessive model TT versus CC/CT (OR 1.45, 95% CI 1.10–1.89).

**Conclusion:** Our study indicates that minor allele T of *prohibitin* 1630 C>T polymorphism is associated with increased susceptibility to breast cancer.

**Keywords:** *prohibitin*, breast cancer, genetic, polymorphisms, risk

## Introduction

*Prohibitin* is a candidate tumor suppressor gene encoding a 30 kDa intracellular protein which regulates cell cycle progression in multiple cell types. It interacts with the retinoblastoma tumor suppressor protein and its family members to suppress E2F-mediated transcription, and binds to p53 protein, increasing p53 transcriptional activity via increased DNA binding.<sup>1,2</sup> The human *prohibitin* gene is located on chromosome 17q21, a region of frequent loss of heterozygosity in breast cancers, spanning approximately 11 kb and consisting of seven exons.<sup>3</sup> In total, 217 single nucleotide polymorphisms have been identified in the *prohibitin* gene region, and 38 nucleotide polymorphisms in the coding region (<http://www.ncbi.nlm.nih.gov/SNP/>). Of these, 14 nucleotide polymorphisms have been reported in the 3'-untranslated region, as shown in Table 1, only five nucleotide polymorphisms (rs6917, rs9893420, rs111398671, rs112294663, rs73324369) have minor allele frequencies available, and the potential microRNA binding sites are summarized in Table 1. The most extensively studied nucleotide polymorphism of *prohibitin* is a C-to-T transition at position 1630 in

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**Table 1** The SNPs of *prohibitin* 3'UTR and MicroRNA binding sites

Name	Chr position	Alleles	MAF	Potential MicroRNA binding sites
rs6917	44836542	C/T	0.1924	hsa-miR-886-5p, hsa-miR-1292
rs9893420	44836887	A/G	0.0151	hsa-miR-15a, hsa-miR-15b, hsa-miR-16 hsa-miR-103, hsa-miR-107, hsa-miR-195 hsa-miR-220c, hsa-miR-217, hsa-miR-424 hsa-miR-497, hsa-miR-873, hsa-miR-933
rs111398671	47481589	C/T	0.0064	NA
rs112294663	47481625	A/G	0.0172	NA
rs73324369	47481676	C/T	0.0115	NA

**Abbreviations:** Chr, chromosome; MAF, Minor Alleles Frequency; Mi, micro; NA, not available; RNA, ribonucleic acid; SNP, single nucleotide polymorphism; UTR, untranslated region.

the 3'-untranslated region, that creates a variant with hsa-miR-1292 and hsa-miR-886-5p as potential binding sites (<http://snpinfo.niehs.nih.gov/cgi-bin/snpinfo/snpfunc.cgi>). This variant lacks antiproliferative activity and significantly reduces cell motility.<sup>4,6</sup>

Recent studies have evaluated the potential role of *prohibitin* in development of breast cancer and risk modification associated with *prohibitin* 1630 C>T polymorphism, but there are still no consistent data to indicate the molecular mechanism of 1630 C>T polymorphism in the regulation of *prohibitin* mRNA expression and its role in carcinogenesis. Although the T allele has been associated with an increased risk of breast cancer in women aged younger than 50 years who have a first-degree relative with breast cancer,<sup>7</sup> there are other studies that have not found an association between this polymorphism and breast cancer. In order to evaluate this potential association more precisely, we identified all published case-control studies, amounting to 5072 cases and 4796 controls, and undertook a quantitative analysis to identify evidence of an association between *prohibitin* 1630 C>T polymorphism and breast cancer risk.

