J Hum Genet (2008) 53:499–507
DOI 10.1007/s10038-008-0277-z

ORIGINAL ARTICLE

The role of *MTHFR* gene in multiple myeloma

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Received: 11 December 2007 / Accepted: 23 February 2008 / Published online: 19 March 2008

Abstract Case-control studies investigating associations between multiple myeloma (MM) and the *C677T* and *A1298C* polymorphisms of the methylenetetrahydrofolate reductase (MTHFR) have provided controversial results. In an attempt to interpret these results, a meta-analysis of all available studies was performed. In the meta-analysis the pooled odds ratios (OR) were estimated using fixed effects (FE) and random effects (RE) models. The heterogeneity between studies, the sources of potential bias and the consistency of genetic effects across ethnicities were explored. Cumulative meta-analysis was also performed. The meta-analysis revealed non-significant heterogeneity between studies ($P_q \geq 0.65$). The dominant model for the effect of *677T* allele produced significant association overall [FE OR = 1.23 (1.04–1.47)] and in Caucasians [FE OR = 1.54 (1.14–2.08)], but not in East Asians [FE OR = 1.05 (0.82–1.34)]. Although the cumulative meta-analysis for the dominant model of *677T* allele showed a downward trend of RE OR for the period 2000–2007, the association still remained significant. Analysis of the *A1298C* polymorphisms revealed lack of association both in Caucasians and in East Asians. There is an indication of potential bias: a differential magnitude of effect in large versus small studies emerged. In conclusion, the accumulated evidence indicated an association between *MTHFR C677T* polymorphism and MM in Caucasians under a dominant model.

Keywords Multiple myeloma · MTHFR · Meta-analysis · Heterogeneity · Gene · Polymorphism

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Introduction

Multiple myeloma (MM) is a plasma cell malignancy accounting for 10% of all hematological malignancies. Although its etiology and pathogenesis remain unclear, genetic and environmental factors have been implicated (Gonzalez Ordonez et al. 2000; Fonseca et al. 2004). Folate and methionine metabolism play an essential role in DNA synthesis and methylation. Methylenetetrahydrofolate reductase (MTHFR), one of the enzymes involved in folate metabolism, catalyzes the irreversible conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. The latter constitutes the major circulating form of folate, which acts as a methyl donor for the remethylation of homocysteine to methionine (Blount et al. 1997). *MTHFR* gene is located to 1p36.3. Two known polymorphisms in the *MTHFR* gene, *C677T* (Ala222Val) and *A1298C* (Glu429Ala), decrease the enzymatic activity of their respective encoded proteins (Frosst et al. 1995; Weisberg et al. 1998). Reduction in MTHFR activity increases 5,10 methylenetetrahydrofolate, resulting in an increased amount of thymidylate available for DNA synthesis. This in turn lowers the incidence of uracil misincorporation, thereby diminishing the risk of chromosomal injury caused during the repair of the abnormal DNA. MTHFR is

implicated in various diseases/disorders, including vascular disease, neural tube defects and malignancies (Zintzaras 2007a; Zintzaras et al. 2005, 2007; Zintzaras 2006a, b, c; Zdoukopoulos and Zintzaras 2008).

The hitherto case-control studies investigating possible associations between MM and the *MTHFR* gene polymorphisms have provided either controversial or inconclusive results, possibly due to the limited number of patients and controls included in these studies (Lima et al. 2007). This is further hampered by the enrolment of different populations, the use of varying sampling strategies, genotyping procedures, as well as the number of loci examined in the analyses. In an attempt to interpret these contradictory results and to overcome the aforementioned limitations, a meta-analysis of all available studies was performed relating the presence of *C677T* and/or *A1298C* polymorphisms of the *MTHFR* gene with the risk of developing MM (Zintzaras and Lau 2008a, b). In addition, any heterogeneity between studies and potential bias was explored. Cumulative and recursive cumulative meta-analyses were also performed in order to investigate the trend of association as evidence accumulated (Lau et al. 1992; Zintzaras and Lau 2008a).

