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ORIGINAL ARTICLE

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The relationship between C677T methylenetetrahydrofolate reductase gene polymorphism and retinopathy in type 2 diabetes: a meta-analysis

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Abstract The association between retinopathy in type 2 diabetes [diabetic retinopathy (DR)] and the C677T polymorphism in the methylenetetrahydrofolate reductase (MTHFR) gene has been investigated in several case-control studies. These studies rendered contradictory results, some indicating that the polymorphism is associated with the risk of developing DR whereas others concluded there is no association. To shed light on these inconclusive findings, a meta-analysis of all available studies relating the C677T polymorphism to the risk of developing DR was conducted. Four out of five identified studies included populations of East Asian descent, and only one involved samples from European descent (Caucasians). Overall, the meta-analysis suggested large heterogeneity between studies ($p = 0.08$, $I^2 = 52\%$) and marginal association between C677T transition and the risk of developing DR: random effects odds ratio (OR) = 1.39 [95% CI (1.05, 1.83)]. The sensitivity analysis [exclusion of one East Asian study with the controls not in Hardy–Weinberg equilibrium (HWE)] showed no heterogeneity ($p = 0.25$, $I^2 = 27\%$) and no significant association: fixed effects OR = 1.22 [95% CI (0.99, 1.51)] and random effects OR = 1.24 [95% CI (0.96, 1.60)]. The sub-group analysis for the East Asian population produced a significant association: fixed effects OR = 1.48 [95% CI (1.20, 1.83)] and random effects OR = 1.52 [95% CI (1.14, 2.03)]. However, sensitivity analysis in East Asians revealed that the association is marginal: fixed effects OR = 1.33 [95% CI (1.04, 1.70)] and random effects OR = 1.36 [95% CI (1.01, 1.83)]. There is a source of bias in the selected studies: the largest studies failed to show association while the smallest study claimed an association. The above findings reinforce the need for larger and more rigourous studies in this area.

Keywords Diabetic retinopathy · MTHFR · C677T · Polymorphism · Meta-analysis · Type 2 diabetes

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Introduction

Diabetic retinopathy (DR) is a serious chronic microangiopathic complication of types 1 and 2 diabetes and represents one of the leading causes of adult-acquired blindness in industrialised countries (Munier et al. 1998; Congdon et al. 2004). Various predisposing factors (Walker et al. 1985; van Leiden et al. 2003; Best and Chakravarthy 1997) have been identified already, but the pathogenesis of DR is not yet fully elucidated. The main risk factors are proven to be poor glycemic control and the duration of diabetes (Yanko et al. 1983; Klein et al. 1992). However, in large surveys, there were sub-groups of patients with type 2 diabetes who did not develop retinopathy despite poor glycemic control while others with fairly good control did develop retinopathy [UK Prospective Diabetes Study (UKPDS) Group 1998; The Diabetes Control and Complications Trial Research Group 1997]. Furthermore, sub-group analysis in the Diabetes Control and Complications Trial showed strong familial transmission for DR, especially in patients with severe proliferative retinopathy (The Diabetes Control and Complications Trial Research Group 1997). These facts represent convincing evidence that genetic factors contribute to the development of DR, but the genes conferring susceptibility remain to be identified (Simonelli et al. 2001; Warpeha and Chakravarthy 2003).

The methylenetetrahydrofolate reductase (MTHFR) gene is located on chromosome 1p36.3 (Goyette et al. 1994). Human MTHFR catalyses the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, a co-substrate for re-methylation of homocysteine to methionine. The C677T transition is a common ubiquitous missense mutation in the coding region of the MTHFR gene, which creates a *Hinf*I recognition sequence and is detectable as a restriction fragment length polymorphism (RFLP). It causes an alanine to valine (Al222Val) amino acid substitution located in the anticipated catalytic domain of the enzyme (Frosst et al. 1995) and results in a thermo-labile MTHFR variant with reduced catalytic activity.

Homozygosity for the mutation (TT genotype) predisposes to significantly elevated plasma homocysteine levels (Frosst et al. 1995; Kang et al. 1991). Hyperhomocysteinemia is recognised as an independent risk factor for macroangiopathy (cerebrovascular or coronary artery disease) and for arterial or venous thrombosis (Bostom et al. 2001; Boushey et al. 1995; McCully 1996). The DR is a micro-angiopathic lesion primarily affecting the retinal capillaries and, therefore, its development might be related to MTHFR gene polymorphisms.

