Association of the C47T polymorphism in *SOD2* with diabetes mellitus and diabetic microvascular complications: a meta-analysis

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Abstract

Aims/hypothesis A meta-analysis was performed to assess the association of C47T (rs4880) (also called Val16Ala) polymorphism in *SOD2* gene with reduced risk of diabetes mellitus, including type 1 and type 2 diabetes, and diabetic microvascular complications (DMI) including diabetic nephropathy, diabetic retinopathy and diabetic polyneuropathy. **Methods** A comprehensive search was conducted to identify all case-control or cohort design studies of the above-mentioned associations. The fixed or random effect pooled measure was selected on the basis of homogeneity test among studies. Heterogeneity among studies was evaluated using the I^2 . Meta-regression and the 'leave one out' sensitive analysis of Patsopoulos et al. were used to explore potential sources of between-study heterogeneity. Publication bias was estimated using modified Egger's linear regression test as proposed by Harbord et al.

Results Seventeen articles were included. After excluding articles that deviated from Hardy-Weinberg equilibrium in cases and/or in controls, and were also the key contributors to between-study heterogeneity, the meta-analysis showed a significant association of the C allele with reduced risk of DMI in dominant (OR 0.788, 95% CI 0.680–0.914), recessive (OR 0.808, 95% CI 0.685–0.953) and codominant (OR 0.828, 95% CI 0.751–0.913) models. It also showed a significant association with reduced risk of diabetic nephropathy in the dominant model (OR 0.801, 95% CI 0.664–0.967), and reduced risk of diabetic retinopathy in the dominant (OR 0.601, 95% CI 0.423–0.855), recessive

(OR 0.548, 95% CI 0.369–0.814) and codominant (OR 0.651, 95% CI 0.517–0.820) models.

Conclusions/interpretation The meta-analysis suggested that C allele of C47T polymorphism in *SOD2* gene has protective effects on risk of DMI, diabetic nephropathy and diabetic retinopathy. This risk needs to be confirmed by further studies.

Keywords Diabetes · Meta-analysis · Microangiopathy · MnSOD · Nephropathy · Oxidative stress · Polymorphism · Polyneuropathy · Retinopathy · SOD2

Abbreviations

DMI	Diabetic microvascular complications
DPN	Diabetic polyneuropathy
FEM	Fixed effect model
HWE	Hardy-Weinberg equilibrium
MnSOD	Manganese superoxide dismutase
REM	Random effect model
SOD	Superoxide dismutase

Introduction

Oxidative stress induced by superoxide anion plays a key role in the initiation as well as progression of diabetes mellitus [1] and diabetic microvascular complications (DMI) [2]. Superoxide dismutase (SOD) 2 (also known as manganese superoxide dismutase [MnSOD]) is an essential defender against mitochondrial superoxide radicals among antioxidative enzymes [3]. The T to C nucleotide polymorphism (rs4880) (previously called C47T) [4], which is located in exon 2 of *SOD2* gene and results in the amino acid substitution of valine with alanine at position 16 of the protein (Val16Ala), is considered the most interesting of

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several polymorphisms in *SOD2* gene, because C allele instead of T allele results in more efficient transport of SOD2 into the mitochondrial matrix [5], which can increase the ability to neutralise superoxide radicals. Since Chistyakov et al first showed the significant association between C47T polymorphism in *SOD2* gene and risk of diabetic polyneuropathy (DPN), and no significant association with type 1 diabetes risk [6], the associations of this polymorphism with diabetes mellitus, including type 1 and type 2 diabetes, and DMI including diabetic nephropathy, diabetic retinopathy and DPN have been investigated. However, the published results are controversial. Hence we conducted a meta-analysis to: (1) assess the effect of C47T polymorphism in *SOD2* gene on risk of diabetes mellitus and of DMI including diabetic nephropathy, diabetic retinopathy and DPN; (2) evaluate the potential heterogeneity among studies; and (3) explore any potential publication bias.

