There is no relationship between SOD2 Val-16Ala polymorphism and breast cancer risk or survival

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Abstract. Breast cancer is the most common diagnosed cancer among females worldwide. Superoxide dismutase 2 (SOD2), an antioxidant enzyme, may break the balance between the oxidant and antioxidant system to induce various diseases. The present study aimed to clarify the association between the SOD2 Val-16Ala polymorphism and breast cancer risk or survival. Thus, a meta-analysis of the relevant articles retrieved from PubMed and EMBASE databases was conducted to illuminate the association with odd ratios (ORs) or hazards ratios (HRs). A total of 26 eligible publications (n=38,008) were available in risk analysis and eight publications (n=5,746) in survival analysis. The results demonstrated a marginal association between breast cancer risk and SOD2 polymorphism in Caucasian patients [TT vs. CT + CC: (OR, 0.94; 95% confidence interval (CI), 0.88-1.00)]. However, no other positive results were observed in risk and survival of breast cancer in the whole study [T vs. C: (OR, 0.99; 95% CI, 0.96-1.02); CT vs. CC: (OR, 1.00; 95% CI, 0.95-1.05); TT vs. CC: (OR, 0.98; 95% CI, 0.92-1.05); TT vs. CT + CC: (OR, 1.00; 95% CI, 0.95-1.05); CT + TT vs. CC: (OR, 0.99; 95% CI, 0.95-1.05)]. The present meta-analysis indicated that there was no significant relationship between SOD2 Val-16Ala polymorphism and breast cancer risk or survival, although in Caucasian patients, the SOD2 TT genotype may marginally decrease the risk of breast cancer in comparison to the CT + CC genotype.

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Abbreviations: SOD, superoxide dismutase; OR, odds ratio; HR, hazards ratio; ROS, reactive oxygen species; CI, confidence interval; SNP, single nucleotide polymorphism

Key words: SOD2, Val-16Ala, breast cancer, risk, survival, meta-analysis

Introduction

Breast cancer, diagnosed in ~1.7 million patients and being the cause of 521,900 mortalities in 2012, is the most common diagnosed cancer and the most notable cause of cancer mortality among females worldwide (1). Various factors have an impact in the incidence of breast cancer, such as family history, gene susceptibility, hormone, diet, lifestyle factors and environmental exposures (2-6).

Increasing research has identified a significant effect of reactive oxygen species (ROS) in breast cancer etiology (7-12). ROS may induce oxidative stress, resulting in DNA sequence changes and damage, such as mutations, rearrangements and DNA strand breaks. ROS may also lead to damage to lipids, proteins, membranes and mitochondria (8,13). In humans, various antioxidant actions may balance the effect of ROS, including antioxidant enzymes and antioxidant agents. Antioxidant enzymes predominantly include glutathione peroxidase, catalase and, most importantly, superoxide dismutase (SOD) (7,8).

There are three types of SOD, which are cytosolic Cu-ZnSOD (SOD1), extracellular Cu-ZnSOD (SOD3) and mitochondrial SOD (SOD2; MnSOD). SOD2 is mostly produced in mitochondria, having a vital effect on balancing mitochondrial oxidant stress and antioxidant defense (14). SOD2 gene, located on 6q25 of chromosome 6, encodes SOD2, whose expression is highly regulated at transcription, translation and posttranslational levels (15-17). SOD2, a polymorphic enzyme, has several structural mutations and single nucleotide polymorphisms (SNPs). The most common SNP is rs4880 SNP, also called rs1799725 SNP, which is a T to C substitution in exon 2, changing the amino acid codon at position 16 from valine to alanine, known as the SOD2 Val-16Ala genotype and also as Ala-9Val as the SNP is 9 amino acids upstream of the cleavage site (18-20).

According to the latest research, various studies have demonstrated an association between the polymorphism of SOD2 and disease, particularly between SOD2 Val-16Ala and cancer. Several meta-analyses have demonstrated that the SOD2 Val-16Ala SNP polymorphism increases susceptibility to various types of cancer, such as prostate cancer (21-23), lung cancer, colorectal cancer and non-Hodgkin lymphoma (24). However, referring to breast cancer, from 1999 to present, there have been many studies and cohorts to investigate the

Table I. Characteristics of studies included in analysis of association between superoxide dismutase 2 Val-16Ala polymorphism (rs4880) and breast cancer risk or survival.