## Materials and methods

### Genotype and mRNA expression data in lymphoblastoid cell lines

We used additional data on *prohibitin* genotypes and mRNA levels available online (<http://app3.titan.uio.no/biotools/help.php?app=snpexp>) for analysis of the genotype-phenotype relationship.<sup>8</sup> We analyzed the variation in gene expression using genome-wide expression arrays (47,294 transcripts) from Epstein-Barr virus-transformed lymphoblastoid cell lines from the same 270 HapMap individuals.<sup>9</sup> The genotyping data were from the HapMap Phase II release 23 data set consisting of 3.96 million single nucleotide polymorphism genotypes from 270 individuals in four populations.<sup>10</sup>

### Publication search and data extraction

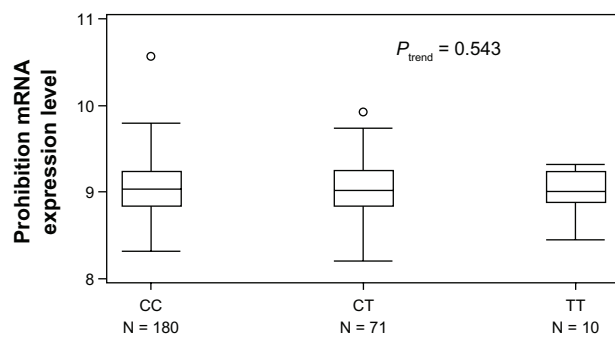
Eligible studies were identified by searching in the PubMed, ISI Web of Knowledge, and Embase databases for relevant reports (last search update, August 2012), using the search terms “PHB” or “prohibitin”, “polymorphism”, and “breast cancer”. We did not define any minimum number of patients to be included for meta-analysis. When multiple studies of the same patient population were identified, we included the published report with the largest sample size.

Inclusion criteria were: evaluation of *prohibitin* 1630 C>T polymorphism and breast cancer risk, case-control study design, and sufficient published data for estimating an odds ratio (OR) with a 95% confidence interval (CI). Only the most recent or complete study was used if the same study subjects were included in more than one publication. The main exclusion criteria were: no control population, no available genotype frequency, and overlapping data.

Two authors reviewed the articles separately and extracted the data from all eligible publications according to the criteria listed above. Any discrepancies between investigators were resolved by discussion and consultation with a third reviewer. The first author's surname, year of publication, country of original ethnicity, study design, genotyping method, and numbers of genotyped cases and controls (CC, CT, and TT genotypes) were recorded for each study.

### Statistical methods

The genotype and phenotype relationship analysis was performed using SAS software (version 9.1, SAS Institute, Cary, NC). A pooled OR and 95% CI were calculated to estimate the risk of breast cancer associated with *prohibitin* 1630 C>T. For all studies, we estimated the association under five different types of OR, namely the allele contrast model (T versus C), homozygote codominant model (TT versus CC), heterozygote codominant model (CT versus CC), dominant model (TT/CT versus CC), and recessive model (TT versus CC/CT). Hardy-Weinberg equilibrium



**Figure 1** mRNA expression level of the *prohibitin* gene in Epstein Barr virus-transformed lymphoblastoid cell lines.

was investigated using the  $\chi^2$  test. The Q-statistic and  $I^2$  test were used to investigate the degree of heterogeneity between studies. When  $P \geq 0.1$  or  $I^2 \leq 50\%$  indicated a lack of heterogeneity, the fixed-effects model (Mantel-Haenszel method) was used. Otherwise, the random-effects model (DerSimonian-Laird method) was chosen. Egger's test and inverted Begg's funnel plots were used to detect any publication bias. A sensitivity analysis was also performed by repeating the meta-analysis and omitting each study at each iteration.<sup>11,12</sup> The data were analyzed using Revman 5.0 software (<http://ims.cochrane.org/revman>).

