Association between manganese superoxide dismutase gene polymorphism and breast cancer risk: A meta-analysis of 17,842 subjects

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Abstract. The aim of this meta-analysis was to explore the association between the manganese superoxide dismutase (MnSOD) gene polymorphism and breast cancer risk, and to investigate the interaction of this gene polymorphism with known risk factors for breast cancer. Crude odds ratios (ORs) with 95% confidence intervals (CIs) for breast cancer risk associated with co-dominant models [valine/alanine (Val/Ala) vs. Val/Val, Ala/Ala vs. Val/Val], a dominant model (Val/Ala + Ala/Ala vs. Val/Val) and a recessive model (Ala/Ala vs. Val/Ala + Val/Val) were statistically estimated. This meta-analysis included 8,102 breast cancer cases and 9,740 controls from 14 published case-control studies. The data revealed no significant association between the MnSOD polymorphism and the risk of developing breast cancer. However, upon subgroup analyses, the risk was significantly increased in premenopausal women with the dominant model of the MnSOD gene polymorphism (OR, 1.15; 95% CI, 1.01-1.31). Statistically significant increased risks were also identified in women with the MnSOD genotypes containing the Ala allele who had a tobacco smoking history (OR, 1.17; 95% CI, 1.02-1.34), a higher body mass index (OR, 1.26; 95% CI, 1.02-1.56) or who used oral contraceptives (OR, 1.98; 95% CI, 1.34-2.93). By contrast, there was no significant association between breast cancer risk and alcohol consumption and ethnicity. This meta-analysis demonstrated no statistically significant association between the MnSOD gene polymorphism and breast cancer susceptibility, except in premenopausal women with certain unhealthy lifestyle habbits.

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Introduction

Breast cancer is the most common malignant disease among women worldwide. Breast cancer accounted for more than 1.3 million new cases and 464,000 mortalities in 2007 (1). A higher risk of breast cancer is associated with individuals who are older, have a family history of breast cancer, are overweight or obese following menopause, have never had children, have recently used oral contraceptives, had their first child after age 30, have gene mutations (including BRCA1 and BRCA2 mutation), have high breast tissue density and have used postmenopausal hormone therapy. However, living a healthy lifestyle, including maintaining a healthy body weight, increasing physical activity and minimizing alcohol intake, may reduce breast cancer risk (1). To date, practical screening to detect the early stages of breast cancer and significant advances in treatment have significantly reduced breast cancer mortality; however, many women still develop metastatic disease and ultimately succumb to the disease. Therefore, there is an urgent requirement to develop more aggressive and useful strategies to identify high-risk populations for the early detection and prevention of breast cancer.

Risk factors contribute to breast cancer development through multiple genetic or epigenetic alterations (1), including the activation of oncogenes or the silencing of tumor suppressor genes (1,2). To date, a large body of evidence has accumulated regarding these gene alterations that are associated with mammary gland carcinogenesis, although the precise mechanism underlying breast cancer development remains to be defined. For example, manganese superoxide dismutase (MnSOD) is essential for mammalian cell vitality and has been considered as a tumor suppressor gene as oxidative stress and mitochondrial DNA damage play a significant role in breast cancer carcinogenesis (2). The MnSOD gene is localized on chromosome 6q25.3 and contains 4 introns and 5 exons, with at least 2 functionally validated single nucleotide polymorphisms in the amino acid codons at 9 and 16. The MnSOD protein is synthesized in the cytoplasm and then transported into the mitochondria via N-terminal mitochondrial targeting sequences. A common

polymorphism of MnSOD is Val16Ala (rs4880) at the 9-amino acid upstream of this cleavage site, a substitution of thymine to cytosine (T to C) at nucleotide 47, resulting in the encoded amino acid from valine (Val, GTT) to alanine (Ala, GCT) on the 16th residue of the 24-amino acid signal sequence (3,4). This particular polymorphism has been designated as the MnSOD Val9Ala (rs4880) polymorphism (4). The Val variant is predicted to form a β-sheet structure, while the Ala variant results in an α-helical conformation. The Ala variant leads to a decreased transport rate into the inner mitochondrial membrane and therefore, reduces MnSOD enzymatic activity compared to the Val variant. Inefficient MnSOD protein in the mitochondria may leave the cell vulnerable to oxidative damage without its full defense against superoxide radicals, resulting in protein oxidation and DNA mutations. There is increasing evidence demonstrating that the MnSOD gene polymorphism is a risk factor for several types of malignancies, including breast (5,6), lung (7), bladder (8), colorectal (9) and prostate (10,11) cancer.