Analysis	Authors, year	Ethnicity	Source of controls	Genotyping method	Case, n			Control, n			(Refs.)
					CC	CT	TT	CC	CT	TT	
Risk analysis	Ambrosone *et al*, 1999	Caucasian	PB	PCR-RFLP	90	137	39	63	169	63	(25)
	Mitrunen *et al*, 2001	Caucasian	PB	PCR-RFLP	100	255	124	98	231	153	(26)
	Egan *et al*, 2003	Caucasian	PB	PCR	118	250	102	127	240	130	(27)
	Cai *et al*, 2004	Asian	PB	PCR-RFLP	28	266	831	23	290	884	(28)
	Knight *et al*, 2004	Mixed	PB	PCR	105	187	107	87	195	90	(29)
	Millikan *et al* (1), 2004	African American	PB	TaqMan	129	372	259	124	357	196	(30)
	Millikan *et al* (2), 2004	Caucasian	PB	TaqMan	311	681	273	283	586	266	(30)
	Tamimi *et al*, 2004	Mixed	HB	PCR	245	468	255	296	612	297	(31)
	Bergman *et al*, 2005	Caucasian	NA	PCR	12	73	33	43	88	43	(32)
	Cheng *et al*, 2005	Asian	HB	PCR	11	115	343	11	183	545	(33)
	Gaudet *et al*, 2005	Mixed	PB	PCR	270	511	253	281	539	264	(34)
	Kocabaş *et al*, 2005	Caucasian	NA	PCR-RFLP	18	38	28	38	39	26	(35)
	Cebrian *et al*, 2006	Caucasian	PB	TaqMan and PCR	1,297	2,237	940	1,328	2,290	962	(36)
	Silva *et al*, 2006	Caucasian	HB	PCR-RFLP	59	146	36	99	276	82	(37)
	Slanger *et al*, 2006	Caucasian	PB	TaqMan and PCR	152	318	144	289	528	263	(38)
	Justenhoven *et al*, 2008	Caucasian	PB	MALDI-TOF MS	133	312	159	145	313	163	(39)
	Bica *et al*, 2009	Mixed	PB	PCR	14	51	24	26	252	94	(40)
	Eras-Erdogan *et al*, 2009	Caucasian	PB	PCR-RFLP	30	113	107	39	141	150	(41)
	Kostrykina *et al*, 2009	Caucasian	HB	TaqMan and PCR	119	233	123	90	183	103	(42)
	Ermolenko *et al*, 2010	Asian	HB	PCR	239	454	228	104	235	121	(43)
	Kim *et al*, 2010	Asian	HB	TaqMan and PCR	4	66	234	7	90	279	(44)
	Cerne *et al*, 2011	Caucasian	HB	PCR-RFLP	143	269	118	71	134	65	(45)
	Tsai *et al*, 2012	Asian	HB	PCR		68	192		86	138	(46)
	Attatippaholkun and Wikainapakul, 2013	Asian	NA	PCR	5	54	82	3	48	84	(47)
	Méplan *et al*, 2013	Danish	PB	PCR	226	485	228	227	494	237	(48)
	Jablonska *et al*, 2015	Caucasian	HB	TaqMan and PCR	29	75	32	50	92	41	(49)
	Kakkoura *et al*, 2015	Danish	PB	TaqMan	212	512	342	252	550	343	(50)
Survival analysis	Ambrosone *et al*, 2005	Caucasian	NA	PCR-RFLP	267			-			(58)
	Udler *et al*, 2007	Caucasian	NA	TaqMan	4,181			-			(59)
	Bewick *et al*, 2008	Caucasian	NA	DNA MiniKit	95			-			(60)
	Glynn *et al*, 2009	Caucasian, mixed	NA	TaqMan and PCR	322			-			(61)
	Yao *et al*, 2010	Mixed	NA	MALDI-TOF	432			-			(62)
	Hubackova *et al*, 2012	Caucasian	NA	TaqMan	59			-			(63)

Table I. Continued.

Analysis	Authors, year	Ethnicity	Source of controls	Genotyping method	Case, n			Control, n			(Refs.)
					CC	CT	TT	CC	CT	TT	
	Cronin-Fenton *et al*, 2014	Danish	NA	TaqMan	326			-			(64)
	Tengström *et al*, 2014	Caucasian	NA	PCR-RFLP	64			-			(65)

PB, population-based; HB, hospital-based; NA, not available; PCR, polymerase chain reaction; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; MALDI-TOF, matrix-assisted laser desorption/ionization-time of flight mass spectrometry.

Figure 1. Flow chart of the literature search and selection of included studies.

relationship between SOD2 Val-16Ala polymorphism and breast cancer; however, these studies cannot reach an agreement and have drawn some conflicting conclusions (25-50). In 2008, the first meta-analysis to investigate the association between Val-16Ala and breast cancer was conducted, with 13 publications including a total of 7,366 cases and 9,102 controls; however, it indicated no overall association with the Val-16Ala polymorphism (51). Subsequently, from 2010-2012, four meta-analyses also demonstrated similar negative effects (52-55). Presently, due to increased individual studies and larger sample sizes, a more accurate estimation may be obtained to judge this association. Thus, an updated meta-analysis was performed to investigate whether SOD2 Val-16Ala polymorphism is a risk factor and/or prognostic factor for breast cancer.

Materials and methods

Search strategy. A comprehensive strategy was used to search PubMed (ncbi.nlm.nih.gov/pubmed) or MEDLINE (medline.com) and EMBASE (embase.com) to obtain the relevant publications about the association between breast cancer risk and SOD2 gene polymorphism. The search terms were 'superoxide dismutase 2,' 'SOD2,' 'MnSOD,' 'ala9val,' 'val16ala,' 'breast cancer,' 'breast carcinoma,' 'breast tumor,' 'breast neoplasm,' 'mammary cancer,' 'polymorphism,' 'mutation' and 'variant,' alone or in combination. The last updated data was October 5, 2016, and with no restriction of the post time. Additional publications listed in references were also retrieved by a computer-aided manual search to gain more information about this field. Furthermore, only publications in the English language were included in the meta-analysis.

Table II. Summary of the included studies in the risk analysis regarding the ethnicity of patients.

Polymorphisms	Ethnicity	Studies, n	Participants, n	Odds ratio	95% confidence interval	P-value	Egger's value	Begg's value
T vs. C	Caucasian	14	20,589	0.98	0.94-1.02	0.357		
	Asian	5	5,867	0.95	0.87-1.05	0.319		
	Danish	2	4,108	1.04	0.95-1.13	0.388		
	African American	1	1,437	1.14	0.98-1.32	0.080		
	Mixed	4	55,23	1.00	0.93-1.08	0.971		
	Total	26	37,524	0.99	0.96-1.02	0.702	0.612	0.865
CT vs. CC	Caucasian	14	15,821	1.03	0.97-1.10	0.351		
	Asian	5	2,236	0.82	0.65-1.03	0.089		
	Danish	2	2,958	1.05	0.89-1.22	0.574		
	African American	1	982	1.00	0.75-1.33	0.991		
	Mixed	4	4,139	0.90	0.79-1.03	0.136		
	Total	26	26,136	1.00	0.95-1.05	0.961	0.523	0.810
TT vs. CC	Caucasian	14	10,142	0.97	0.89- 1.05	0.409		
	Asian	5	4,066	0.80	0.62-1.03	0.090		
	Danish	2	2,067	1.08	0.91-1.29	0.386		
	African American	1	708	1.27	0.93-1.73	0.129		
	Mixed	4	2,708	0.99	0.85-1.15	0.850		
	Total	26	19,691	0.98	0.92-1.05	0.601	0.440	0.514
TT vs. CT + CC	Caucasian	14	20,589	0.94	0.88-1.00	0.059		
	Asian	6	6,351	1.03	0.92-1.16	0.558		
	Danish	2	4,108	1.05	0.91-1.20	0.505		
	African American	1	1,437	1.27	1.01-1.59	0.037		
	Mixed	4	5,523	1.07	0.94-1.21	0.312		
	Total	27	38,008	1.00	0.95-1.05	0.954	0.755	0.904
CT + TT vs. CC	Caucasian	14	21,068	1.01	0.95-1.08	0.742		
	Asian	5	5,867	0.82	0.65-1.02	0.073		
	Danish	2	4,108	1.06	0.91-1.23	0.456		
	African American	1	1,437	1.10	0.84-1.44	0.505		
	Mixed	4	5,523	0.93	0.82-1.06	0.280		
	Total	26	37,524	0.99	0.95-1.05	0.839	0.737	0.261

