REVIEW

CASP8 -652 6N del polymorphism and breast cancer risk: a systematic review and meta-analysis

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ABSTRACT

Purpose: Many studies have investigated the association between CASP8 -652 6N del polymorphism and the risk of breast cancer, but the result is still unclear owing to the obvious inconsistence among those studies. This study aims to quantify the strength of association between CASP8 -652 6N del polymorphism and risk of breast cancer.

Methods: We searched the electronic MEDLINE database for studies relating to the association between CASP8 -652 6N del polymorphism and risk of breast cancer. We estimated summary odds ratios (ORs) with their 95% confidence intervals (95% CIs) to assess the association. Ten case-control studies with 13,220 cases and 13,750 controls were included into this meta-analysis.

Results: Meta-analysis of a total of ten studies showed that reduced breast cancer risk was associated with CASP8 -652 6N del polymorphism (homozygous: OR=0.85, 95% CI 0.93-0.98). After adjustment for heterogeneity, meta-analysis showed that reduced breast cancer risk was also associated with CASP8 -652 6N del polymorphism (homozygous: OR=0.78, 95% CI 0.63-0.95, dominant: OR=0.93, 95% CI 0.88-0.99). For Caucasians, CASP8-652 6N del was associated with reduced breast cancer risk at a borderline level (homozygous: OR=0.94, 95% CI 0.86-1.02, heterozygous: OR=0.96, 95% CI 0.90-1.03, recessive: OR=0.96, 95% CI 0.90-1.03, dominant: OR=0.94, 95% CI 0.88-1.01). No evidence of publication bias was observed.

Conclusion: Meta-analyses of the available data suggest that CASP8 -652 6N del polymorphism is associated with reduced breast cancer risk.

KEYWORDS

CASP8 -652 6N del polymorphism, breast cancer risk, meta-analysis

INTRODUCTION

Apoptosis, also called programmed cell death, is important to maintain internal homeostasis by removing irreparable damaged cells. Defects in apoptosis machinery may lead to cancer.[1] The Caspase-8 (CASP8) protein regulates apoptosis, and it stimulates cell proliferation, malignant transformation and tumour progression as a result of its dysfunction or reduced activity.[2] CASP8 is encoded by the CASP8 gene. The human CASP8 gene contains at least 11 exons spanning ~30 kb on the highly polymorphic chromosome 2q33-34.[3] Several studies have confirmed that in addition to rare mutations, a few common variants of the CASP8 gene disrupt the apoptotic mechanism and thus impact the risk of developing various types of cancer, including breast cancer,[4] prostate cancer[5] and several other cancers.[6] Previous studies have largely focused on two variants of the CASP8 gene: D302H (rs1045485) and -652 6N del (rs3834129). Although the results of studies on the D302H variant have been generally consistent, conclusions on the -652 6N del variant remain inconsistent and inconclusive; some studies have demonstrated reduced susceptibility,[7] whereas other studies did not detect any association.[8-10] Zhang *et al.*[11] found that CASP8 -652 6N del polymorphism was not associated with breast cancer risk. However, their meta-analysis was only a small part of their original paper. When they performed the meta-analysis, the pooled sample size was relatively small and not enough information was available for more exhaustive subgroup analysis. Since then, several additional studies about this polymorphism and breast cancer risk, with large sample sizes, have been reported, which would greatly improve the power of the meta-analysis of this polymorphism. Subgroup analyses performed by ethnicity were also possible now. Therefore, we performed an updated meta-analysis on all the available case-control studies to access the breast cancer risk with CASP8 -652 6N del.

METHODS

Identification and eligibility of relevant studies

We searched for relevant papers published before 24 May 2013 in the English literature by using the electronic MEDLINE database with the following terms "CASP8", "caspase 8", "-652 6N del", "rs3834129", "breast cancer", "polymorphism" and "variant". References of the retrieved articles were also screened for original studies. We included all the case-control studies and cohort studies that investigated the association between CASP8-652 6N del polymorphisms and breast cancer risk with genotyping data. Abstracts, unpublished reports and articles not written in the English language were not considered. Additionally, when a case-control study was included by more than one article using the same case series, we selected the study that included the largest number of individuals.

Data extraction

We extracted the following information from each manuscript: author, year of publication, country of origin, ethnicity and genotyping information. For studies including subjects of different ethnicities, data were extracted separately and categorised as Asians, Caucasians and mixed-race individuals.