There are a number of recent case-control studies that investigated the association between the C677T polymorphism in the MTHFR gene and the diabetes-related micro-angiopathic complications, i.e. diabetic nephropathy and DR (Fujita et al. 1999; Yoshioka et al. 2003; Moczulski et al. 2003; Shpichinetsky et al. 2000). Results concerning the relationship between the risk of developing DR and the C677T mutation are inconclusive. Concretely, some of the studies indicate that the T allele of the MTHFR gene relative to the C allele is a risk factor for developing DR (Neugebauer et al. 1997; Sun et al. 2003; Maeda et al. 2003c) whereas other investigators report no genetic association (Yoshioka et al. 2003; Santos et al. 2003). However, the significant associations described in single studies between the T allele and DR are rather marginal and have, therefore, become subject to controversy (Yoshioka et al. 2003; Maeda et al. 2003b).

To provide an answer to these contradictory results, a meta-analysis (Lau et al. 1997) of all available studies relating the C677T polymorphism of the MTHFR gene to the risk of developing DR in patients with type 2 diabetes was conducted. In this meta-analysis, the estimates of the genetic association of each individual study and a pooled estimate of this association were obtained. In addition, the heterogeneity between studies and the existence of bias were investigated.

Materials and methods

Selection of studies

All studies that investigate the association of the C677T polymorphism in the MTHFR gene with the development of DR published before November 2004 were considered in the meta-analysis. The studies were identified by extensive computer-based searches of the PubMed and EBSCO databases. As a search criterion, we used the following: (MTHFR or ''homocysteine metabolism'') and ''DR'' and ''polymorphism''.

The retrieved publications were then read in their entirety in order to assess their appropriateness for inclusion in this meta-analysis. All references cited in the studies were also reviewed to identify additional published work not indexed by PubMed and EBSCO databases. Abstracts, case reports, editorials, and review articles were excluded. The search was restricted to articles in English. Case-control studies that determine the distribution of the C677T genotypes in cases with DR (proliferative and non-proliferative) and in controls free of DR were eligible for inclusion. Both cases and controls were patients with type 2 diabetes.

The distribution of genotypes in the control group was tested whether it is in Hardy–Weinberg equilibrium (HWE) using an exact test (Weir 1996) implemented by GDA software (Lewis and Zaykin 2001). Studies with controls not in HWE were subjected to a sensitivity analysis. Studies based on pedigree data were excluded since they investigated linkage (Zintzaras and Ioannidis 2005) and not association.

Data extraction

From each study, the following information was extracted: first author, journal, year of publication, racial descent of study population, demographics, matching, validity of the genotyping method, and the number of cases and controls for each C677T genotype. Frequencies of alleles were calculated, for the cases and the controls, from the corresponding genotype distributions. In addition, it was recorded whether the genotyping in each study was blinded to clinical status.

Meta-analysis

The meta-analysis examined the overall association of T allele with the risk of diabetic nephropathy relative to the C allele; and the contrast of homozygotes TT versus CC, the contrast TT versus (TC + CC), and the contrast (TT + TC) versus CC. All associations were indicated as odds ratios (ORs) with the corresponding 95% confidence interval (CI). Then, based on the individual ORs, a pooled OR was estimated.

Heterogeneity between studies was tested using the Q-statistic, which is a weighted sum of squares of the deviations of individual study OR estimates from the overall estimate (Cochran 1954). When the ORs were homogeneous, Q follows a chi-squared distribution with r-1 (r is the number of studies) degrees of freedom (d.f.). If $p < 0.10$, then the heterogeneity was considered statistically significant. Heterogeneity was quantified with the I^2 metric ($I^2 = (Q\text{–d.f.})/Q$), which is independent of

the number of studies in the meta-analysis (Higgins et al. 2003). The I^2 takes values between 0% and 100%, with higher values denoting greater degree of heterogeneity (I^2 = 0–25%, no heterogeneity; I^2 = 25–50%, moderate heterogeneity; I^2 = 50–75%, large heterogeneity; I^2 = 75–100%, extreme heterogeneity).