Inclusion and exclusion criteria. In the present meta-analysis, eligible publications had to be randomized controlled trials, cohort studies or case-control studies that investigated the association between breast cancer and SOD2 gene polymorphism. The publications meeting the following inclusion criteria were retained: i) The cases were diagnosed with breast cancer that was pathologically confirmed and the controls were free of breast cancer; ii) had sufficient data, such as size of the sample, alleles and genotypes, to calculate the odd ratios (ORs) or hazards ratios (HRs) with 95% confidence intervals (95% CIs); and iii) preferably used subgroup analysis. The exclusion criteria were as follows: i) Studies had no control individuals; ii) studies were about the activity of the SOD2 enzyme; iii) the study was not about the rs4880 or rs1799725 SNP. If there were some duplicated publications, the latest studies were retained. If several different publications had the same patient source, the studies with the largest number of individuals were reserved. Furthermore, two cooperators reviewed the publications independently to ensure that the appropriate studies were chosen.

Data extraction. Available data were extracted and collected by two investigators independently from all of the included publications, following the same standard protocol. If there were any inconsistencies between the data obtained by the two reviewers, the problem was solved through a careful discussion. If an agreement could not be reached, a third reviewer would take part in this to make everyone satisfied. Data information from the publications were about the first author, publish year, ethnicity, sources of controls, genotyping methods, total number of cases and controls, distribution of alleles and genotypes and the HR with 95% confidence interval (CI) of relative polymorphism.

Statistical analysis. The crude ORs with 95% CIs for alleles and genotypes were used to estimate the association between breast cancer risk and SOD2 gene polymorphism, and HRs with 95% CIs were used for survival analysis. The pooled ORs and HRs were calculated for the genotypes of T vs. C, CT vs. CC, TT vs. CC, TT vs. CT + CC and CT + TT vs. CC,

Table III. Summary of the included studies in the risk analysis regarding the menopausal status of patients.

Polymorphisms	Menopausal status	Studies, n	Participants, n	Odds ratio	95% confidence interval	P-value	Egger's value	Begg's value
T vs. C	Premenopausal	8	3,962	0.93	0.85-1.01	0.095	0.138	0.101
	Postmenopausal	9	6,182	0.97	0.90-1.04	0.417	0.404	0.341
CT vs. CC	Premenopausal	8	2,924	0.94	0.80-1.10	0.419	0.621	0.566
	Postmenopausal	9	4,604	0.99	0.87-1.12	0.846	0.404	0.225
TT vs. CC	Premenopausal	8	1,993	0.87	0.72-1.04	0.136	0.458	0.204
	Postmenopausal	9	3,110	0.94	0.82-1.09	0.433	0.144	0.128
TT vs. CT + CC	Premenopausal	8	3,962	0.89	0.77-1.04	0.135	0.138	0.227
	Postmenopausal	9	6,182	0.95	0.85-1.07	0.389	0.095	0.029
CT + TT vs. CC	Premenopausal	9	5,475	0.89	0.77-1.03	0.121	0.144	0.242
	Postmenopausal	10	6,982	0.98	0.87-1.10	0.706	0.929	0.381

Table IV. Summary of the included studies in the survival analysis.

Model	Variables	Studies, n	Hazard ratio (95% confidence interval)	P-value	I-squared, %
Dominant	CC	3	Reference	0.001	51.6
	CT/TT		0.67 (0.29-1.06)		
Recessive	CC/CT	2	Reference	0.03	81.4
	TT		1.00 (0.10-1.90)		
Homozygote	CC	4	Reference	0.001	86.5
	TT		1.06 (0.45-1.68)		
Heterozygote	CC	2	Reference	<0.001	0.0
	CT		1.21 (0.96-1.45)		
Allelic	C	1	Reference	-	-
	T		1.06 (0.94-1.20)		

which assumed the allele contrast model, two co-dominant models, one recessive model and one dominant model of the SOD2 rs4880 variant, respectively. Subgroup analyses were also conducted according to ethnicity and menopausal status using the ORs.

To assess the heterogeneity of the publications, Chi-square (X^2) tests were carried out. At first, if I-squared ≤50%, the ORs with 95% CI were calculated using the fixed effects model (Mantel-Haenszel) for meta-analysis (56). If I-squared >50%, the fixed effects model could not be applied, and so the random effects model (DerSimonian and Laird) was used (57). Conventionally, pooled OR ≠1 revealed the existent association between breast cancer risk and SOD2 gene polymorphism, and pooled HR ≠1 revealed association between cancer survival and polymorphism. If 95% CI did not overlap 1, P<0.05 was considered to indicate a statistically significant difference. The pooled ORs and HRs with 95% CI were presented in the form of forest plots, using Stata version 12.0 (StataCorp LP, College Station, TX, USA).