Statistics

Based on the genotype frequencies in cases and controls, crude odds ratios (ORs) as well as their standard errors (SEs) were calculated. Pooled ORs were calculated for the homozygous genetic model, heterozygous genetic model, dominant genetic model and recessive genetic model, respectively. The fixed effects model (Mantel-Haenszel method) as well as the random effects (DerSimonian Laird) model were used to calculate the pooled OR. Between-study heterogeneity and between-study inconsistency were assessed by using Cochran Q statistic and by estimating I^2, respectively.[12] Heterogeneity was considered to be significant when $p<0.10$ and $I^2>50\%$. When significant heterogeneity was detected, the random effects model was chosen; nevertheless, the fixed effects estimates are also secondarily reported as an alternative approach. To study the source of between-study heterogeneity, the Galbraith plot was used to spot the outliers as possible major sources of between-study heterogeneity.[13] Evidence of publication bias was determined using Begg's[14] and Egger's[15] formal statistical test and by visual inspection of the funnel plot. All statistical tests were conducted with Review Manager downloaded from the Cochrane Collaboration website (Version 5.1). A p value of 0.05 for any test or model was considered to be statistically significant.

RESULTS

Study characteristics

A total of six publications met the inclusion criteria.[7,10,16-19] In two of these studies, the ORs were presented separately according to the different subgroups.[17,18] Therefore, each group in one publication was considered separately for subgroup analysis. Hence, a total of ten studies including 13,220 cases and 13,750 controls were used in the meta-analysis. *Table 1* lists the studies identified and their main characteristics. Among these studies, there were seven studies of Caucasians, one study of Asians and two studies of mixed populations. Almost all of the cases were pathologically confirmed. Controls were mainly healthy populations and matched for age.

Main results

Table 2 lists the main results of this meta-analysis. There was obvious between-study heterogeneity among these ten studies (homozygous: $I^2=67.3\%$, heterozygous: $I^2=64\%$, recessive: $I^2=63.9\%$, dominant: $I^2=71\%$), thus the random effects model was used to pool data. Meta-analysis showed that reduced breast cancer risk was associated with CASP8 -652 6N del polymorphism (homozygous: OR=0.85, 95% CI 0.93-0.98). After adjustment for heterogeneity by the Galbraith plot, there was no between-study heterogeneity among the remaining studies, thus the fixed effects model was used to pool the ORs. Meta-analysis showed that reduced breast cancer risk was also associated with CASP8 -652 6N del polymorphism (homozygous: OR=0.78, 95%

Table 1. *Main characteristics of all the studies included in the meta-analysis*

Study	Country	Ethnicity	Case	Control	Genotype method
Sun 2007[7]	China	Asian	1119	1004	PCR-RFLP
Cybulski 2008[16]	Poland	Caucasian	618	965	PCR-RFLP
Frank 2008[17]	Germany	Caucasian	1110	1108	Fluorescent analysis
Frank 2008[17]	UK	Caucasian	1212	1184	Fluorescent analysis
Frank 2008[17]	Germany	Caucasian	1143	1155	Fluorescent analysis
Frank 2008[17]	Germany	Caucasian	4470	4560	Fluorescent analysis
Haiman 2008[18]	USA	Mixed	2029	2245	TaqMan
Haiman 2008[18]	USA	Mixed	703	920	TaqMan
De Vecchi 2009[10]	Italy	Caucasian	580	406	PCR-RFLP
Hashemi 2012[19]	Iran	Caucasian	236	203	PCR premix

Table 2. *Main results of pooled ORs in the meta-analysis*

	Studies	OR (95% CI)	P_{OR}	Model	I^2 (%)	P_H
Homozygous	10	0.85 (0.73-0.98)	0.028	Random	67.3	0.001
Homozygous (adjustment for heterogeneity)	2	0.78 (0.63-0.95)	0.003	Fixed	0	0.915
Heterozygous	10	0.93 (0.84-1.02)	0.052	Random	64	0.003
Heterozygous (adjustment for heterogeneity)	8	0.94 (0.89-1.00)	0.067	Fixed	0	0.951
Recessive	10	0.89 (0.78-1.00)	0.053	Random	63.9	0.003
Recessive (adjustment for heterogeneity)	8	0.96 (0.90-1.03)	0.246	Fixed	32.1	0.172
Dominant	10	0.91 (0.81-1.01)	0.074	Random	71	0
Dominant (adjustment for heterogeneity)	8	0.93 (0.88-0.99)	0.022	Fixed	0	0.681