Methods

Selection of studies

PubMed database was used to locate all relevant studies published up to November 2007. As a search criterion the following keywords were used: "MTHFR" and "multiple myeloma." The retrieved studies were then assessed for inclusion in the meta-analysis. All references cited in the studies were also reviewed to identify additional published work not indexed by the PubMed database. Case reports, editorials and review articles were excluded. The search was restricted to articles in English. Case-control studies that employed validated genotyping methods and examined the prevalence of *C677T* and *A1298C* genotypes in MM patients and appropriate controls were eligible for inclusion. Family studies were excluded owing to different design considerations.

Data extraction

The information extracted included: first author, journal, year of publication, racial descent of the study population, demographics, matching, genotyping method and the number of cases and controls for each *C677T* and *A1298C* genotype. We also recorded whether the genotyping in each study was blinded to clinical status. Where studies involved the investigation of more than one polymorphism, information on linkage disequilibrium and combined genotypes was also recorded.

Meta-analysis

The meta-analysis examined the overall association of *T* allele and risk of MM development compared with *C* allele, the recessive model (*TT* vs. *TC* + *CC*) and dominant model (*TC* + *TT* vs. *CC*) for *T* allele. All associations were indicated as odds ratios (OR) with the corresponding 95% confidence interval (CI). Subsequently, a pooled OR was estimated based on the individual ORs. The heterogeneity between studies was tested using the *Q* statistic (Zintzaras and Ioannidis 2005a, b; Ioannidis et al. 2006). Heterogeneity was considered statistically significant if $P_q < 0.10$. Heterogeneity was quantified with the I^2 metric, a measure that is independent of the number of studies included in the meta-analysis (Zintzaras and Hadjigeorgiou 2004; Zintzaras and Lau 2008a). I^2 measures values between 0 and 100% with higher values denoting a greater degree of heterogeneity. The pooled OR was estimated using fixed effect (Mantel–Haenszel) and random effect (DerSimonian and Laird) models (DerSimonian and Laird 1986). Random effect modeling assumes a genuine diversity in the results of various studies incorporating study variance. Therefore, in cases of heterogeneity between studies the pooled OR was estimated using the RE model (Whitehead 2002).

A cumulative and recursive cumulative meta-analysis was carried out for each polymorphism to evaluate the trend of RE OR over time (Lau et al. 1992). In cumulative meta-analysis, studies were first chronologically ordered by publication year, and the pooled ORs were then obtained at the end of each year, i.e., at each information step. Cumulative meta-analysis shows the evolution of summary effect (OR) over time. In recursive cumulative meta-analysis, the relative change in pooled OR at each information step (OR in next year/OR in current year) was calculated (Zintzaras et al. 2006b). Thus, cumulative and recursive cumulative meta-analyses provide a framework for updating a genetic effect from all studies and a measure to what extent this genetic effect alters as evidence accumulates (Zintzaras 2007b).

The differential magnitude of effect in large versus small studies was assessed using the Egger regression test for funnel plot asymmetry (Egger et al. 1997; Zintzaras and Stefanidis 2005) and the Begg–Mazumdar test, which is based on Kendall's tau (Begg and Mazumdar 1994). Given that these tests are underpowered, they were considered statistically significant for $P < 0.10$. z-statistic was used to assess whether the OR of the first study versus the pooled OR of the subsequent studies were different beyond chance ($P < 0.05$) (Zintzaras et al. 2005). In addition to the main

(or overall) analysis, which included all available data, a subgroup analysis for each ethnicity was also performed (Zintzaras and Lau 2008b). The distribution of the genotypes in the control group was tested for Hardy–Weinberg equilibrium using an exact test (HWE; $P \geq 0.05$) (Weir 1996). Studies with controls not in HWE were subjected to a sensitivity analysis in which the effect of excluding specific studies was examined (Zintzaras et al. 2006c; Zintzaras and Hadjigeorgiou 2005). Analyses were performed using Meta-Analyst (Joseph Lau, Boston, MA, 1998) and Compaq Visual Fortran90 with IMSL library.