To assess potential publication bias, graphical funnel plots were used, and Egger's and Begg's linear regression methods were also utilized to estimate the funnel plot asymmetry. An asymmetric funnel plot demonstrated possible publication bias and P<0.05 was considered to indicate a statistically significant publication bias.

Results

Characteristics of the studies. Through the primary search algorithm, 189 publications were acquired, which consisted of 79 studies from PubMed or MEDLINE and 110 studies from EMBASE. Only 45 candidate studies were retrieved for more detailed evaluation. By reading the full texts, 11 studies were out of scope as they did not satisfy the inclusion criteria (one meeting abstract, three animal experiments or cell cultures and seven without available data). Finally, a total of 34 publications were available for the present meta-analysis, 26 for risk analysis (25-50) and eight for survival analysis (58-65). The flow chart of study search and inclusion was demonstrated in Fig. 1.

For the risk analysis, the 26 included studies were published between 1999 and 2015 and consisted of 18,481 cases and 19,527 controls. The total sample size of the patients was 38,008, ranging from 187-9054 per cohort. As an article had

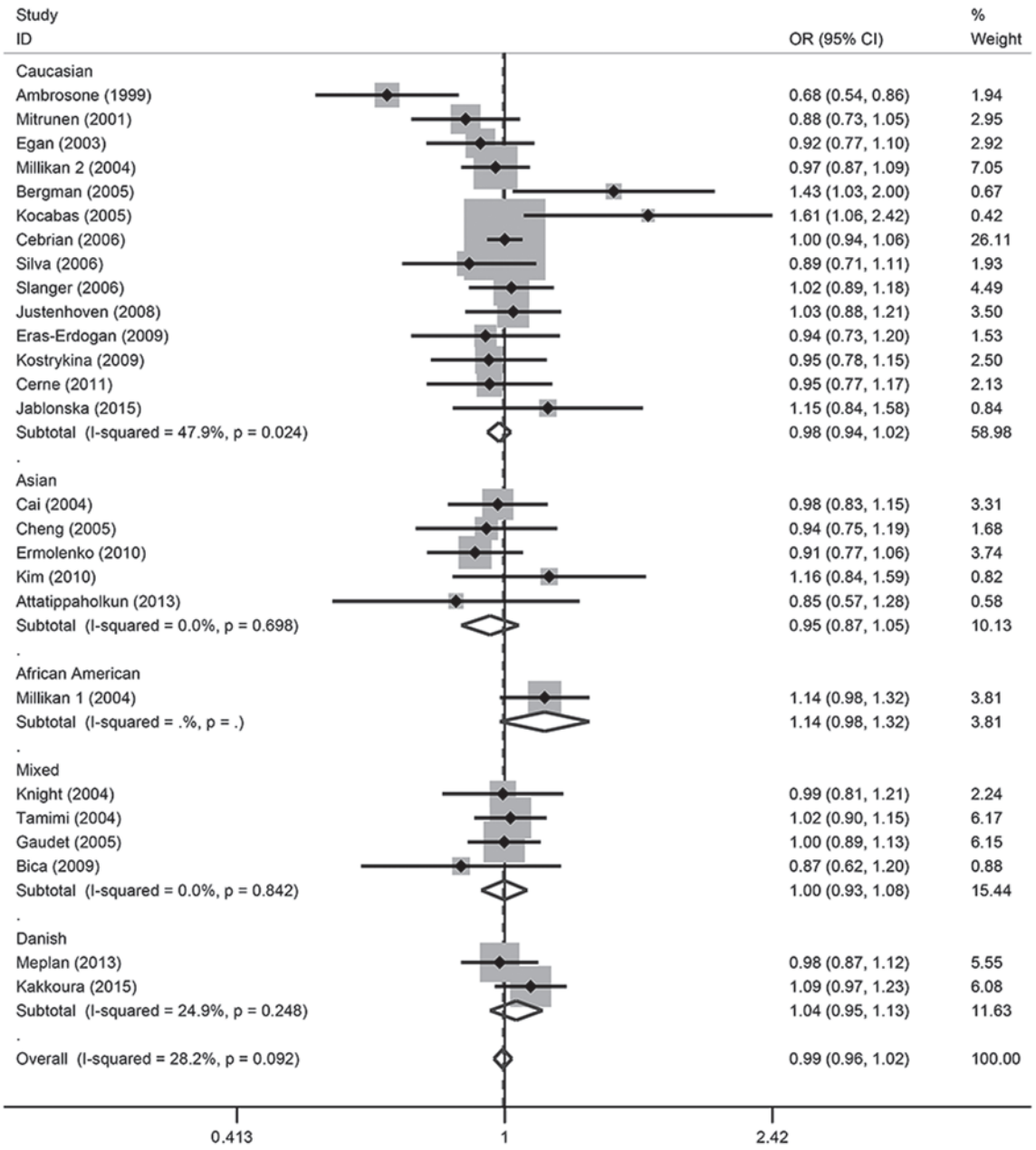

Figure 2. Forrest plots of OR for T vs. C. OR, odd ratio; CI, confidence interval.

two cohorts and ethnicities (30), there were 27 cohorts. A total of 14 were conducted in Caucasian patients, five in Asian patients, two in Danish patients, one in African American patients and four in mixed races. All of the case patients were confirmed by histological or pathological methods. The controls were healthy or free of breast cancer, and matched for age, ethnicity or area to cases. The majority of the cohorts examined the blood sample using polymerase chain reaction (PCR) genotyping methods. For the survival analysis, the included eight studies contained 5746 participants, published between 2005 and 2014. The primary characteristics of included cohorts were summarized in Table I.