In breast cancer, a number of individual studies have been conducted to explore the association between the MnSOD gene polymorphism and breast cancer susceptibility; however, the results remain inconclusive. This may be due to the possibly small effect of this gene polymorphism on breast cancer risk, or the relatively small sample sizes of each published study. Thus, in the present study, we performed a meta-analysis of the published data to obtain a more precise estimation of this gene polymorphism as a risk factor for breast cancer.

Materials and methods

Identification and eligibility of relevant studies. In this study, we attempted to include all case-control studies published to date that have examined the association between the MnSOD gene polymorphism and breast cancer. We searched the PubMed database using the search terms 'manganese superoxide dismutase', 'MnSOD', 'polymorphism(s)' and 'breast cancer'. Additional articles were identified from the references of original studies or reviews. Our meta-analysis included only full-text publications written in English, and we did not define an inclusion criteria for a minimum number of subjects. In addition, in case of multiple studies published using the same group of subjects, we only selected the largest and/or the most recent publication.

Inclusion and exclusion criteria. In this meta-analysis, the selected publications had to meet the following criteria: i) have individuals with a pathologically confirmed diagnosis of breast cancer and confirmed control individuals which were free of any cancer; ii) be an independent case-control or cohort study that quantitatively evaluated the association between breast cancer risk and MnSOD gene polymorphism; iii) have sufficient data to calculate odds ratios (ORs) with 95% confidence intervals (CIs); and iv) have sufficient subgroup information for more detailed analyses. By contrast, the major exclusion criteria were: i) studies that had no control population, ii) studies that had no available genotype frequency, or iii) studies that were duplicated from a previous study.

Data extraction. We followed a standard protocol for data extraction. Two independent investigators extracted data and then reached a consensus on all of the items through discussion. In the event when no consensus could be reached, a third investigator was then consulted to resolve the dispute, and a final decision was formulated by the majority. The following information was recorded for each publication: first author, publication date, ethnicity of subjects, criteria for matching controls, total number of cases and controls, genotype frequencies of the cases and controls (e.g., Val/Val, Val/Ala, Ala/Ala) and result of the Hardy-Weinberg equilibrium calculations.

Statistical analysis. Crude ORs with 95% CIs were used to estimate the relative risk of breast cancer associated with MnSOD gene polymorphism. For all subjects, we evaluated the risk of Val/Ala vs. Val/Val, Ala/Ala vs. Val/Val, Ala/Ala vs. Val/Ala + Val/Val, and Val/Ala + Ala/Ala vs. Val/Val, assuming 2 co-dominant models, 1 recessive model, and 1 dominant model, respectively. An online Chi-square (χ^2) test program (http://ihg2.helmholtz-muenchen.de/cgi-bin/hw/hwa1.pl) was used to determine whether the reported genotype distribution in the control population conformed to the Hardy-Weinberg equilibrium. $P<0.05$ was considered to indicate a statistically significant difference.

Subgroup analyses were then conducted for factors related to menopausal status, lifestyle and ethnicity. Heterogeneity was determined using the χ^2-based Q-test using the formula: $Q = \sum weight_i \times (lnOR_{MH} - lnOR_i)^2$, where $weight_i = 1/variance_i$. $P>0.10$ for the Q test was considered to indicate a lack of heterogeneity among the studies. Furthermore, the fixed-effects model (Mantel-Haenszel) and the random-effects model (DerSimonian and Laird) were also used to combine values from each of the studies. Use of the random-effects model was more appropriate when heterogeneity was present. If the controls in the studies were found to not be in the Hardy-Weinberg equilibrium, sensitivity analysis was performed with and without these studies to test the robustness of the findings. Similarly, sensitivity analysis was also conducted by omitting each study in turn to identify potential outliers. Potential publication bias was estimated using Begg's funnel plots and Egger's test, in which an asymmetric plot suggested a possible publication bias and $P<0.05$ indicated a statistically significant publication bias. All statistical analyses were performed using Review Manage version 4.2 (Oxford, England) and STATA version 11.0 (Stata Corporation, College Station, TX, USA). All P-values were two-sided.