The pooled OR was estimated using fixed effects (Mantel–Haenszel) and random effects (DerSimonian and Laird) models. Random effects modelling assumes a genuine diversity in the results of various studies, and it incorporates into the calculations a between-study variance. Therefore, when there is heterogeneity between studies, then the pooled OR is estimated using the random effects model (Whitehead 2002). Adjusted estimates of OR were considered whenever possible in a separate analysis. A cumulative meta-analysis (Lau et al. 1992; Whitehead 1997) and recursive meta-analysis were carried out in order to evaluate the trend of pooled OR for the allele contrast (T versus C) in time. A differential magnitude of effect in large versus small studies, for the allele contrast, was checked using the Egger regression test for funnel plot asymmetry (Egger et al. 1997; Whitehead 2002). The test is based on the linear regression model: $y_i = \alpha + \beta x_i$, where the dependent variable y_i is the standardised estimate of $\theta_i = \ln(\mathrm{OR}_i)$: $y_i = \theta_i\sqrt{w_i}$, $w_i = 1/\mathrm{variance}(\theta_i)$, and the independent variable x_i is the precision: $x_i = \sqrt{w_i}$, i.e. the study magnitude. A test of a differential magnitude of effect in large versus small studies would be a test of the null hypothesis that α is equal to zero (Ioannidis et al. 2003).

Whether the OR in the first study versus the pooled OR of the subsequent studies was different beyond chance ($p < 0.05$) was assessed using the z-statistic, i.e. the difference of the natural logarithm of the ORs divided by the standard error of this difference (Ioannidis et al. 2003). In addition, the association between the C677T MTHFR gene polymorphism and DR was assessed with or without the first study.

The meta-analysis consisted of the main analysis, which includes all available data; the sub-group analysis of each population race; and sensitivity analyses, which examines the effect of excluding specific studies. Analyses were performed using Meta-Analyst (Joseph Lau, Boston, MA, USA), SAS software, and CVF90 with IMLS library (Zintzaras and Hadjigeorgiou 2004; Zintzaras and Stefanidis 2005; Whitehead 2002).

Results

Eligible studies

The literature review identified ten titles in PubMed and five titles in EBSCO that met the search criteria. After review, seven titles were judged to be potentially relevant. The abstracts of these articles were further reviewed, and full articles of selected studies were read to assess their appropriateness for meta-analysis. Five studies investigating the association between the MTHFR gene C677T polymorphism and DR met the inclusion criteria (Table 1).

Four studies were published in 2003 and one study in 1997. In four studies, the subjects were of East Asian descent (Neugebauer et al. 1997; Yoshioka et al. 2003; Sun et al. 2003; Maeda et al. 2003c), and in one study, the subjects were of European descent (Caucasians) (Santos et al. 2003). In four studies (Neugebauer et al. 1997; Sun et al. 2003; Santos et al. 2003; Yoshioka et al. 2003), the subjects' ages (cases and controls) ranged from an average of 50.5 to 60 years, and gender was not evenly distributed; the fifth study does not report any demographic details (Maeda et al. 2003c).

In all studies, the cases, namely patients with type 2 diabetes showing DR, were well defined following similar inclusion criteria. In two studies (Sun et al. 2003; Santos et al. 2003) the existence of DR was assessed by means of ophthalmoscopy and/or biomicroscopy and/or fluorescein angiography while in the remaining studies the assessment method was not reported. In two studies, it was specified that cases and controls had no evidence of nephropathy (Maeda et al. 2003c; Yoshioka et al. 2003).

The controls were patients with type 2 diabetes without evidence for DR (Table 1). The duration of type 2 diabetes reported ranged in from 10 (Santos et al. 2003) to 12 years (Yoshioka et al. 2004). In one study (Sun et al. 2003), a duration of type 2 diabetes in controls longer than 10 years was reported.

In all studies genotypes were analysed using a validated genotyping method: PCR and digestion of the 198 bp PCR amplification product by *Hinf*I, a restriction enzyme (Frosst et al. 1995).

Statistics summary

In total, the studies included 435 cases with type 2 diabetes and DR, and 620 controls with type 2 diabetes without DR.