Data synthesis

SOD2 polymorphism and risk analysis. To estimate the association between breast cancer risk and SOD2 gene polymorphism, the ORs and their corresponding 95% CIs were reconstructed from the 27 cohorts. As demonstrated in Table II and Fig. 2, in all of the cohorts included in the present analysis, there were no significant relationships found in any of the genetic models. For the allele contrast, the wild-type allele did not increase or decrease the risk of breast cancer compared with the variant allele (T vs. C: OR, 0.99; 95% CI, 0.96-1.02). There was no association between breast cancer risk and SOD2 gene polymorphism in two co-dominant models (CT vs. CC: OR, 1.00; 95% CI, 0.95-1.05; TT vs. CC: OR, 0.98; 95% CI, 0.92-1.05), one recessive model (TT vs. CT + CC: OR, 1.07; 95% CI, 0.94-1.21) or one dominant model (CT + TT vs. CC: OR, 0.99; 95% CI, 0.95-1.05).

In the subgroup analysis, there was also no significant association detected in different ethnicities or menopausal status, except for TT vs. CT + CC in Caucasian patients,

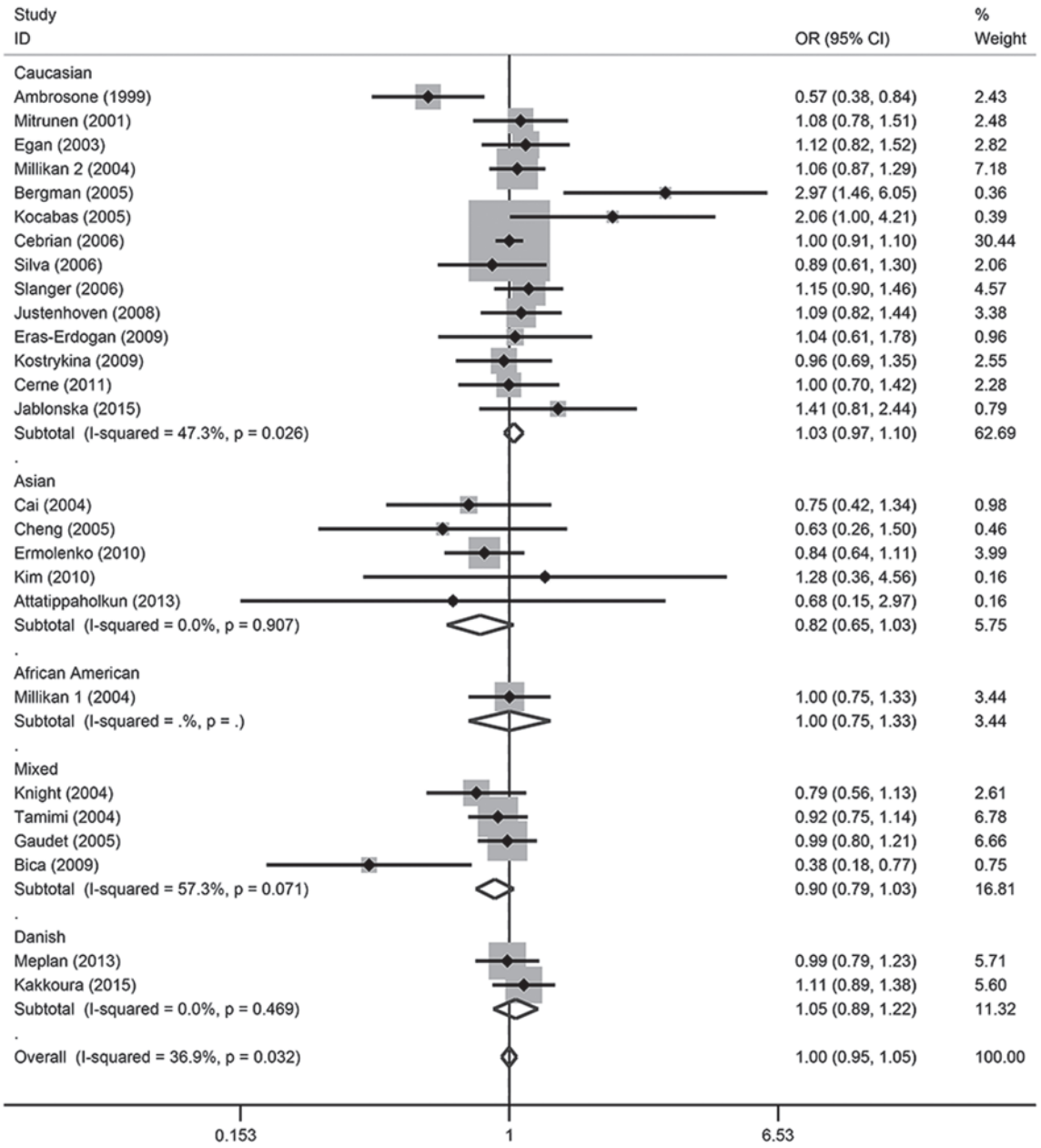

Figure 3. Forrest plots of OR for CT vs. CC. OR, odd ratio; CI, confidence interval.

which demonstrated a marginal association (OR, 0.94; 95% CI, 0.88-1.00); however, this association was not significant (P>0.05). These details were listed in Tables II and III, and Figs. 2-6.

SOD2 polymorphism and survival analysis. No significant relationship was detected from the present meta-analysis, which included eight studies that investigated the association between SOD2 polymorphism and breast cancer overall survival (OS). There were no significant differences between patients with the T carrier and CC genotype (CT + TT vs. CC: HR, 0.67; 95% CI, 0.29-1.06), or between TT and CC + CT genotype (TT vs. CT + CC: HR, 1.00; 95% CI, 0.10-1.90). When compared with the CC genotype, it was not demonstrated that TT or CT genotypes had a better outcome (TT vs. CC: HR, 1.06; 95% CI, 0.45-1.68; CT vs. CC: HR, 1.21; 95% CI, 0.96-1.45). In addition, due to the limited number of included studies, only one cohort compared the T allele with C allele, so the OS could not be evaluated (Table IV).

Sensitivity analysis. Sensitivity analyses were carried out to determine whether there was association between breast cancer risk and SOD2 gene polymorphism. The inclusion criteria were altered to fit the Hardy-Weinberg equilibrium (HWE) and nine cohorts were excluded due to not meeting the HWE in the distribution of the genotype among the controls. However, all the ORs and corresponding 95% CIs were not substantially altered, suggesting that the results were statistically robust (data not shown).