## Results

### *Prohibitin* mRNA expression by genotype in lymphoblastoid cell lines

We used the available HapMap-cDNA expression database for correlation analysis of prohibitin genotype and mRNA expression in 270 HapMap lymphoblastoid cell lines. Except for nine cell lines with no available values, 180 (68.9%) cell lines had the CC genotype, 71 (27.3%) had the CT genotype, and 10 (3.8%) had the TT genotype. Figure 1 shows *prohibitin* mRNA expression according to 1630 C>T genotype for the lymphoblastoid cell lines. There was no significant difference in *prohibitin* mRNA expression level between cell lines carrying the TT genotype ( $9.05 \pm 0.31$ ),

TC genotype ( $9.04 \pm 0.33$ ), or CC genotype ( $8.96 \pm 0.29$ ,  $P_{\text{trend}} = 0.543$ , Figure 1).

### Study characteristics

After careful examination according to the inclusion criteria, six publications on polymorphisms of *prohibitin* 1630 C>T and breast cancer risk were eligible,<sup>4,7,13–16</sup> of which the study by Jakubowska et al<sup>14</sup> was reported twice. For the overlapping studies, only the one with the largest sample numbers was included. Jupe et al<sup>7</sup> only provided information on C/T or T/T versus C/C. Hence, a total of five publications including 5072 cases and 4796 controls were used in the present meta-analysis. Table 2 lists the main characteristics of these studies. All cases were histologically confirmed as breast cancer, and controls were cancer-free and hospital-based populations matched for age and gender. The genotype distribution of the controls was in Hardy-Weinberg equilibrium, except for one study.<sup>7</sup>

### Meta-analysis results

The results of the meta-analysis are shown in Table 3. Because the between-study heterogeneity of each study included in our meta-analysis was not statistically significant, all pooled ORs were derived from fixed-effects models. We observed that the *prohibitin* 1630 C>T polymorphism was significantly correlated with risk of breast cancer in the allele contrast model T versus C (OR 1.09, 95% CI 1.01–1.18, Figure 2), the homozygote codominant model TT versus CC (OR 1.47, 95% CI 1.12–1.92, Figure 3), and the recessive model TT versus CC/CT (OR 1.45, 95% CI 1.10–1.89, Figure 4). However, no significant association was detected for the heterozygote codominant model CT versus CC (OR 1.04, 95% CI 0.95–1.14, Figure 5) or the dominant model TT/CT versus CC (OR 1.08, 95% CI 0.99–1.18, Figure 6).

### Publication bias

Funnel plots and Egger's test were used to assess publication bias in the literature. There was no evidence of publication

**Table 2** Characteristics of the studies included in the meta-analysis

First author	Year	Country	Genotyping method	Source	Genotypes distribution (cases/controls)						HWE
					CC	CT	TT	CC	CT	TT	
Jupe et al <sup>7</sup>	2001	USA	PCR-RFLP	PB	128	77*		709	337*		NA
Spurdle et al <sup>13</sup>	2002	Australia	PCR-RFLP	PB	992	416	38	533	235	18	0.18
Campbell et al <sup>4</sup>	2003	UK	PCR-RFLP	PB	188	93	10	170	61	7	0.59
Karakus et al <sup>15</sup>	2008	Turkey	PCR-RFLP	PB	67	36	3	101	47	6	0.86
Jakubowska et al <sup>16</sup>	2012	Poland	iPLEX PCR-RFLP Taqman	PB	2029	891	104	1771	747	54	0.02

**Note:** \*For these just presenting the information for genotypes of CC and CT + TT, dominant model is calculated only.

**Abbreviations:** HWE, Hardy-Weinberg equilibrium; NA, not available; PB, population-based study; PCR, polymerase chain reaction.

**Table 3** Result of meta-analysis for *prohibitin* 3'UTR 1630 C>T polymorphism and breast cancer risk

Study	T vs C		TT vs CC		CT vs CC		CT/TT vs CC		TT vs CT/CC	
	OR (95% CI)	Ph	OR (95% CI)	Ph	OR (95% CI)	Ph	OR (95% CI)	Ph	OR (95% CI)	Ph
Total	1.09 (1.01–1.18) <sup>a</sup>	0.43	1.47 (1.12–1.92) <sup>a</sup>	0.51	1.04 (0.95–1.14)	0.38	1.08 (0.99–1.18)	0.4	1.45 (1.10–1.89) <sup>a</sup>	0.49

Notes: <sup>a</sup>Statistically significant result; Ph: P value of Q test for heterogeneity.