Results

Study characteristics. The main characteristics of this meta-analysis are shown in Table I. We identified 14 eligible studies that were published between 1999 and 2009 and consisted of 8,102 breast cancer cases and 9,740 controls. Sample sizes for these 14 studies ranged from 187 to 3,837. Seven studies had been conducted on mixed-ethnicity populations, 5 studies on Caucasian and 2 studies on Asian populations. All studies, except 4, indicated that the distribution of the genotypes in the controls was consistent with the Hardy-Weinberg equilibrium. The controls in all studies were primarily healthy and were matched for age to the breast cancer cases.

Table I. Characteristics of the published studies included in the meta-analysis.

Authors/(Refs.)	Year	Ethnicity	Source	Case/control	Case			Control			HWE
					VV	VA	AA	VV	VA	AA	
Ambrosone *et al* (12)	1999	Mixed	PB	266/295	39	137	90	63	169	63	0.012[a]
Mitrunen *et al* (5)	2001	Caucasian	HB	479/482	124	255	100	153	231	98	0.526
Egan *et al* (13)	2003	Mixed	PB	470/497	102	250	118	130	240	127	0.446
Cai *et al* (6)	2004	Asian	PB	1125/1197	831	266	28	844	290	23	0.741
Millikan *et al* (14)	2004	Mixed	PB	2025/1812	532	1053	440	462	943	407	0.075
Tamimi *et al* (15)	2004	Mixed	HB	968/1205	255	468	245	297	612	296	0.584
Bergman *et al* (16)	2005	Caucasian	PB	118/174	12	73	33	43	88	43	0.879
Cheng *et al* (17)	2005	Asian	PB	469/739	343	115	11	545	183	11	0.322
Gaudet *et al* (18)	2005	Mixed	PB	1034/1084	253	511	270	264	539	281	0.862
Kocabas *et al* (19)	2005	Caucasian	PB	84/103	23	32	29	25	40	38	0.032[a]
Slanger *et al* (20)	2006	Caucasian	PB	614/1080	144	318	152	263	528	289	0.477
Bica *et al* (21)	2007	Mixed	HB	100/370	29	56	15	94	252	24	<0.001[a]
Bica *et al* (22)	2009	Mixed	HB	100/372	15	29	56	26	94	252	<0.001[a]
Eras-Erdogan *et al* (23)	2009	Caucasian	PB	250/330	107	113	30	150	141	39	0.508

VV, Val/Val; VA, Val/Ala; AA, Ala/Ala; HB, hospital-based control; PB, population-based control; HWE, Hardy-Weinberg equilibrium; [a]P<0.05.

Table II. Odds ratios for MnSOD genotypes and breast cancer according to menopausal status.

MnSOD genotypes	Case	Control	OR	95% CI	χ^{2a}	P-value[a]	Z-value[b]	P-value[b]
Premenopausal women								
Val/Ala vs. Val/Val	1215	1409	1.09	0.95-1.25	6.35	0.50	1.25	0.21
Ala/Ala vs. Val/Val	565	652	1.09	0.93-1.28	8.80	0.27	1.02	0.31
Ala/Ala vs. Val/Val + Val/Ala	655	652	1.06	0.85-1.32	15.22	0.03[c]	0.52	0.60
Val/Ala + Ala/Ala vs. Val/Val	1781	1977	1.15	1.01-1.31	4.06	0.77	2.13	0.03
Postmenopausal women								
Val/Ala vs. Val/Val	1790	1838	1.11	0.91-1.35	17.22	0.02b	1.02	0.31
Ala/Ala vs. Val/Val	839	872	1.02	0.89-1.16	5.33	0.62	0.29	0.77
Ala/Ala vs. Val/Val + Val/Ala	839	872	1.00	0.90-1.12	9.76	0.20	0.06	0.95
Val/Ala + Ala/Ala vs. Val/Val	2629	2710	1.03	0.92-1.14	10.98	0.14	0.46	0.65

[a]Test for heterogeneity. [b]Test for overall effect. [c]A random-effects model was used when for heterogeneity the P< 0.10 value; otherwise, a fixed-effects model was used. MnSOD, manganese superoxide dismutase; OR, odds ratio.