Bias analysis. To assess the potential publication bias, Egger's and Begg's linear regression methods were applied for this analysis. As demonstrated in Tables II and III, there was no evidence of statistically significant differences among the

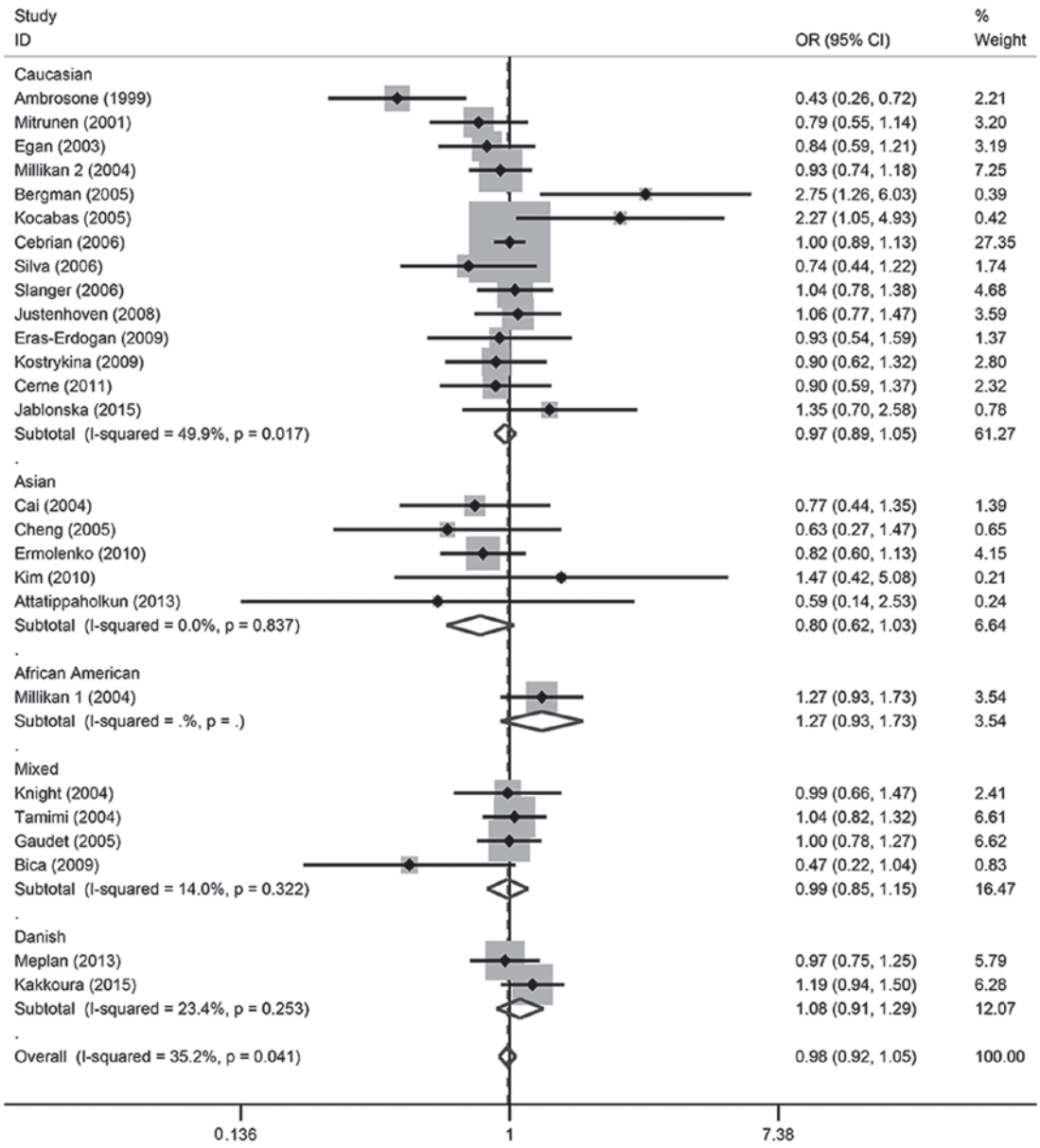

Figure 4. Forrest plots of OR for TT vs. CC. OR, odd ratio; CI, confidence interval.

whole analysis, except for the TT vs. CT + CC in the subgroup of postmenopausal status, which had a Begg's value of 0.029 but an Egger's value of 0.095 (Table III). However, this result had little impact on the present analysis.

Discussion

Due to the electron transport chain and environment exposure in mitochondrion, many ROS are formed as a by-product during cell metabolic or oxidative phosphorylation processes, such as hydrogen peroxide, superoxide anion radical and hydroxyl radical (66). As a very important part of the anti-oxidant defense system, SOD2 has a crucial role in balancing oxidant stress and ROS in mitochondria (67). In an animal experiment, a murine model with SOD2 gene deficiency demonstrated neurodegeneration, myocardial injury and perinatal death due to the impaired SOD2 activity (68). As a polymorphic enzyme, the SOD2 gene has a series of SNPs, such as Val-16Ala and Ile58Thr polymorphisms (20,69), and the SNPs in the oxidative stress-related genes have some links with cancer risk (70). In a review, several studies demonstrated that, in cancer cells, the activity and expression of SOD2 was usually lower compared with the normal cells (71). Thus, various studies have focused on illuminating the association between SOD2 polymorphism and breast cancer; however, the results have remained controversial and uncertain.