Table 3. *Main results of pooled ORs in the meta-analysis of the Caucasian populations*

Studies with poor design	Studies	OR (95% CI)	P_{OR}	Model	I^2 (%)	P_H
Studies with poor design	7	0.86 (0.74-1.01)	0.062	Random	60.4	0.019
Studies with poor design	6	0.94 (0.86-1.02)	0.116	Fixed	20.6	0.278
Heterozygous	7	0.39 (0.33-0.47)	0	Random	71.6	0.002
Heterozygous (adjustment for heterogeneity)	6	0.96 (0.90-1.03)	0.277	Fixed	0	0.967
Recessive	7	0.89 (0.77-1.02)	0.091	Random	65	0.009
Recessive (adjustment for heterogeneity)	6	0.96 (0.90-1.03)	0.244	Fixed	42.3	0.123
Dominant	7	0.94(0.88-1.01)	0.089	Random	0	0.623

CI 0.63-0.95, dominant: OR =0.93, 95% CI 0.88-0.99) (*figures 1 and 2*).

In the subgroup analysis by ethnicity, CASP8 -652 6N del polymorphism was associated with reduced breast cancer risk at a borderline level after adjustment for heterogeneity (homozygous: OR=0.94, 95% CI 0.86-1.02, heterozygous: OR=0.96, 95% CI 0.90-1.03, recessive: OR=0.96, 95% CI 0.90-1.03, dominant: OR=0.94, 95% CI 0.88-1.01, *table 3*). The borderline character of the association may be due to relatively inadequate overall power.

Publication bias

Begg's funnel and Egger's test were performed to access the publication bias in this meta-analysis. The shape of the funnel plots did not reveal obvious evidence of asymmetry, and the p value of Egger's test was >0.05 (homozygous: p=0.79, heterozygous: p=0.788, recessive: p=0.245, dominant: p=0.531), providing statistical evidence of funnel plot symmetry (*figure 3*). Thus, the results above suggest that publication bias was not evident in this meta-analysis.

DISCUSSION

It is well recognised that there is individual susceptibility to the same kind of cancer even with the same environmental exposure. Host factors, including polymorphisms of genes involved in carcinogenesis, may have accounted for this difference. Therefore, genetic susceptibility to cancer has been a research focus in the scientific community; CASP8, encoded by the CASP8 gene, has a central function in apoptotic pathways and changes in the genetically determined structure of this enzyme can influence the rate of apoptosis. More specifically, a six nucleotide deletion polymorphism (-652 6N del) has been identified in the promoter region of the CASP8 gene and is associated with decreased RNA expression in lymphocytes due to the altering of an Sp1 binding site.[20] This variant has been found to decrease CASP8 activity and apoptotic reactivity of T lymphocytes through the cancer cell *ex vivo* model. Recently, due to the functional significance of the CASP8 -652 6N del variant, genetic variants of the CASP8 gene in the aetiology of several cancers have drawn increasing attention. A growing number of studies have suggested that -652 6N del in the promoter region of the CASP8 gene was associated with decreased breast cancer risk. However, the results are inconclusive. To better understand the association between this polymorphism and breast cancer risk, a pooled analysis with a large sample and heterogeneity explored is necessary.

Thus, we performed this meta-analysis by critically reviewing ten individual case-control studies with a total of 13,220 breast cancer cases and 13,750 controls. Meta-analysis of the ten studies showed that reduced breast cancer risk was associated with CASP8 -652 6N del polymorphism (homozygous: OR=0.85, 95% CI 0.93-0.98). Heterogeneity is a potential problem when interpreting the results of all meta-analyses, and finding the sources of heterogeneity is

Figure 1. *Forest plots showing associations between CASP8 -652 6N del polymorphism and risk of breast cancer. A) Analysis of all the included studies. B) Analysis of all the studies after adjustment for heterogeneity*