Association between MnSOD gene polymorphism and breast cancer risk. To determine the association between the MnSOD gene polymorphism and breast cancer risk, we recalculated the ORs and their corresponding 95% CIs from the 14 studies (Fig. 1). However, there was no significant association between the MnSOD gene polymorphism and breast cancer risk analyzed in the different genetic models. As shown in Fig. 1, neither variant heterozygotes (Val/Ala) nor variant homozygotes (Ala/Ala) led to an increased risk of breast cancer compared to the wild-type Val/Val homozygotes (Val/Ala vs. Val/Val: OR, 1.04; 95% CI, 0.93-1.17; Ala/Ala vs. Val/Val: OR, 1.12; 95% CI, 0.95-1.33). Furthermore, there was no significant association between the MnSOD gene polymorphism and breast cancer risk in either the recessive model (Ala/Ala vs. Val/Ala + Val/Val: OR, 1.06; 95% CI, 0.93-1.20) or the dominant model (Val/Ala + Ala/Ala vs. Val/Val: OR, 1.06; 95% CI, 0.94-1.18).

However, in the subgroup analysis of menopausal status using the dominant model, a statistically significant increased risk of breast cancer was found in premenopausal women (Val/Ala + Ala/Ala vs. Val/Val: OR, 1.15; 95% CI, 1.01-1.31). However, there was no significant association identified in postmenopausal women among the different genetic models (Fig. 2; Table II). Additional analysis was conducted to evaluate the association of the MnSOD gene polymorphism with breast cancer risk stratified by lifestyle factors (Table III). We found that there was significant evidence demonstrating that a history of smoking tobacco was associated with an increased risk of breast cancer in carriers of the Ala allele (OR, 1.17; 95% CI, 1.02-1.34). The OR was significant for women who

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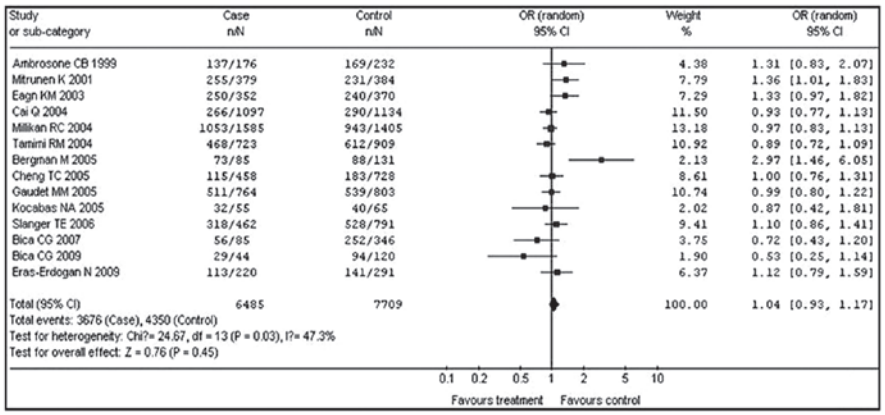

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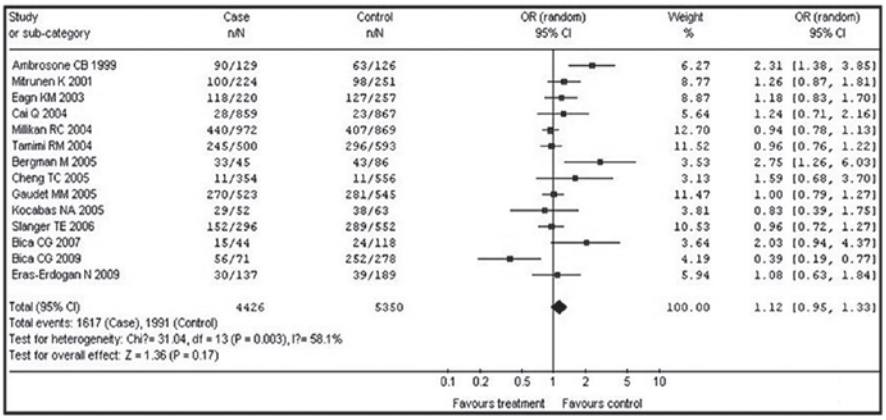