Methods

Search strategy A search was conducted for relevant available articles published in English or Chinese from five databases: (1) PubMed (1990–2010); (2) China National Knowledge Infrastructure (CNKI) (1990–2010); (3) Database of Chinese Scientific and Technical Periodicals (VIP) (1990–2010); (4) China Biology Medical literature database (CBM) (1990–2010); and (5) Web of Science (ISI) (1990–2010). The search strategy used the following keywords: ‘diabetes’ and ‘C47T’, ‘Val16Ala’, ‘MnSOD’, ‘SOD2’, ‘manganese superoxide dismutase’, ‘superoxide dismutase’, ‘polymorphism’, ‘mut*’ and ‘varia*’. Additional studies not captured by our database searches were identified by reviewing the bibliographies of relevant articles as well as those of relevant studies.

Inclusion criteria All identified studies were carefully reviewed independently by two investigators to determine whether an individual study was eligible for inclusion in this meta-analysis. The inclusion criteria were as follows: (1) case–control or cohort study published as an original study to evaluate the association between C47T (Val16Ala) polymorphism in *SOD2* gene and risk of diabetes mellitus and of DMI including diabetic nephropathy, diabetic retinopathy and DPN; (2) numbers in case and control groups or exposed and unexposed groups reported for each genotype, or data provided from which numbers could be calculated; (3) case and control groups in case–control study or exposed and unexposed groups in cohort study unrelated and drawn from the same temporally and geographically defined underlying population. If the two investigators disagreed about the eligibility of an article, it was resolved by consensus with a third reviewer.

Data extraction Data were independently extracted by two investigators who reached a consensus on all of the items. The most recent and complete article was chosen, if a study had been published more than once. Information extracted from each study was as follows: publication year, name of first author, country, ethnic origin of the studied population, number in case (exposed) and control (unexposed) groups, genotype and allele distributions, mean age, male sex percentage in case (exposed) and control (unexposed) groups.

Statistical analysis We used χ^2 analysis with exact probability to test departure from Hardy–Weinberg equilibrium (HWE) for the C47T genotype distribution of *SOD2* gene in case and control groups. Pooled measure was calculated as the inverse variance-weighted mean of the logarithm of OR with 95% CI to assess the strength of association between C47T polymorphism in *SOD2* gene and risk of diabetes mellitus and of DMI including diabetic nephropathy, diabetic retinopathy and DPN for dominant (CC+TC vs TT), recessive (CC vs TC+TT) and codominant (C vs T) models, respectively. The I^2 of Higgins and Thompson was used to assess heterogeneity among studies [7]. I^2 describes the proportion of total variation attributable to between-study heterogeneity as opposed to random error or chance. In the presence of substantial heterogeneity ($I^2 > 50\%$) [8], the DerSimonian and Laird random effect model (REM) was adopted as the pooling method; otherwise, the fixed effect model (FEM) was used as the pooling method. Meta-regression with restricted maximum likelihood estimation was performed to assess the potentially important covariates exerting substantial impact on between-study heterogeneity. The ‘leave one out’ sensitive analysis [9] was carried out using $I^2 > 50\%$ as the criteria to evaluate the key studies with substantial impact on between-study heterogeneity. Publication bias was estimated using modified Egger’s linear regression test, as proposed by Harbord et al [10]. An analysis of influence was conducted [11], which describes how robust the pooled estimator is to removal of individual studies. An individual study is suspected of excessive influence, if the point estimate of its omitted analysis lies outside the 95% CI of the combined analysis. All statistical analyses were performed with STATA version 9.2 (Stata Corporation, College Station, TX, USA). All reported probabilities (p values) were two-sided, with $p < 0.05$ considered statistically significant.

Results

Characteristics of studies We found 17 published articles [6, 12–27] with 31 outcomes eligible for this meta-analysis on the relation of C47T polymorphism in *SOD2* gene to

diabetes mellitus and DMI including diabetic nephropathy, diabetic retinopathy and DPN. All 17 eligible studies were case–control designs. General characteristics, the C47T allele and genotype distributions in the published articles included in this meta-analysis are showed in Tables 1 and 2.