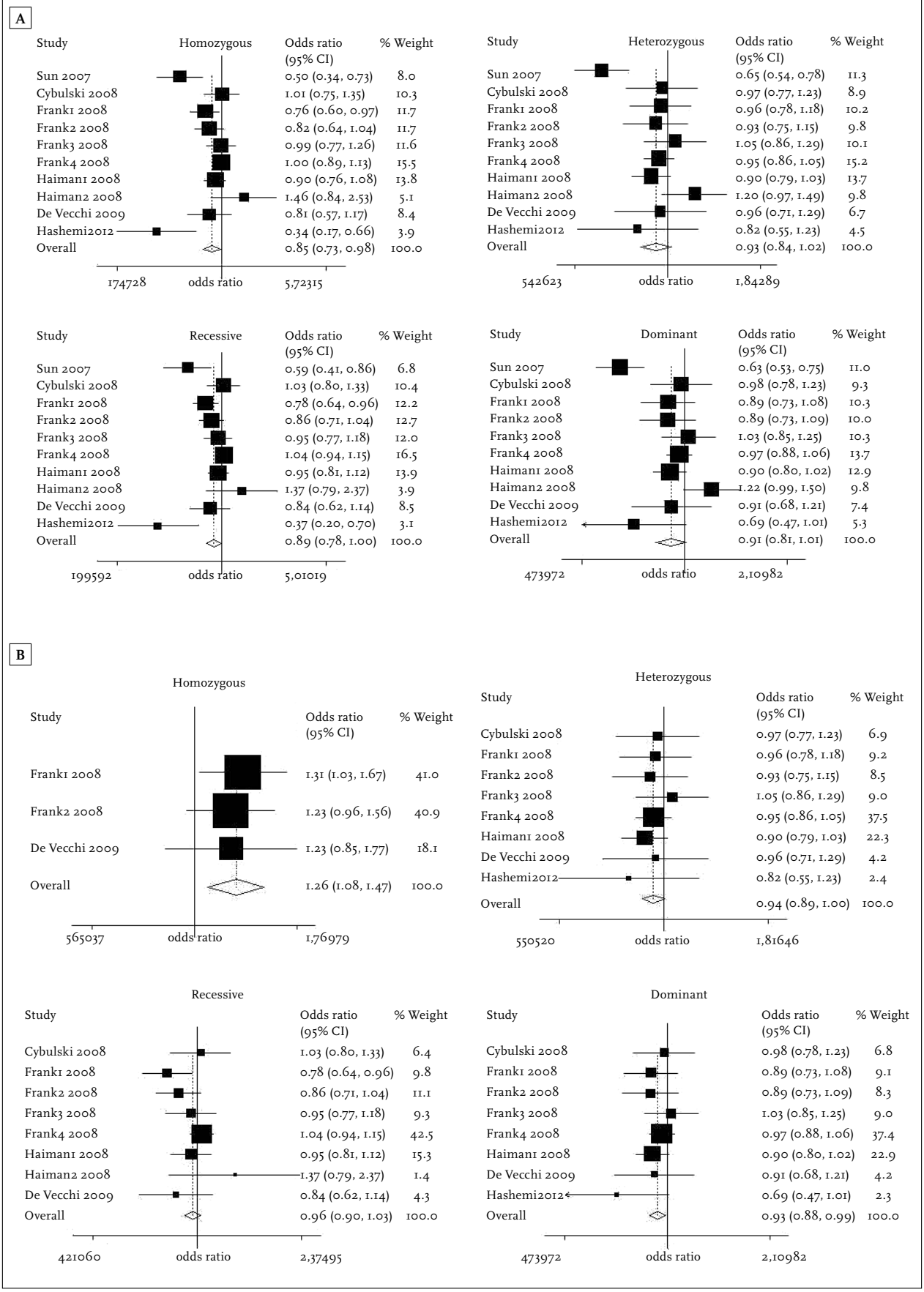


Figure 2. *Galbraith plots of association between CASP8 -652 6N del polymorphism and breast cancer risk. A) Galbraith plot of meta-analysis of all the included studies. B) Galbraith plot of meta-analysis of the Caucasian studies*