Abbreviations: CI, Confidence Interval; OR, Odds Ratio; UTR, Untranslated Regions.

bias for *prohibitin* 1630 C>T polymorphism, and the results of the Egger's test suggested no publication bias for the allele contrast model ( $P = 0.685$ ), homozygote codominant model ( $P = 0.810$ ), heterozygote codominant model ( $P = 0.926$ ), dominant model ( $P = 0.639$ ), or recessive model ( $P = 0.846$ ).

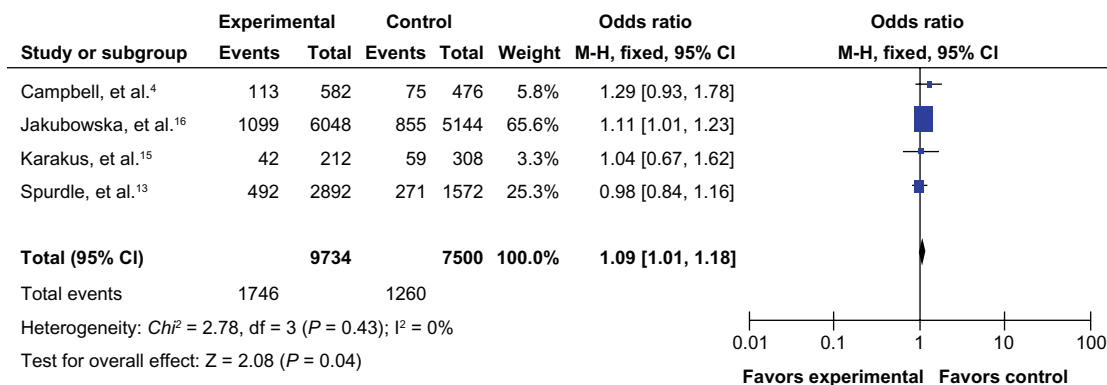
## Discussion

The 3' untranslated region of the *prohibitin* gene which encodes a transacting regulatory RNA molecule arrests cell proliferation between the G<sub>1</sub> and S phases of the cell cycle.<sup>17</sup> Jupe et al confirmed the antiproliferative activity of prohibitin by microinjection of prohibitin mRNA and protein into normal and immortalized cancer cell lines.<sup>18</sup> The protein-encoding region of the prohibitin gene was not found to be mutated, but the 3'-untranslated region of prohibitin mutations inhibited cell cycle progression in loss of human cancer cell lines.<sup>5</sup> The investigators confirmed that a single nucleotide polymorphism (C–T transition) in the prohibitin 3'-untranslated region creates a null (T) allele whereby the RNA product has lost its antiproliferative activity.<sup>17</sup> The results of our study are consistent with the functional *prohibitin* 3'-untranslated region 1630 C>T polymorphism resulting in increased risk of breast cancer, although there was no significant association between *prohibitin* 1630 C>T polymorphism and mRNA expression in lymphoblastoid cell lines from the HapMap database. Data being collected from different studies without stratification/adjustment for differences between studies and inconsistent