Quantitative synthesis Results of pooled analysis are summarised in detail in Table 3. This meta-analysis showed a significant protective effect of C allele on risk of diabetes mellitus, including type 1 and type 2 diabetes, in the recessive (REM OR 0.509, 95% CI 0.300–0.861) and codominant (REM OR 0.672, 95% CI 0.459–0.984) models, but this was only marginally significant in the dominant model (REM OR 0.626, 95% CI 0.391–1.002). However, no significant association was found between C allele and reduced risk of diabetes mellitus after excluding articles that deviated from HWE in cases and/or in controls for dominant (REM OR 0.594, 95% CI 0.325–1.086), recessive (REM OR 0.430, 95% CI 0.162–1.141) and codominant (REM OR 0.637, 95% CI 0.371–1.093) models.

The C allele was found to be significantly associated with a reduced risk of diabetic nephropathy in the dominant (REM OR 0.734, 95% CI 0.559–0.964) model, with marginal significance in codominant (REM OR 0.820, 95% CI 0.670–1.003) models, and no significant association in the recessive model (REM OR 0.948, 95% CI 0.725–1.239). After exclusion of articles deviating from HWE in cases and/or in controls, the associations in the above-mentioned inherited models were not significant.

With regard to diabetic retinopathy, no significant association with the C allele was found in dominant (REM OR 0.685, 95% CI 0.444–1.057), recessive (REM OR 0.773, 95% CI 0.408–1.463) and codominant (REM OR 0.759, 95% CI 0.503–1.147) models. After exclusion of articles deviating from HWE in cases and/or in controls, the associations in the above-mentioned inherited models remained non-significant.

The meta-analysis also showed a significant association between the C allele and reduced risk of DPN in dominant (FEM OR 0.197, 95% CI 0.101–0.383), recessive (FEM OR 0.367, 95% CI 0.246–0.549) and codominant (FEM OR 0.506, 95% CI 0.397–0.644) models. However, all relevant articles regarding the association between C47T polymorphism and risk of DPN deviated from HWE in cases and/or in controls.

In addition, when we combined diabetic nephropathy, diabetic retinopathy and DPN into overall DMI, we found a significant effect of C allele on reduced risk of DMI in dominant (REM OR 0.649, 95% CI 0.520–0.809), recessive (REM OR 0.733, 95% CI 0.573–0.940) and codominant (REM OR 0.739, 95% CI 0.631–0.866) models. However, no significant association was found between C allele and reduced risk of DMI after excluding articles that

deviated from HWE in cases and/or in controls with regard to the above-mentioned inherited models.

Sources of heterogeneity and sensitive analysis As seen in Table 3, before the ‘leave one out’ sensitive analysis [9], strong evidence of heterogeneity among studies was demonstrated in the above-mentioned inherited models for risk of diabetes mellitus, DMI, diabetic nephropathy and diabetic retinopathy, with the exception in the recessive model for diabetic nephropathy risk. No significant heterogeneity among studies was found in the above-mentioned inherited models for DPN risk.

After excluding the articles deviating from HWE in cases and/or in controls, univariate meta-regression analysis, with the covariates publication year, ethnicity (categorised as Europeans, East Asians, South Asians and North Africans), sex (ratio of males in per cent in case group to that in control group), age (ratio of mean age in case group to that in control group) and sample size, showed that no covariates had a significant impact on between-study heterogeneity.

The key contributors of the articles to between-study heterogeneity assessed by the ‘leave one out’ sensitive analysis [9] are presented as ‘articles excluded’ in Table 3.

After exclusion of articles that deviated from HWE in cases and/or in controls, and were the key contributors to between-study heterogeneity, the meta-analysis showed a significant association of the C allele with reduced risk of DMI in dominant (OR 0.788, 95% CI 0.680–0.914) (Fig. 1), recessive (OR 0.808, 95% CI 0.685–0.953) and codominant (OR 0.828, 95% CI 0.751–0.913) models. It also showed a significant association of the C allele with reduced risk of diabetic nephropathy in the dominant model (OR 0.801, 95% CI 0.664–0.967) (Fig. 2), as well as with reduced risk of diabetic retinopathy in dominant (OR 0.601, 95% CI 0.423–0.855), recessive (OR 0.548, 95% CI 0.369–0.814) and codominant (OR 0.651, 95% CI 0.517–0.820) models.