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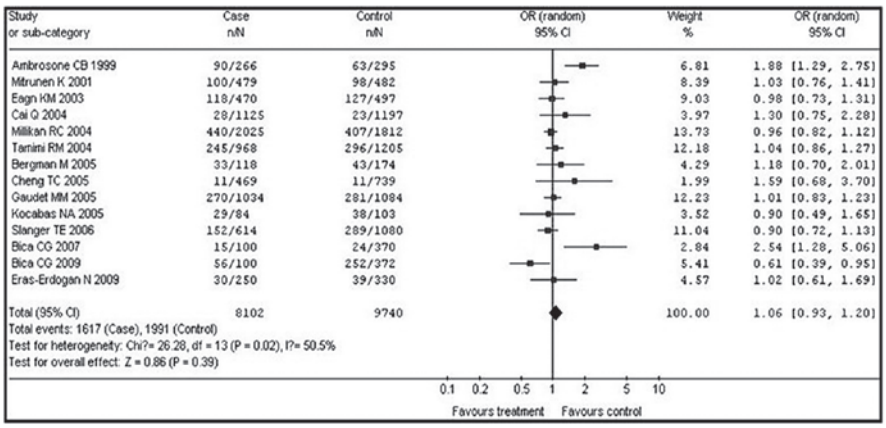

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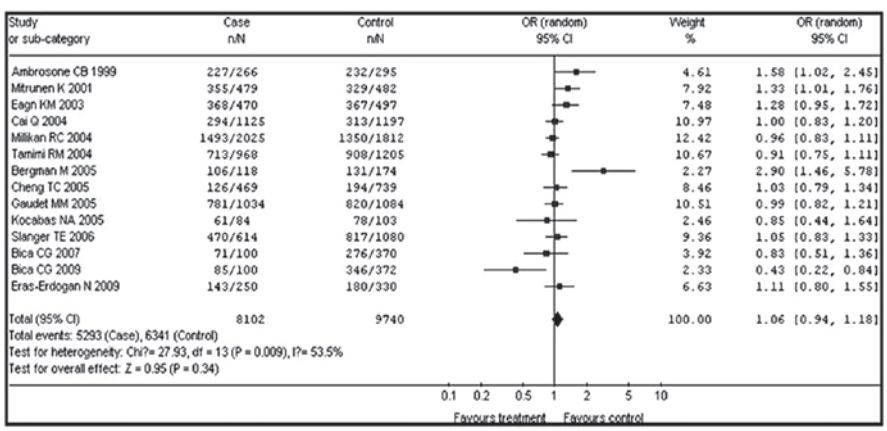

Figure 1. Forest plot odds ratios with 95% CI between the MnSOD gene polymorphism and breast cancer risk. (A) Val/Ala vs. Val/Val. (B) Ala/Ala vs. Val/Val. (C) Ala/Ala vs. Val/Val + Val/Ala. (D) Val/Ala + Ala/Ala vs. Val/Val. OR, odds ratio; MnSOD, manganese superoxide dismutase.

Table III. Correlation between MnSOD genotypes and breast cancer according to lifestyle.

	MnSOD genotypes (Val/Ala + Ala/Ala vs. Val/Val)							
Lifestyle factors	Case	Control	OR	95% CI	χ^{2a}	P-value[a]	Z-value[b]	P-value[b]
History of smoking								
Never	1156	1337	1.07	0.84-1.36	11.32	0.05[c]	0.61	0.54
Ever	1450	1798	1.17	1.02-1.34	5.00	0.08[c]	2.18	0.03
Alcohol consumption								
Never	834	821	0.97	0.80-1.18	2.32	0.31	0.27	0.78
Ever	467	942	1.42	0.89-2.26	7.47	0.02[c]	1.46	0.14
Body mass index (kg/m^2)								
<25	389	471	0.94	0.78-1.15	0.91	0.63	0.58	0.56
≥25	363	331	1.26	1.02-1.56	2.34	0.31	2.12	0.03
Oral contraceptives								
Never	376	331	1.13	0.85-1.51	0.45	0.50	0.83	0.40
Ever	226	221	1.98	1.34-2.93	1.26	0.26	3.45	0.0006

[a]Test for heterogeneity. [b]Test for overall effect. [c]A random-effects model was used when the P-value for heterogeneity was <0.10; otherwise, a fixed-effects model was used. MnSOD, manganese superoxide dismutase; OR, odds ratio.