In 1999, the first research reporting the relationship between SOD2 Val-16Ala polymorphism and breast cancer in a Caucasian population was conducted by Ambrosone *et al* (25), which drew a conclusion that SOD2 had a significant role in breast cancer risk, particularly in premenopausal women. More specifically, premenopausal women with homozygous CC demonstrated a 4-fold higher risk of developing breast cancer compared with heterozygote CT or homozygous TT (OR, 4.3; 95% CI, 1.7-10.8). In 2005, Bergman *et al* (32) conducted a case-control study that included 118 women with early onset

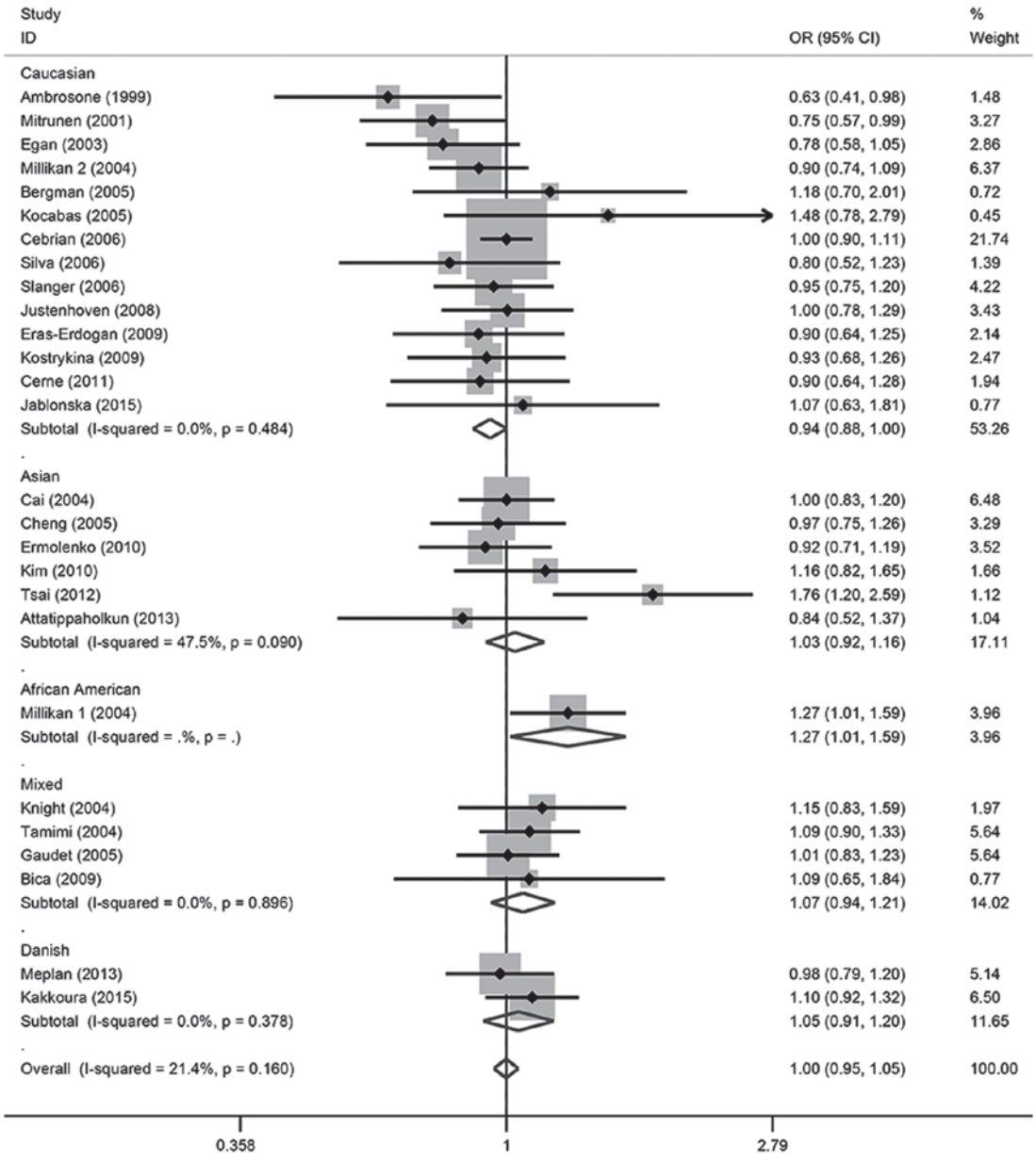

Figure 5. Forrest plots of OR for TT vs. CT + CC. OR, odd ratio; CI, confidence interval.

breast cancer and 174 age-matched controls, which indicated that SOD2 TT and CT genotype could increase the prevalence rate of breast cancer (OR, 2.7; 95% CI, 2.2-5.5; OR, 3.0; 95% CI, 1.4-6.5). In the same year, Kocabaş *et al* (35) also carried out another case-control study, including 103 patients and 84 controls, demonstrating a similar result to Bergman *et al* (32); however, no significant difference about the risk of allele T and C was observed. Furthermore, the majority of other publications suggested that the SOD2 Val-16Ala could not increase or decrease breast cancer risk and survival (26-31,33,34,36-50).

It is widely accepted that meta-analyses have been the gold standard to judge the association between risk factors and diseases (72). In 2008, Bag and Bag (51) conducted the first meta-analysis investigating the association between Val-16Ala and breast cancer, with 13 publications that included 7,366 cases and 9,102 controls. Their findings indicated no overall association with the Val-16Ala polymorphism (25). Two years later, Ma *et al* (53) and Qiu *et al* (55) conducted two independent meta-analyses, respectively, and obtained the same negative conclusion. Although the analysis of Qiu *et al* (55) involved 58,448 subjects of 26,022 cases and 32,426 controls, the patient resources of several case-control studies were mixed and unavailable at present. Subsequently, two relative meta-analyses were published in 2011 and 2012, but all of the included studies and cohorts were carried out before 2009 (52,54). From 2010-2016, there were another eight studies published, and so the present meta-analysis was conducted (43-50).