Influence analysis After excluding articles that deviated from HWE in cases and/or in controls, and were the key contributors to between-study heterogeneity, no individual study was found to have excessive influence on the pooled effect in the above-mentioned inherited models with regard to diabetes mellitus, DMI, diabetic nephropathy and diabetic retinopathy, with the exception of one study [19] on reduced risk of diabetes mellitus in the recessive model (OR of this study was 0.701, with 95% CI for pooled effect of 0.757–1.452).

Publication bias evaluation After excluding articles that deviated from HWE in cases and/or in controls, and were the key contributors to between-study heterogeneity, no significant publication bias was detected in the above-mentioned inherited models for diabetes mellitus, DMI,

Table 1 Characteristics of *SOD2* gene C47T polymorphism genotype distributions for diabetes mellitus risk in studies included in the meta-analysis

Authors [ref.]	Year	Population	Case	Control	Genotypes (TT / TC / CC)		C allele frequency (%)			% of male (case/control)	Mean age (case/control)			
					Case		p_h value ^a	Case	Control			p value	OR ^b	
					Number	Number								
Chistyakov et al. [6]	2001	Russian	DM1	NC	15/104/47	0.001	5/47/36	0.053	59.6	67.6	0.084	0.708	58/67	25.0/28.3
el-Masry et al. [12]	2005	Egyptian	DM1	NC	13/88/49	0.003	3/21/16	0.478	62.0	66.3	0.517	0.831	58/63	16.6/19.0
Flekac et al. [13] ^d	2008	Czech	DM1	NC	79/36/5	0.767	52/90/38	1.000	19.2	46.1	0.000	0.277	48/52	40.0/39.0
Flekac et al. [13] ^d	2008	Czech	DM2	NC	220/80/6	0.825	52/90/38	1.000	15.0	46.1	0.000	0.207	51/52	57.0/39.0
Nomiyama et al. [14] ^c	2003	Japanese	DM2	NC	364/111/3	0.089	190/67/4	0.620	12.2	14.4	0.257	0.831	66/60	59.8/48.1
Lee et al. [15] ^c	2006	Korean	DM2	NC	243/57/4	0.761	152/37/3	0.712	10.7	11.2	0.835	0.949	59/58	53.4/52.1
Yang et al. [16] ^c	2007	Chinese	DM2	NC	114/67/6	0.384	66/27/4	0.503	21.1	18.0	0.440	1.217	52/55	55.0/42.0
Ye et al. [17] ^c	2008	Chinese	DM2	NC	192/18/54	0.000	133/15/50	0.000	23.9	29.0	0.082	0.766	49/54	55.0/52.0
Liu et al. [18] ^c	2009	Chinese	DM2	NC	214/39/4	0.244	134/35/3	0.712	9.1	11.9	0.207	0.744	54/52	65.2/59.6
Kangas-Kontio et al. [19]	2009	Finnish	DM1 and DM2	NC	59/105/58	0.423	132/246/115	1.000	49.8	48.3	0.607	1.062	56/49	58.1/51.4

^a Exact p value for HWE test^b OR ratio calculated by C vs T allele^c Genotype counts were calculated by the genotype frequency data in the publication^d One study with different types of diseases

DM1, type 1 diabetes mellitus; DM2, type 2 diabetes mellitus; NC, normal controls; ref., reference number

Table 2 Characteristics of *SOD2* gene C47T polymorphism genotype distributions for DM1 risk in studies included in the meta-analysis