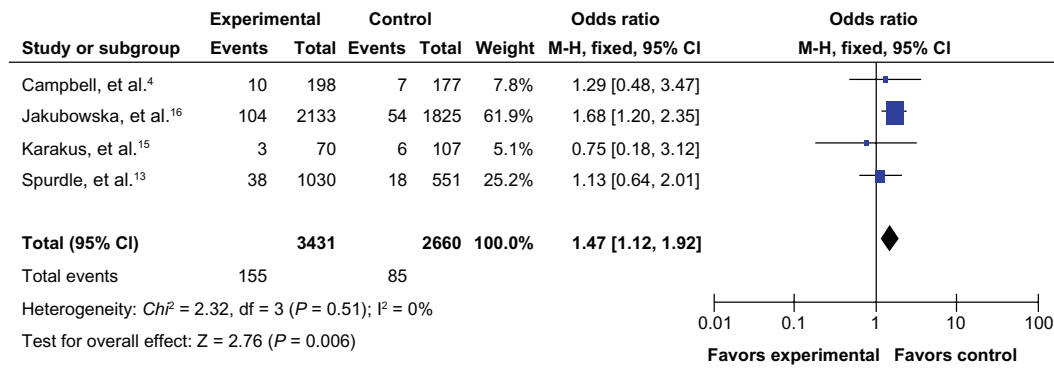
use of selection criteria are possible explanations for this. Further investigations should be done in breast cancer tissue or cells to determine if a correlation exists between genotype and mRNA expression.

In this study, we investigated 5072 cases and 4796 controls, the allele contrast model, the homozygote codominant and the recessive model of *prohibitin* 1630 C>T polymorphism were found to be significantly associated with influencing the risk of breast cancer. Heterogeneity and publication bias were not observed in this study. Our findings suggest that *prohibitin* 1630 C>T polymorphism increases the risk of breast cancer.

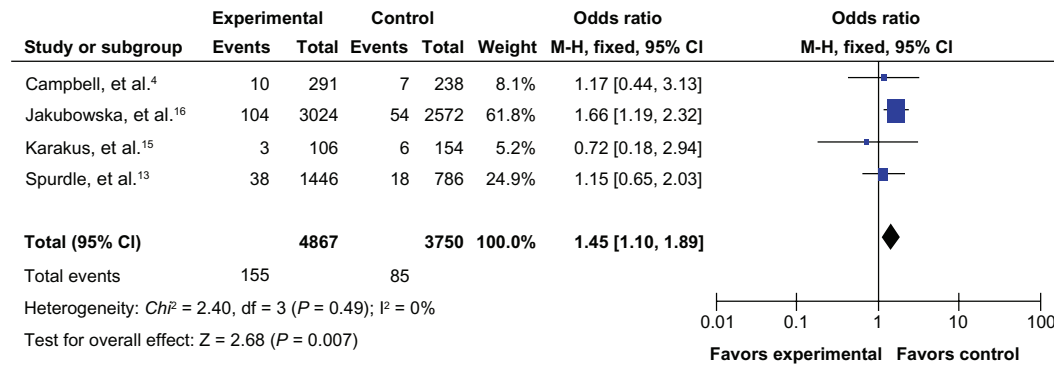
Some limitations of this meta-analysis need to be acknowledged when interpreting its findings. First, we presumed that ethnicity status and family history play diverse roles in the risk of breast cancer. In our study, we considered the possibility that the effect of *prohibitin* 1630 C>T polymorphism might be ethnicity-specific in mixed populations, but we did not perform subgroup analysis to detect an association between this polymorphism and ethnicity. Second, our results were based on unadjusted estimates, so a more precise analysis should be done when more detailed individual data become available. A recent study evaluated the association between genetic variants of *prohibitin* and breast cancer risk in BRCA1 or BRCA2 mutation carriers, and the findings showed that the *prohibitin* 1630TT genotype may modify breast cancer risk in these women.<sup>16</sup> Third, as we all know, cancer is a complicated disease, different genetic backgrounds may contribute to the discrepancy, and it is still

**Figure 2** Forest plot for the association between the *prohibitin* 1630 C>T polymorphism and breast cancer risk (for T versus C) in a fixed-effects model.