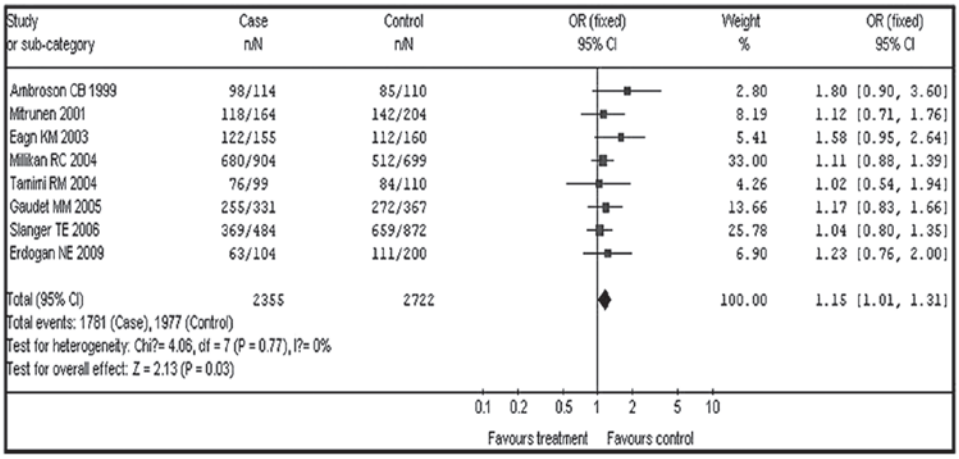

Figure 2. Forest plot odds ratios with 95% CI between the MnSOD gene polymorphism and breast cancer risk in premenopausal women for the dominant model (Val/Ala + Ala/Ala vs. Val/Val). OR, odds ratio; MnSOD, manganese superoxide dismutase.

carried the Val/Ala or Ala/Ala genotypes and had a body mass index ≥25 kg/m^2 (OR, 1.26; 95% CI, 1.02-1.56). Moreover, a significant risk of breast cancer was identified in women who carried the MnSOD Val/Ala or Ala/Ala genotypes and used oral contraceptives (OR, 1.98; 95% CI, 1.34-2.93). By contrast, there was no significant effect of alcohol consumption on breast cancer risk in women carrying the Ala allele (OR, 1.42; 95% CI, 0.89-2.26). In addition, when the controls were stratified by ethnicity, there was no significant association of the MnSOD gene polymorphism with breast cancer risk observed among Caucasian, mixed ethnicity or Asian women for any comparison model (Table IV).

Analysis of sensitivity for association of MnSOD gene polymorphism with breast cancer risk. To analyze the sensitivity of the association between the MnSOD gene polymorphism and breast cancer risk, we identified 4 studies (12,19,21,22) that did not follow the Hardy-Weinberg equilibrium in the distribution of the genotype among the controls. However, the significance of the corresponding recalculated ORs and 95% CIs was not changed regardless of whether these studies were included in the analysis [co-dominant models (Val/Ala vs. Val/Val: OR, 1.01; 95% CI, 0.93-1.09; Ala/Ala vs. Val/Val: OR, 1.06; 95% CI, 0.90-1.26), recessive model (Ala/Ala vs. Val/Ala + Val/Val: OR, 1.00; 95% CI, 0.92-1.09) and dominant model (Val/Ala + Ala/Ala vs. Val/Val: OR, 1.04; 95% CI, 0.90-1.18)]. Furthermore, upon sensitivity analyses stratified by menopausal status, lifestyle factors and ethnicity, pooled estimates for all genetic models were insensitive to the removal of individual studies, and the corresponding pooled ORs were not substantially changed (data not shown), indicating that the results were statistically robust.

Table IV. Odds ratios for MnSOD genotypes and breast cancer according to ethnicity.

MnSOD genotypes	Case	Control	OR	95% CI	χ^{2a}	P-value[a]	Z-value[b]	P-value[b]
Caucasian								
Val/Ala vs. Val/Val	791	1028	1.26	0.98-1.63	8.27	0.08[c]	1.80	0.07
Ala/Ala vs. Val/Val	344	507	1.11	0.92-1.35	7.20	0.13	1.10	0.27
Ala/Ala vs. Val/Val + Val/Ala	344	507	0.97	0.83-1.13	1.20	0.88	0.41	0.68
Val/Ala + Ala/Ala vs. Val/Val	1135	1535	1.23	0.95-1.58	9.28	0.05[c]	1.58	0.11
Mixed								
Val/Ala vs. Val/Val	2504	2849	0.98	0.89-1.08	9.83	0.13	0.44	0.66
Ala/Ala vs. Val/Val	1234	1450	1.08	0.84-1.38	21.95	0.001[c]	0.61	0.55
Ala/Ala vs. Val/Val + Val/Ala	1234	1450	1.08	0.89-1.32	22.84	0.0009[c]	0.79	0.43
Val/Ala + Ala/Ala vs. Val/Val	3738	4299	0.99	0.85-1.17	14.41	0.03[c]	0.07	0.95
Asian								
Val/Ala vs. Val/Val	381	473	0.95	0.82-1.12	0.17	0.68	0.60	0.50
Ala/Ala vs. Val/Val	39	43	1.34	0.84-2.14	0.22	0.64	1.22	0.22
Ala/Ala vs. Val/Val + Val/Ala	39	43	1.38	0.87-2.20	0.15	0.70	1.36	0.17
Val/Ala + Ala/Ala vs. Val/Val	420	507	1.01	0.87-1.17	0.04	0.84	0.13	0.90