Authors [ref.]	Year	Population	Case	Control	Genotypes (TT/TC/CC)		C allele frequency (%)			% of male (case/control)	Mean age (case/control)			
					Case	Control	p _h value ^a	p _h value ^a	p value			OR ^b		
el-Masry et al. [12] ^d	2005	Egyptian	DN+/DM1	DN- and DPN-/DM1	4/24/17	2/37/26	0.347	0.019	64.4	68.5	0.562	0.835	62/51	16.0/18.0
Mollsten et al. [20] ^d	2007	Finnish	DN+/DM1	DN-/DM1	212/392/197	91/183/84	0.572	0.751	49.1	49.0	1.000	1.002	59/39	38.7/41.3
Mollsten et al. [20] ^d	2007	Swedish	DN+/DM1	DN-/DM1	43/65/42	39/101/56	0.104	0.667	49.7	54.3	0.249	0.829	51/45	48.7/43.8
Mollsten et al. [21] ^e	2009	Danish	DN+/DM1	DN-/DM1	115/220/106	69/151/94	1.000	0.571	49.0	54.0	0.060	0.818	62/55	42.2/43.7
Hovnik et al. [22] ^d	2009	Slovene	DN+/DM1	DN- and DR-/DM1	7/23/7	20/42/25	0.198	0.830	50.0	52.9	0.781	0.891	56/56	27.4/26.8
Nomiyama et al. [14] ^e	2003	Japanese	DN+/DM2	DN-/DM2	158/28/1	206/83/2	1.000	0.038	8.0	14.9	0.002	0.496	66/66	60.6/59.3
Lee et al. [23] ^e	2006	Korean	DN+/DM2	DN-/DM2	111/13/3	203/24/17	0.020	0.000	7.5	11.9	0.075	0.599	68/56	53.2/53.9
Yang et al. [16] ^e	2007	Chinese	DN+/DM2	DN-/DM2	91/34/12	23/25/2	0.004	0.183	21.2	29.0	0.129	0.657	47/50	Na/Na
Tiwari et al. [24] ^d	2009	South Indians	DN+/DM2	DN-/DM2	13/42/51	34/68/47	0.374	0.325	67.9	54.4	0.002	1.778	76/69	56.0/60.5
Tiwari et al. [24] ^d	2009	North Indians	DN+/DM2	DN-/DM2	21/34/29	19/28/28	0.122	0.037	54.8	56.0	0.910	0.951	87/53	53.6/61.0
Liu et al. [18] ^e	2009	Chinese	DN+/DM2	DN-/DM2	136/17/1	78/22/3	0.446	0.390	6.2	13.6	0.005	0.418	58/49	64.3/66.6
Hovnik et al. [22] ^d	2009	Slovene	DR+/DM1	DN- and DR-/DM1	11/15/6	16/50/26	1.000	0.403	42.2	55.4	0.082	0.587	56/56	27.4/26.8
Lee et al. [15] ^e	2006	Korean	DR+/DM2	DR-/DM2	105/23/2	138/34/2	0.626	1.000	10.4	10.9	0.895	0.945	59/59	53.1/53.7
Petrovic et al. [25]	2008	Slovene	DR+/DM2	DR-/DM2	80/140/63	23/69/51	0.905	1.000	47.0	59.8	0.001	0.596	47/40	66.0/66.9
Ye et al. [17] ^e	2008	Chinese	DR+/DM2	DR-/DM2	99/8/18	93/10/36	0.000	0.000	17.6	29.5	0.002	0.511	Na/Na	Na/Na
Kangas-Kontio et al. [19]	2009	Finnish	DR+/DM1 and DM2	DR-/DM1 and DM2	30/56/40	29/49/18	0.281	0.837	54.0	44.3	0.045	1.476	60/51	59.6/56.1
Chistyakov et al. [6]	2001	Russian	DPN+/DM1	DPN-/DM1	13/55/14	2/49/33	0.004	0.002	50.6	68.5	0.001	0.472	59/58	23.5/32.1
Strokov et al. [26]	2003	Russian	DPN+/DM1	DPN-/DM1	11/35/8	3/34/17	0.054	0.018	47.2	63.0	0.028	0.526	Na/Na	23.0/31.0
Zotova et al. [27]	2003	Russian	DPN+/DM1	DPN-/DM1	16/53/17	5/57/32	0.051	0.003	50.6	64.4	0.010	0.567	60/36	25.5/27.5
el-Masry et al. [12] ^d	2005	Egyptian	DPN+/DM1	DN- and DPN-/DM1	7/27/6	2/37/26	0.055	0.019	48.8	68.5	0.006	0.438	65/51	15.0/18.0
Flekae et al. [13] ^e	2008	Czech	DR+ and DPN+/DM1 and DM2	DR- and DPN-/DM1 and DM2	84/91/24	84/109/34	1.000	1.000	34.9	39.0	0.228	0.840	Na/Na	Na/Na