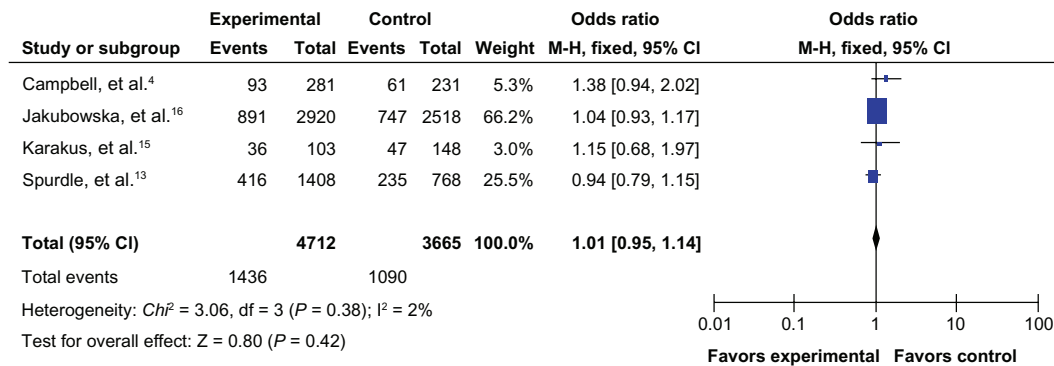
Abbreviations: CI, Confidence Interval; M-H, Mantel-Haenszel.



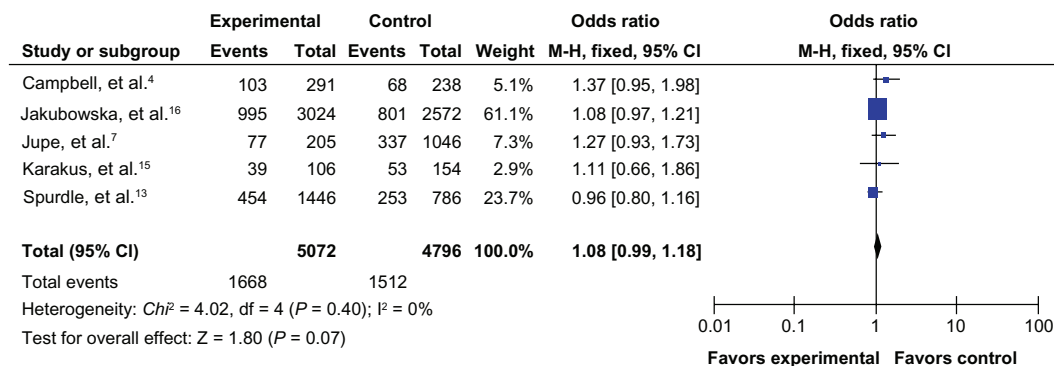
**Figure 3** Forest plot for the association between the *prohibitin* 1630 C>T polymorphism and breast cancer risk (for TT versus CC) in a fixed-effects model. **Abbreviations:** CI, Confidence Interval; M-H, Mantel-Haenszel.



**Figure 4** Forest plot for the association between the *prohibitin* 1630 C>T polymorphism and breast cancer risk (for TT versus CC/CT) in a fixed-effects model. **Abbreviations:** CI, Confidence Interval; M-H, Mantel-Haenszel.



**Figure 5** Forest plot for the association between the *prohibitin* 1630 C>T polymorphism and breast cancer risk (for CT versus CC) in a fixed-effects model. **Abbreviations:** CI, Confidence Interval; M-H, Mantel-Haenszel.



**Figure 6** Forest plot for the association between the *prohibitin* 1630 C>T polymorphism and breast cancer risk (for TT/CT versus CC) in a fixed-effects model. **Abbreviations:** CI, Confidence Interval; M-H, Mantel-Haenszel.

necessary to conduct larger sample studies considering gene-gene and gene-environment interactions, which may be an important component of the association between *prohibitin* 1630 C>T polymorphism and risk of breast cancer. In conclusion, the results of this meta-analysis suggest that the *prohibitin* 1630 C>T variant was associated with a significant increase in the risk of breast cancer.

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## Disclosure

The authors report no conflicts of interest in this work.

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