[a]Test for heterogeneity. [b]Test for overall effect. [c]A random-effects model was used when the P<0.10 for heterogeneity; otherwise, a fixed-effects model was used. MnSOD, manganese superoxide dismutase; OR, odds ratio.

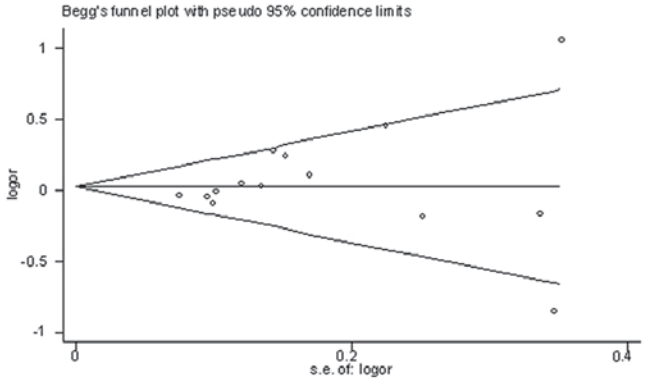

Figure 3. Funnel plot for odds ratios of dominant model in overall association.

Analysis of bias for the published data. Next, we performed statistical analysis for any bias produced in these publications and the shapes of the Begg's funnel plots demonstrated no evident asymmetry for any of the different models in the overall analysis (Fig. 3). The results of Egger's test also indicated no evidence of publication bias in any comparison model (P=0.39 for Val/Ala vs. Val/Val, P=0.37 for Ala/Ala vs. Val/Val, P=0.56 for Ala/Ala vs. Val/Ala + Val/Val, P=0.32 for Val/Ala + Ala/Ala vs. Val/Val). Furthermore, the Begg's funnel plots and Egger's test did not reveal any evidence of asymmetry in the 3 other subgroups (data not shown).

Discussion

In the present study, we performed a meta-analysis of 14 published studies in order to determine the association between the MnSOD gene polymorphism and breast cancer risk. Our data demonstrated that there was no significant association between polymorphisms of the MnSOD gene and breast cancer risk. However in subgroup analyses, the risk was significantly increased in premenopausal women with the dominant model of the MnSOD gene polymorphism. It was identified that there was a statistically significant increased risk in women with the Ala allele of the MnSOD gene who had a history of smoking tobacco, had a higher body mass index or used oral contraceptives. By contrast, there was no significant association between breast cancer risk and the dominant model of the MnSOD gene polymorphism and alcohol consumption or ethnicity groups. In conclusion, the present study demonstrates no statistically significant association between the MnSOD gene polymorphism and breast cancer susceptibility. In subgroup analyses, the MnSOD gene polymorphism increased breast cancer risk in premenopausal women with particular lifestyles. Future studies should focus on the function of the MnSOD protein in breast cancer.

MnSOD is a mitochondrial enzyme which converts the superoxide radical O_2- into H_2O_2, and then subsequently into water and oxygen under the combined action of glutathione peroxidase and catalase. Thus, MnSOD is a signficant antioxidant enzyme in human cells. Previous studies have demonstrated that low levels of MnSOD expression lead to an increase in reactive oxygen species (ROS) in the mitochondria. These can damage DNA, proteins and lipids, and also increase the instability of the genome, causing the transformation of normal cells (24,25). Other studies have revealed that MnSOD expression is absent or reduced in breast (26), prostate (27) or lung (28) cancers. These studies indicate that the alteration of MnSOD expression in different types of cancer. However, the current study does not demonstrate any statistical association between the MnSOD gene polymorphism and breast cancer risk, although data from subgroup analyses revealed a slight association between the polymorphism and breast cancer risk