^a Exact p value for HWE test^b OR ratio calculated by C vs T allele^c Genotype counts were calculated by the genotype frequency data in the publication^d One study with different types of diseases or populations

DM1, diabetic microvascular complications; DM1, type 1 diabetes mellitus; DM2, type 2 diabetes mellitus; DN, diabetic nephropathy; DPN, diabetic polyneuropathy; DN+/DM1: DM1 patients with DN; DN-/DM1, DM1 patients without DN; DN+/DM2, DM2 patients with DN; DN-/DM2, DM2 patients without DN; DR+/DM1, DM1 patients with DR; DR-/DM1, DM1 patients without DR; DR+/DM2, DM2 patients with DR; DR-/DM2, DM2 patients without DR; DPN+/DM1, DM1 patients with DPN; DPN-/DM1, DM1 patients without DPN; DPN+/DM2, DM2 patients with DPN; DPN-/DM2, DM2 patients without DPN

Na, not available; ref., reference number

Table 3 Pooled measures on the relation of *SOD2* gene C47T polymorphism with DM, DMI, DN, DR and DPN

Disease	Data	Inherited model	Before HETRED analysis			After HETRED analysis			Articles Excluded [ref.]
			Numbers of cases/controls	FEM Pooled OR (95% CI)	<i>I</i> ² (%)	REM Pooled OR (95% CI)	<i>I</i> ² (%)	Pooled OR (95% CI)	<i>I</i> ² (%)
DM	All relevant articles	Dominant	2,454/1,901	0.632 (0.546–0.731)**	89.4	0.626 (0.391–1.002)	89.4	0.894 (0.758–1.054)	0.0
		Recessive	2,454/1,901	0.675 (0.546–0.834)**	78.6	0.509 (0.300–0.861)*	78.6	0.839 (0.671–1.050)	0.0
		Codominant	2,454/1,901	0.678 (0.608–0.755)**	91.6	0.672 (0.459–0.984)*	91.6	0.895 (0.793–1.010)	8.3
	Excluded for DHWE	Dominant	1,874/1,575	0.611 (0.521–0.716)**	92.8	0.594 (0.325–1.086)	92.8	0.935 (0.777–1.126)	0.0
		Recessive	1,874/1,575	0.664 (0.496–0.888)**	85.5	0.430 (0.162–1.141)	85.5	1.049 (0.757–1.452)	0.0
		Codominant	1,874/1,575	0.652 (0.575–0.739)**	94.3	0.637 (0.371–1.093)	94.3	0.968 (0.836–1.121)	0.0
DMI	All relevant articles	Dominant	3,426/3,100	0.714 (0.631–0.808)**	61.1	0.649 (0.520–0.809)**	61.1	0.638 (0.554–0.734)**	49.6