The prevalence of allele T was 43% and 37% for the DR and control groups, respectively. The prevalence of homozygotes TT among patients with DR and controls was 19% and 14%, respectively. The prevalence of CC among patients with DR and controls was 33% and 40%, respectively. The prevalence of heterozygotes TC among patients with DR and controls was 48% and 46%, respectively (Table 2).

In one study (Sun et al. 2003), the distribution of the genotypes in the control group was not in HWE ($p < 0.01$), indicating genotyping errors and population stratification (Silverman and Palmer 2000; Xu et al. 2002). Therefore, a sensitivity analysis was carried out for this study; however, the produced results should be interpreted with caution.

Main results, sub-group and sensitivity analyses

The main analysis for investigating the association of the allele T and the risk of developing DR relative to the

Table 1 Characteristics of the case-control studies considered in the meta-analysis. *DR* diabetic retinopathy

First author, year	Country	Racial descent	Selection criteria and demographic data of cases	Selection criteria and demographic data of controls	Cases (*n*)	Controls (*n*)
Sun, 2003	China	East Asians	Type 2 diabetes, 64 males and 46 females with DR, mean age 55.6 ± 6.7 years, duration of diabetes < 5 years. DR assessed by fundus photography or fundus fluorescein angiography	Type 2 diabetes, 56 males and 42 females without DR, mean age 54.7 ± 7.1 years, duration of diabetes > 10 years	110	98
Santos, 2003	Brazil	Caucasians	Type 2 diabetes, 99 patients with DR (cases and controls: 68 male, 142 female, mean age 58.7 ± 12 years, duration of diabetes 10.5 ± 9.7 years). DR assessed by ophthalmoscopy and/or biomicroscopy, and fluorescein angiography when indicated	Type 2 diabetes, 110 patients without DR	99	111
Yoshioka, 2003	Japan	East Asians	Type 2 diabetes, 52 patients with non-proliferative DR and 46 with proliferative DR all without overt nephropathy. Patients with urinary albumin excretion > 300 mg/g creatinine were excluded (cases and controls: mean age 60 years, mean duration of diabetes 11.7 years, HbA_{1c} 7.3%, serum creatinine 0.71 mg/dl)	Type 2 diabetes, 268 patients without DR, and without overt nephropathy	98	268
Maeda, 2003	Japan	East Asians	Type 2 diabetes, 19 patients with non-proliferative DR and 33 with proliferative DR and without serum creatinine elevation	Type 2 diabetes, 107 patients without DR and without serum creatinine elevation	52	104
Neugebauer, 1997	Japan	East Asians	Type 2 diabetes, 76 patients with DR and without any clinical or laboratory evidence of diabetic nephropathy, creatinine 70 ± 7.6 μmol/l, HbA_{1c} 7.7 ± 1.1%, mean age 50.5 ± 9.7 years, duration of diabetes 11.2 ± 4.2 years	Type 2 diabetes, 36 patients without DR, some of them with diabetic nephropathy, mean age 55.5 ± 7.9 years, creatinine 266.7 ± 243.8 μmol/l, HbA_{1C} 7.5 ± 1.3%, duration of diabetes 16.5 ± 5.1 years	76	36

allele C showed that there was large heterogeneity (p = 0.08, I^2 = 52%) between the five studies, then the random effects pooled OR was marginally significant OR = 1.39 [95% CI (1.05, 1.83)] (Table 3 and Fig. 1).

In sub-group analysis, there was moderate heterogeneity (p = 0.16, I^2 = 42%) between the studies performed in the East Asian population. The random effects and fixed effects pooled ORs were significant, i.e. OR = 1.52 [95% CI (1.14, 2.03)] and OR = 1.48 [95% CI (1.20, 1.83)], respectively (Table 3).

In sensitivity analysis (exclusion of the study with the controls not in HWE), there was no between-study heterogeneity either for all studies in HWE (p = 0.25, I^2 = 27%) or for the sub-group analysis (East Asians in HWE) (p = 0.28, I^2 = 22%) (Table 3). Then, in the analysis for all studies in HWE, the fixed and random effects ORs were not significant: OR = 1.22 [95% CI (0.99, 1.51)] and OR = 1.24 [95% CI (0.96, 1.60)], respectively, and in East Asians, they were marginally significant: OR = 1.33 [95% CI (1.04, 1.70)] and OR = 1.31 [95% CI (1.01, 1.83)], respectively.