The present updated meta-analysis consisted of 18,481 cases and 19,527 controls from 27 cohorts or case-control studies for risk analysis and 5,746 cancer patients from eight studies for survival analysis. It aimed to more accurately estimate and investigate the association between the SOD2 Val-16Ala polymorphism and breast cancer risk or survival. From the present analysis, a marginal association between breast cancer risk and SOD polymorphism was demonstrated in terms of TT

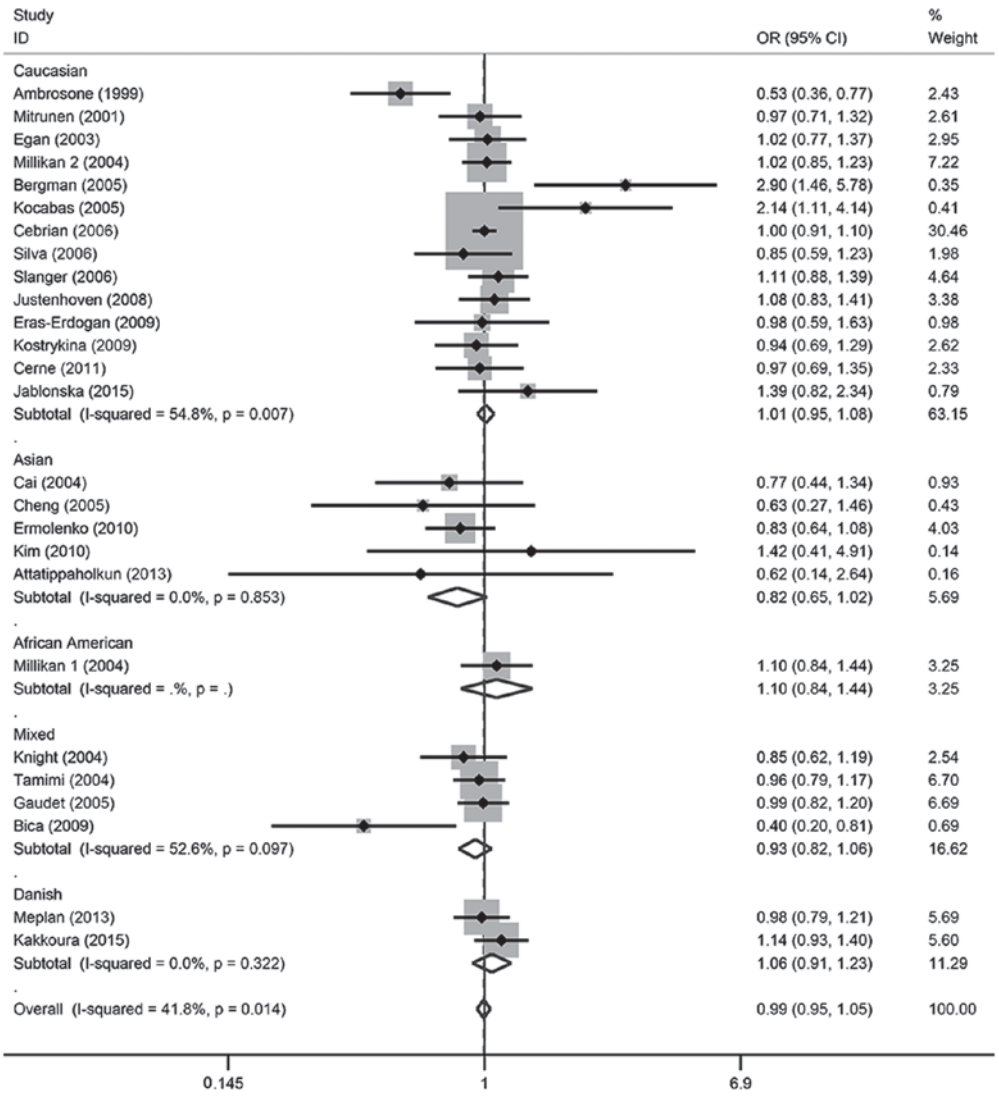

Figure 6. Forrest plots of OR for CT + TT vs. CC. OR, odd ratio; CI, confidence interval.

vs. CT + CC genotype in Caucasian patients, which means the TT genotype may slightly decrease the risk of breast cancer compared with CT + CC. However, no other positive results were observed in the risk and survival analysis of breast cancer, which demonstrated no direct relationship between SOD2 polymorphism and breast cancer.

There were some limitations in the present meta-analysis. Given these, it is necessary to carefully analyze some considerable issues that may affect the study conclusion, to obtain a more cautious result. First, the quantity of the included studies cannot satisfy the condition of meta-analysis for the survival analysis, although every effort was made to search carefully on the PubMed and EMBASE databases with the various combinations of search terms by a computer-aided bibliographic technology. Therefore, the power about the SOD2 polymorphism and breast cancer survival may not be sufficient to make a statistical statement, and the conclusion is also limited, which requires more trails and larger sample sizes to clarify the relationship. Second, the included participants coming from hospital or population may have had some underlying diseases, which may influence the health of participants and the conclusion of the present study. For example, three studies had unclear expression about the underlying diseases in the controls (29,35,43). There was no evidence of statistically significant publication bias according to the graphical funnel plots, and Egger's and Begg's linear regression methods; however, the potential bias cannot be ignored, and this may have affected the final conclusion. Only English publications were available and included in this meta-analysis and the rest were out of scope because the investigators could not understand the language. Last but not least, several different genotyping methods were applied in the studies used, such as PCR-restriction fragment length polymorphism, TaqMan and matrix-assisted laser desorption/ionization-time of flight mass spectrometry, which maybe make a difference to the present conclusion.

In conclusion, the present meta-analysis indicated that there was no significant relationship between SOD2 Val-16Ala polymorphism and breast cancer risk or survival, although in Caucasian patients, the SOD2 TT genotype may marginally decrease the risk of breast cancer in comparison to the CT + CC genotype. Given this conclusion, more multicenter high-quality epidemiological studies or randomized controlled trials with a larger sample size should be conducted to clarify the association between the SOD2 Val-16Ala polymorphism and breast cancer risk or survival.

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