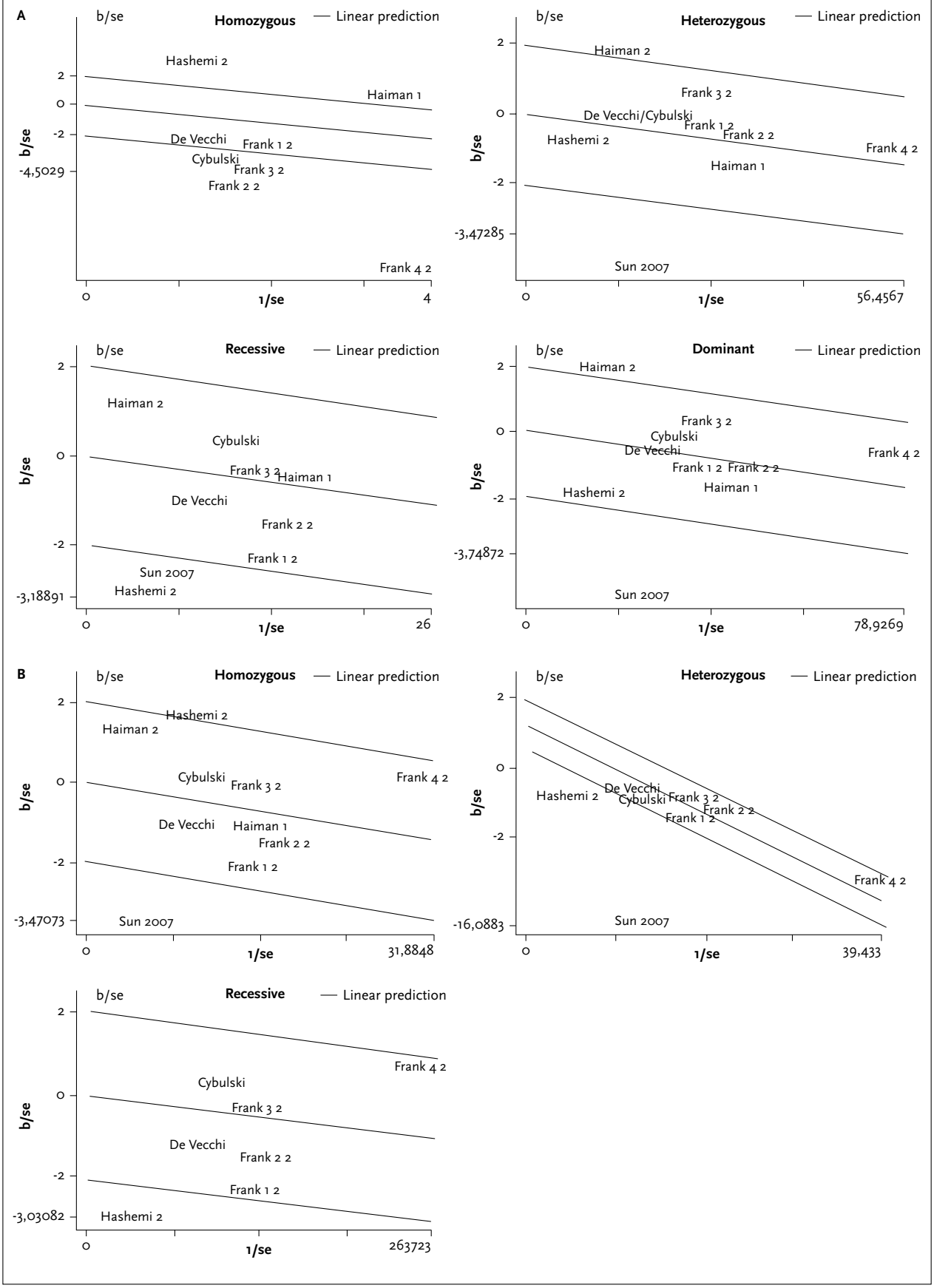
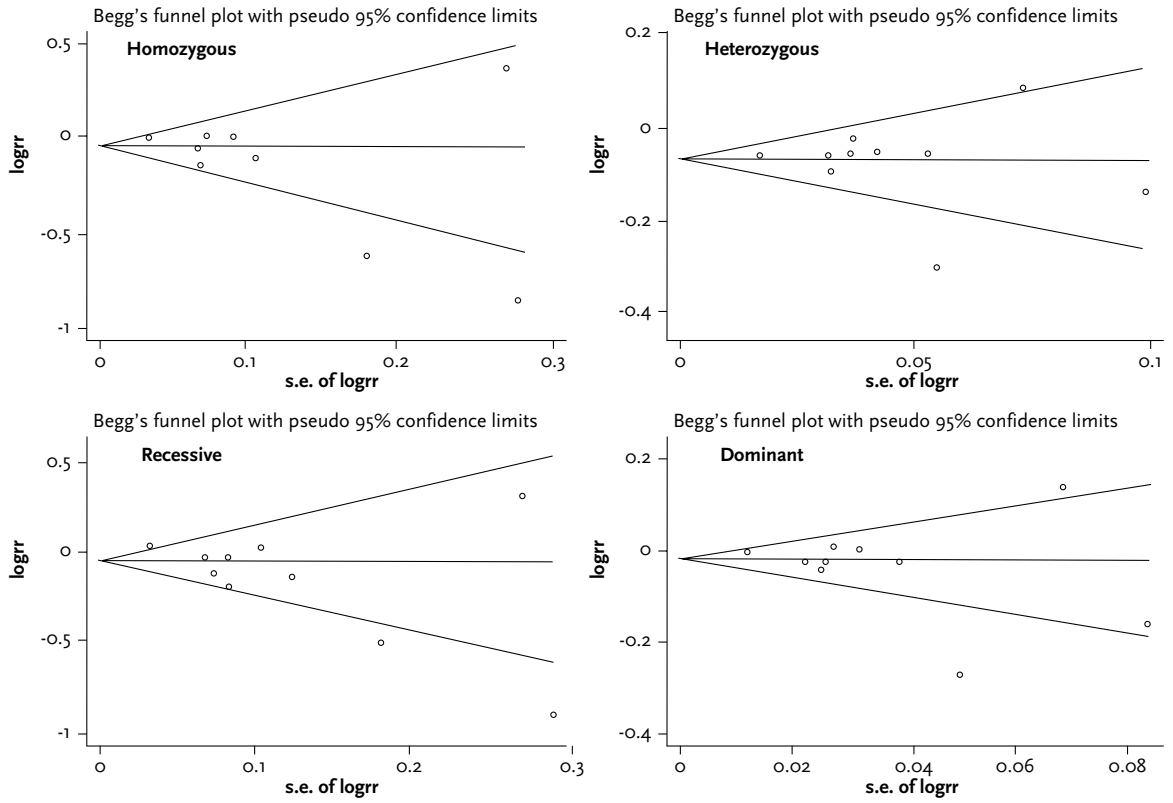