The genotype contrast of the homozygotes produced the same pattern of association with the allele contrast and no heterogeneity. The dominant model for the effect of T allele in the main analysis showed lack of association and for the East Asians significant association. However, sensitivity analyses for both dominant and recessive models showed no significant associations (Table 3).

Potential bias

None of the studies reported that genotyping was blinded to clinical status.

Cumulative meta-analysis and recursive meta-analysis for the allelic contrast showed that random effects pooled OR declined from 1.92 in 1997 (first study) to 1.35 in 2003 (five studies). The four studies published in 2003 produced a random effects OR of 1.32. There was also a suggestion that the two largest studies (Yoshioka et al. 2003; Santos et al. 2003) produced no significant associations and, in contrast, the smallest study produced significant association. The Egger test indicated that there is a differential magnitude of effect in large versus small studies. However, this result might not be so reliable since the number of studies is small (Ioannidis et al. 2003). There is no statistical difference between the OR of the first study versus the pooled OR of the subsequent studies (z = 1.10, $p \geq 0.05$). The random effects pooled OR without the first study was OR = 0.76 [95% CI (0.56, 1.02)], and the between-study heterogeneity was moderate (p = 0.07, I^2 = 56%).

Discussion

The MTHFR is involved in the re-methylation of homocysteine to methionine, and its C677T polymorphism yields a thermo-labile MTHFR variant with reduced enzymatic activity. This mutation is a genetic determinant of hyperhomocysteinemia in healthy subjects (Kang et al. 1991; Frosst et al. 1995) and also in patients with diabetes (Buysschaert et al. 2004). Hyperhomocysteinemia induces endothelial dysfunction (Constans et al. 1999) and has been implicated as a risk factor for atherosclerosis and atherothrombosis (McCully 1969; Frosst et al. 1995) but also for retinopathy in type 1 and in type 2 diabetes patients (Hoogeveen et al. 2000; Goldstein et al. 2004). Recent in vitro studies indicate that homocysteine and other thiol-containing reductive compounds (i.e. thiolactone) increase the expression of the vascular endothelial growth factor (VEGF) in cell cultures via activation of its transcription (Maeda et al. 2003a; Roybal et al. 2004). The VEGF is a pro-angiogenic factor known to play a key role in the development and progression of DR (Roybal et al. 2004; Ray et al. 2004). For these reasons, it may be readily postulated that the C677T MTHFR gene polymorphism might be involved in the development of DR.

The meta-analysis presented here included data from five case-control association studies that investigated the relation between the MTHFR polymorphism and DR in type 2 diabetes. Overall, 435 subjects who developed DR

Table 2 The distribution of the methylenetetrahydrofolate reductase (MTHFR) genotypes and the allelic frequency for type 2 diabetic patients with diabetic retinopathy (DR) and without diabetic retinopathy (NDR) are shown [in parentheses are the corresponding percentages (%)]

First author	Year	Racial decent	Distribution of MTHFR genotypes						Frequency of MTHFR alleles			
			CC		CT		TT		C		T	
			DR	NDR	DR	NDR	DR	NDR	DR	NDR	DR	NDR
Sun	2003	East Asian	33 (30)	51 (52)	46 (42)	29 (30)	31 (28)	18 (18)	112 (51)	131 (67)	108 (49)	65 (33)
Santos	2003	Caucasian	34 (34)	41 (37)	53 (54)	53 (48)	12 (12)	17 (15)	121 (61)	135 (61)	77 (39)	87 (39)
Yoshioka	2003	East Asian	33 (34)	100 (37)	50 (51)	132 (49)	15 (15)	36 (13)	116 (59)	332 (62)	80 (41)	204 (38)
Maeda	2003	East Asian	18 (35)	37 (36)	20 (38)	58 (56)	14 (27)	9 (9)	56 (54)	132 (63)	48 (46)	76 (37)
Neugebauer	1997	East Asian	26 (34)	20 (56)	38 (50)	13 (36)	12 (16)	3 (8)	90 (59)	53 (74)	62 (41)	19 (26)