		Recessive	3,426/3,100	0.805 (0.705–0.919)**	62.4	0.733 (0.573–0.940)*	62.4	0.718 (0.624–0.827)**	41.0
		Codominant	3,426/3,100	0.801 (0.740–0.866)**	71.6	0.739 (0.631–0.866)**	71.6	0.689 (0.628–0.756)**	34.7
	Excluded for DHWE	Dominant	2,459/1,939	0.822 (0.712–0.950)**	57.8	0.812 (0.638–1.033)	57.8	0.788 (0.680–0.914)**	44.6
		Recessive	2,459/1,939	0.924 (0.793–1.076)	63.9	0.922 (0.688–1.236)	63.9	0.808 (0.685–0.953)*	24.6
		Codominant	2,459/1,939	0.898 (0.819–0.984)*	74.1	0.883 (0.725–1.075)	74.1	0.828 (0.751–0.913)**	49.9
DN	All relevant articles	Dominant	2,269/1,932	0.760 (0.651–0.887)**	60.2	0.734 (0.559–0.964)*	60.2	0.721 (0.615–0.844)**	45.0
		Recessive	2,269/1,932	0.963 (0.815–1.138)	44.1	0.948 (0.725–1.239)	44.1	–	–
		Codominant	2,269/1,932	0.879 (0.796–0.971)*	69.1	0.820 (0.670–1.003)	69.1	0.831 (0.750–0.922)**	47.4
	Excluded for DHWE	Dominant	1,689/1,207	0.856 (0.714–1.026)	64.4	0.860 (0.610–1.212)	64.4	0.801 (0.664–0.967)*	43.4
		Recessive	1,689/1,207	0.984 (0.821–1.179)	63.1	0.982 (0.694–1.389)	63.1	0.890 (0.734–1.079)	20.8
		Codominant	1,689/1,207	0.937 (0.838–1.047)	76.5	0.917 (0.707–1.190)	76.5	0.903 (0.802–1.018)	0.0
DR	All relevant articles	Dominant	696/644	0.690 (0.528–0.901)**	60.5	0.685 (0.444–1.057)	60.5	0.580 (0.431–0.782)**	16.7
		Recessive	696/644	0.706 (0.525–0.950)*	72.2	0.773 (0.408–1.463)	72.2	0.528 (0.378–0.738)**	0.0
		Codominant	696/644	0.747 (0.625–0.892)**	79.5	0.759 (0.503–1.147)	79.5	0.615 (0.502–0.752)**	13.0
	Excluded for DHWE	Dominant	571/505	0.745 (0.550–1.010)	66.7	0.731 (0.425–1.258)	66.7	0.601 (0.423–0.855)**	42.3
		Recessive	571/505	0.789 (0.564–1.104)	76.0	0.903 (0.393–2.075)	76.0	0.548 (0.369–0.814)**	0.0
		Codominant	571/505	0.814 (0.668–0.991)*	80.8	0.841 (0.520–1.359)	80.8	0.651 (0.517–0.820)**	18.2
DPN ^a	All relevant articles	Dominant	262/297	0.197 (0.101–0.383)**	0.0	0.197 (0.101–0.383)**	0.0	–	–
		Recessive	262/297	0.367 (0.246–0.549)**	0.0	0.367 (0.246–0.549)**	0.0	–	–
		Codominant	262/297	0.506 (0.397–0.644)**	0.0	0.506 (0.397–0.644)**	0.0	–	–