Figure 3. *Begg's funnel plots for assessing the risk of publication bias*



one of the most important goals of meta-analysis.[21] In this present meta-analysis, we found obvious heterogeneity in the meta-analysis of the ten studies (homozygous: I^2=67.3%, heterozygous: I^2=64%, recessive: I^2=63.9%, dominant: I^2=71%). Heterogeneity may also come from the studies with a poor design, because these studies usually do not exclude possible factors that may bias the estimate of the real effects, and may result in incorrect conclusions.[22] Thus, the Galbraith plot was used to spot the outliers as possible studies with low quality design. After excluding those studies, the between-study heterogeneity decreased and there was no obvious heterogeneity among the remaining studies, which further suggested the heterogeneity might come from those studies. After adjustment for heterogeneity, meta-analysis showed that reduced breast cancer risk was also associated with CASP8 -652 6N del polymorphism (homozygous: OR=0.78, 95% CI 0.63-0.95, dominant: OR=0.93, 95% CI 0.88-0.99). Thus, meta-analyses of the available data supported an association between the CASP8 -652 6N del polymorphism and reduced breast cancer risk. So, the outcomes above provide further evidence for the association between CASP8 -652 6N del genotype and decreased risk of breast cancer.

Some limitations of this meta-analysis should be acknowledged. First, in the subgroup analyses, the number of Asians was relatively small, not having enough statistical power to explore the real association. Additionally, no data were available about Africans. Second, our results were based on unadjusted estimates, while a more precise analysis could have been conducted if individual data had been available, which would allow for the adjustment by other co-variants including age, ethnicity, menopausal status, smoking status, drinking status, obesity, environmental factors, and other lifestyle.

In conclusion, this meta-analysis suggests that the CASP8 -652 6N del polymorphism is associated with decreased breast cancer risk. However, it is necessary to conduct large sample studies using standardised unbiased genotyping methods, homogeneous breast cancer patients and well-matched controls. Moreover, gene-gene and gene-environment interactions should also be considered in the analysis.[23] Such studies taking these factors into account may eventually lead to our better, comprehensive understanding of the association between the CASP8 -652 6N del polymorphism and breast cancer risk.

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