Table 3 The distribution of the methylenetetrahydrofolate reductase (MTHFR) genotypes and the allelic frequency for type 2 diabetic patients with diabetic retinopathy (DR) and without diabetic retinopathy (NDR) are shown. *HWE* Hardy–Weinberg equilibrium

Genetic contrasts	Population and sub-groups under analysis	Studies (*n*)	Alleles/ genotypes (*n*)	Fixed effects [OR (95% CI)]	Random effects [OR (95% CI)]	I^2(%)	*Q* test *p* value
T versus C	All	5	2104	1.35 (1.12, 1.63)	1.39 (1.05, 1.83)	52	0.08
	All in HWE	4	1688	1.22 (0.99, 1.51)	1.24 (0.96, 1.60)	27	0.25
	East Asians	4	1684	1.48 (1.20, 1.83)	1.52 (1.14, 2.03)	42	0.16
	East Asians in HWE	3	1268	1.33 (1.04, 1.70)	1.36 (1.01, 1.83)	22	0.28
TT versus CC	All	5	560	1.78 (1.22, 2.60)	1.82 (1.08, 3.05)	40	0.15
	All in HWE	4	427	1.53 (0.98, 2.39)	1.61(0.87, 2.98)	40	0.17
	East Asians	4	456	2.14 (1.40, 3.28)	2.15 (1.36, 3.40)	9	0.35
	East Asians in HWE	3	323	1.91 (1.13, 3.23)	2.00 (1.04, 3.86)	26	0.26
TT versus (TC + CC)	All	5	1052	1.50 (1.06, 2.12)	1.56 (0.93, 2.62)	49	0.10
	All in HWE	4	844	1.41 (0.94, 2.12)	1.56 (0.76, 3.10)	61	0.05
	East Asians	4	842	1.77 (1.20, 2.61)	1.85 (1.12, 3.05)	33	0.22
	East Asians in HWE	3	634	1.79 (1.11, 2.88)	1.99 (0.90, 4.45)	55	0.11
(TT + TC) versus CC	All	5	1052	1.46 (1.12, 1.91)	1.49 (1.02, 2.17)	47	0.11
	All in HWE	4	844	1.25 (0.92, 1.69)	1.25 (0.92, 1.69)	0	0.40
	East Asians	4	842	1.58 (1.16, 2.13)	1.62 (1.02, 2.56)	54	0.09
	East Asians in HWE	3	634	1.31(0.91, 1.87)	1.34 (0.87, 2.07)	28	0.25

were analysed, along with their respective controls (617 diabetic subjects without DR). These numbers are relatively small and therefore any inferences have to be cautious (Ioannidis et al. 2003). The strength of the present analysis, however, is based on the aggregation of published case-control studies, thus there is more information for investigating the effect of the allele under investigation (Zintzaras and Hadjigeorgiou 2004; Muncer 2002).

The overall results indicated a marginal association of the C677T MTHFR polymorphism with DR and large heterogeneity between study results. Sensitivity analysis, however, showed that there was no heterogeneity and no significant association when the analysis was restricted to studies with the controls in HWE. Although the sub-group analysis in East Asians showed a significant association, the subsequent sensitivity analysis produced a marginal association and no heterogeneity. The study with the controls not in HWE (Sun et al. 2003) (East Asians) supported an association, and therefore, this result can be considered dubious. Among studies with the controls in HWE, the two largest studies (Santos et al. 2003; Yoshioka et al. 2003) (one in Caucasians and one in East Asians) failed to show association while only the smallest and first published study (Neugebauer et al. 1997) (East Asians) claimed a susceptibility effect to DR. This trend of results and the difference in results between the large and small studies might be a source of potential bias in the published studies.