in premenopausal women with particular lifestyles. The reason for this is unclear, although previous data have demonstrated that the MnSOD gene polymorphism affects the normal localization of the MnSOD protein in the mitochondria, and therefore, reduces the MnSOD enzymatic activity and leads to a reduced ability to remove ROS, increasing tumor formation (29). Zhao *et al* (30) demonstrated that ROS play a role in regulating cell proliferation concerning signal transduction pathways. Therefore, a high number of the Ala variant of the MnSOD gene may result in a high risk of developing breast cancer, particularly when cells are exposed to higher ROS stress. One explanation for the lack of an association between the MnSOD gene polymorphism and breast cancer risk could be that other mitochondrial enzymes may compensate the activity of MnSOD to maintain normal levels of ROS activity in the cells. However, the effects of MnSOD mutations on breast cancer risk and the mechanistic basis for these effects requires further examination.

Meta-analysis is a powerful and useful tool to combine the data from several studies that address a set of related research hypotheses, e.g., the study of gene polymorphisms for cancer risk. The advantage of this analysis is it's ability to combine several studies to achieve a high number of cases, and therefore, be less affected by local findings than single studies. Meta-analysis also makes it possible to identify publication bias, help researchers to investigate a large number of individuals and estimate the effect of a certain genetic factor on cancer risk. Although a number of studies have reported an important role of MnSOD gene polymorphism in breast cancer risk, this meta-analysis did not demonstrate any association.

However, in the subgroup analyses, we found a significantly increased risk in premenopausal women with the Ala variant of the MnSOD gene. However, it is unclear why the Ala variant of MnSOD gene contributed to breast cancer risk among premenopausal women. Previous studies have revealed that in other malignancies MnSOD overexpression reduced levels of ROS and in turn decreased the doubling time of osteosarcoma SaOS2 cells and plating efficiency (31,32). MnSOD overexpression inhibited the malignant phenotype and growth of SV40 human fibroblasts (33), malignant melanoma tumors (34) and gliomas (35). These data may indirectly suggest that ROS may play a more significant role in breast carcinogenesis among younger women than that of postmenopausal women. Furthermore, although Slanger *et al* (20) did not identify a difference in ORs of MnSOD Ala/Ala and Ala/Val genotypes in cigarette smokers, this meta-analysis did reveal that women who carried at least one copy of the Ala allele and who smoked were at higher risk of developing breast cancer than that of non-carriers. Mitrunen *et al* (5) demonstrated that women who consumed alcohol and who had the Val/Ala or Ala/Ala genotype had a 2.2-fold greater risk of developing breast cancer than those with the Val/Val genotype. Nevertheless, our results did not reveal a significant change in breast cancer risk by MnSOD genotype with alcohol consumption, which is consistent with other studies (13-15,18,20). Similarly, ORs based on three parallel studies (6,13,23) identified a significant association with breast cancer risk for women with a body mass index ≥25 kg/m^2. In addition, by combining data from three similar studies (5,13,14), we revealed a higher risk of breast cancer among women who used oral contraceptives and who carried at least one copy of the Ala allele. Mitrunen *et al* (5) and Egan *et al* (13) reported markedly elevated ORs for the MnSOD genotype in women who used oral contraceptives. We did not find any significant associations between the MnSOD gene polymorphism and breast cancer risk by different ethnic groups of women with at least 1 copy of the Ala allele for any comparison model.

However, when interpreting the results in the present study several limitations require consideration. Firstly, although the majority of the controls were selected from healthy individuals, specific individuals may have been affected by benign disease. Secondly, the sample sizes for a few of the subgroup analyses were still relatively small. Thirdly, our data were based on unadjusted published estimates; a more precise analysis should be performed to allow for adjustment by other co-variates, including age, family history, environmental factors and other lifestyle factors. Despite these limitations, our meta-analysis also contains strengths, e.g., i) a substantial number of cases and controls were pooled from different studies, resulting in 17,842 subjects, which significantly increased the statistical power of these analyses; ii) no publication biases were detected, indicating that the pooled results may be unbiased.

In conclusion, this meta-analysis suggests that the Ala allele of the MnSOD gene is associated with breast cancer risk among premenopausal women, particularly when combined with certain lifestyle factors. However, it remains necessary to conduct large-sample studies on gene-gene and gene-environment interactions in breast cancer susceptibility by using standardized unbiased genotyping methods, homogeneous breast cancer patients, and sufficiently matched controls to elucidate the effect of the MnSOD polymorphism on breast cancer risk.

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