Dominant model: CC+TC vs TT; recessive model: CC vs TC+TT; codominant model: C vs T

DHWE: deviated from HWE in cases and/or in controls

HETRED: sensitivity analysis for reducing heterogeneity by omitting study using the STATA module of HETRED when $I^2 \geq 50\%$ ^a All articles for DPN were DHWE in cases and/or in controls* $p < 0.05$, ** $p < 0.01$

DM, diabetes mellitus including type 1 and type 2 diabetes; DMI, diabetic microvascular complications; DN, diabetic nephropathy; DR, diabetic retinopathy; DPN, diabetic polyneuropathy; FEM, fixed effect model; ref., reference number; REM, random effect model

Fig. 1 Forest plot of ORs for DMI in dominant model (CC+TC vs TT) of *SOD2* gene C47T polymorphism. White diamond denotes the pooled OR. Black squares indicate the OR in each study, with square sizes inversely proportional to the standard error of the OR. Horizontal lines represent 95% CI. ^aOne study with different types of diseases or populations. Ref., reference number

diabetic nephropathy and diabetic retinopathy (data not shown).

Discussion

Clinical and experimental studies indicate that oxidative stress is associated with diabetes mellitus [1], diabetic nephropathy [28], diabetic retinopathy [29] and DPN [30]. The *SOD2* encoded by the T allele, which disrupts the alpha-helix structure of the targeting sequence, is retained in the mitochondrial inner membrane and induces 30 to 40% lower activity and oxidative stress level. Chistyakov et al [6] first showed the significant protective effect of C47T polymorphism in *SOD2* gene on DPN risk in 82 DPN cases and 84 non-DPN controls in type 1 diabetes mellitus patients, but no association with risk of type 1 diabetes mellitus was found in 166 type 1 diabetes mellitus cases and 88 normal controls in a Russian population. Nevertheless,

results of subsequent studies about the effect of this polymorphism on risk of diabetes mellitus, including type 1 and type 2 diabetes, and of DMI including diabetic nephropathy, diabetic retinopathy and DPN were conflicting.

Because of the above-mentioned inconsistent results from relatively small studies underpowered to detect the effect, a meta-analysis is the appropriate approach to obtain a more definitive conclusion regarding the role of *SOD2* gene C47T polymorphism on risk of diabetes mellitus and DMI including diabetic nephropathy, diabetic retinopathy and DPN. Our meta-analysis, of 17 published articles [6, 12–27] with 31 outcomes from different ethnic origins, allowed a much greater possibility of reaching reasonably strong conclusions.

As mentioned in one paper [31], between-study heterogeneity is common in meta-analysis for genetic association studies. Our meta-analysis also showed significant between-study heterogeneity in most of the inherited models regarding diabetes mellitus, DMI, diabetic nephrop-

Fig. 2 Forest plot of ORs for diabetic nephropathy in dominant model (CC+TC vs TT) of *SOD2* gene C47T polymorphism. White diamond denotes the pooled OR. Black squares indicate the OR in each study, with square sizes inversely proportional to the standard error of the OR. Horizontal lines represent 95% CI. ^aOne study with different types of populations. Ref., reference number

athy and diabetic retinopathy. An indeterminate number of characteristics that vary among studies could be the cause of between-study heterogeneity, e.g. design quality, population stratification, characteristics of the sample, non-comparable measure of genotyping, variation of the covariate, deviation from HWE in some studies, etc. Thus we used meta-regression and 'leave one out' sensitive analysis [9], which aims to reduce between-study heterogeneity and explore the potential important causes of between-study heterogeneity for both covariates and studies. Our meta-analysis of the studies that were in HWE in cases and controls did not identify any of the aforementioned covariates as being an important contributor to between-study heterogeneity. When, after the 'leave one out' sensitive analysis using $I^2 > 50\%$ as the criteria, we performed data analysis on the studies that were in HWE in cases and controls, our results showed that the C allele of C47T polymorphism in *SOD2* gene had a significant effect on reduced risk of DMI, diabetic nephropathy and diabetic retinopathy, but not of diabetes mellitus.

According to another article [32] and the SNP database at the NIH (www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=4880, accessed 24 September 2010), the C allele frequency in the Asian population is significantly lower than in most European populations. Such heterogeneous genetic backgrounds might be, in part, responsible for the heterogeneity of effect on the disease across the ethnicity, even though we could not detect this in our meta-analysis. Diabetes mellitus and DMI including diabetic nephropathy, diabetic retinopathy and DPN, have a complex aetiology and pathophysiology generated by the combined effects of genes and environment factors. Although ethnicity, sex and age were not found to be important sources of disease–effect heterogeneity across the studies in this meta-analysis, other genetic and environment variables, as well as their possible interaction, may well be potential contributors to this disease–effect unconformity. In this respect, the lack of relevant study-level covariates in the reported articles precluded a more robust assessment of sources of this heterogeneity. Other possibilities related to disease–effect diversity, such as variations in design quality, variations in genotyping, etc. could not be ruled out.

In this meta-analysis, we could not find any significant publication bias in the above-mentioned inherited models for diabetes mellitus, including type 1 and type 2 diabetes, DMI, diabetic nephropathy and diabetic retinopathy, this may be due to the small number of studies in those meta-analyses, especially for diabetic retinopathy risk evaluation.

In conclusion, this meta-analysis suggested that the C allele of C47T polymorphism in *SOD2* gene had significant protective effects on risk of DMI, diabetic nephropathy and diabetic retinopathy. Since potential biases and confounders could not be ruled out completely in this meta-analysis, further studies are needed to confirm these results.

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Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

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