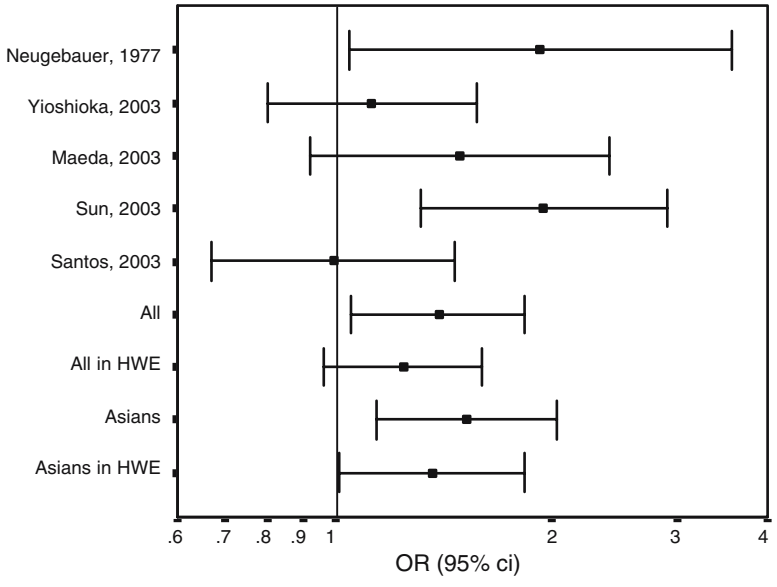

Fig. 1 C677T methylenetetrahydrofolate reductase (MTHFR) polymorphism and the risk of diabetic retinopathy (DR): contrast of allele T against C. Each study is shown by an odds ratio (OR) estimate with the corresponding 95% confidence interval (CI). The random effects pooled ORs are shown. The *horizontal axis* is plotted on a log scale

None of the studies reported a polymorphism to be in linkage disequilibrium with the MTHFR gene polymorphism. However, the discrepancy of results might be due to other locus that are probably in linkage disequilibrium and affect the susceptibility to DR. In linkage studies, the MTHFR gene region (1p36.3) has not been reported to be an important susceptibility locus in DR or any other form of diabetic micro-angiopathy (Imperatore et al. 1998).

Variability of the case inclusion criteria is a central possible confounding factor in all studies on the role of genetic markers, and therefore, the strict selection criteria ensures a clear case, control, and definition for meta-analysis. In the meta-analysis presented here, the cases and controls were well defined with similar inclusion criteria, albeit they unavoidably cover a wide spectrum of disease, in terms of duration and other manifestations.

In the sub-group analysis by racial descent, Chinese and Japanese were analysed together since these two populations are more homogeneous to each other than to Caucasians. In investigating the genetic effects for complex diseases in sub-group meta-analyses, Ioannidis et al. (2004) categorised the racial descent into three main categories: (1) European descent (populations from Europe and subjects of European descent from Oceania, North America, and South America), (2) African descent (populations of sub-Saharan Africa and African Americans), and (3) East Asian descent (populations from China, Japan, Korea, Indochina, and Philippines). These traditionally defined groups are typically used in sub-group meta-analyses by racial descent (Ioannidis et al. 2004; Thomas and White 2002; Zintzaras and Stefanidis 2005; Zintzaras and Hadjigeorgiou 2004). However, the consistency of genetic effects across the traditionally defined racial groups does not necessarily mean that race-specific genetic effects are exactly the same (Ioannidis et al. 2004).

The first case-control association study (Neugebauer et al. 1997) that investigated the relation between the MTHFR polymorphism and DR in type 2 diabetes showed the existence of an association. Patients with retinopathy in that study had diabetes-induced advanced renal failure. This fact was criticised by Maeda et al. (2003a–c) and Yosioka et al. (2003), as renal disease per se causes enhanced homocysteine levels and accelerated atherosclerosis (Perna et al. 1999). However, the TT genotype in type 2 diabetes is associated with enhanced homocysteine levels (Sun et al. 2003) independently from the co-existence of nephropathy (Sun et al. 2004). Furthermore, in a previous study (Fujita et al. 1999), there was no nephropathy effect in association with the MTHFR genotype in patients with type 2 diabetes and DR.

In conclusion, the meta-analysis and the subsequent sensitivity analyses supported marginal association between the MTHFR gene C677T polymorphism and DR. This conclusion is based on a relatively small number of studies and participants, and any inferences have to be cautious. Taking also into account that DR is a complex disease with multi-factorial aetiology, a minor contributing pathogenetic role of the C677T MTHFR gene polymorphism in specific cases, and in co-operation with other factors, cannot be totally excluded. Therefore, the relationship between the C677T MTHFR polymorphism and DR still remains an unresolved issue, and long-term prospective studies are required.

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