Results

Eligible studies

The literature review identified nine titles in PubMed that met the search criteria (Lima et al. 2007; Chiusolo et al. 2006; Kim 2007; Moon et al. 2007; Chen et al. 2006; Gonzalez Ordonez et al. 2000; Gonzalez-Fraile et al. 2002; Lincz et al. 2003; Yanamandra et al. 2003). The full articles were analyzed to assess their appropriateness for meta-analysis. Data from seven articles met the inclusion criteria and were included in the meta-analysis. Two studies were excluded: one was in the Chinese language (Chen et al. 2006) and the other did not provide comprehensive data (Yanamandra et al. 2003). The studies were published between 2000 and 2007. All seven studies dealt with the *C677T* polymorphism, whereas six also investigated the *A1298C* polymorphism. Only one provided data with combined genotypes and reported significant linkage disequilibrium (Moon et al. 2007). The genotyping methods used were: polymerase chain reaction (PCR) and restriction fragment length polymorphism or PCR with allele-specific hybridization probes (Moon et al. 2007). None of the studies provided genotypes according to gender. Controls were reported to be age, sex or ethnicity matched in three studies (Chiusolo et al. 2006; Gonzalez Ordonez et al. 2000; Gonzalez-Fraile et al. 2002). Four studies involved Caucasians (Chiusolo et al. 2006; Gonzalez Ordonez et al. 2000; Gonzalez-Fraile et al. 2002; Lincz et al. 2003), two studies involved East Asians (Kim et al. 2007; Moon et al. 2007), and one study involved mixed population (Lima et al. 2007). The cases were well defined with similar inclusion criteria, although they unavoidably cover a wide spectrum of disease, in terms of clinico-laboratory data and molecular subtypes. The information extracted from the studies included in the meta-analysis is provided in Tables 1 and 2.

Summary statistics

The studies provided 798/3,000 cases/controls for *C677T* and 780/2,795 cases/controls for *A1298C*. Allele *C* and genotype *CT* were the most commonly associated with *C677T* polymorphism in both cases and controls, whereas the genotype *TT* was the least associated. Allele *A* and genotype *AA* were most commonly related to *A1298C* polymorphism in both cases and controls, while genotype

Table 1 Characteristics of eligible studies considered in the meta-analysis

First author, year	Ethnicity	*MTHFR* polymorphisms studied	Selection and characteristics of cases	Selection and characteristics of controls
Gonzalez Ordonez, 2000	Caucasian	*C677T*	26 Patients, mean age = 71 ± 9 years	200 Healthy controls, 25–75 years, ethnicity-matched
Gonzalez-Fraile, 2002	Caucasian	*C677T*, *A1298C*	107 Patients, 8 in stage I, 31 in stage II, 69 in stage III	86 Healthy controls, sex-, age-, ethnicity-matched
Lincz, 2003	Caucasian	*C677T*, *A1298C*	90 Patients (M/F = 62/28), median age = 47 (41–95) years	299 Controls, 18–65 years
Chiusolo, 2006	Caucasian	*C677T*, *A1298C*	100 Patients (M/F = 55/45), median age = 64.5 (36–87) years	100 Healthy controls (M/F = 55/45), median age = 64(35–88) years, age-, sex-matched
Moon, 2007	East Asian	*C677T*, *A1298C*	196 Patients (M/F = 125/71), mean age = 59.1 ± 11.8 years	432 Healthy controls (M/F = 195/239), mean age = 47.3 ± 15.3 years
Kim, 2007	East Asian	*C677T*, *A1298C*	173 Patients, 17 in stage I, 39 in stage II, 103 in stage III, median age = 64(30–77) years	1,700 Population-based controls (M/F = 821/879), mean age = 52.2 ± 14.3 years
Lima, 2007	Mixed (Caucasians and African-Americans)	*C677T*, *A1298C*	123 Patients (M/F = 70/53), 31 in stages I and II, 82 in stage III, 10 with not defined stage, mean age = 57.2 ± 11.4 years	188 Healthy controls (M/F = 121/67), mean age = 53.8 ± 2.9 years

Table 2 The distribution of (a) *MTHFR C677T* and (b) *MTHFR A1298C* genotypes and allele frequencies for cases with multiple myeloma and controls (the respective percentages are shown in parenthesis)

(a)

All studies	Distribution of *MTHFR C677T* genotype						Frequency of *MTHFR C677T* alleles			
First author, year	*TT*		*CT*		*CC*		*T*		*C*	
	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls
Gonzalez Ordonez, 2000			21 (80)[a]	108 (54)[a]	5 (19)	92 (46)				
Gonzalez-Fraile, 2002	11 (12)	9 (11)	48 (53)	32 (40)	31 (34)	38 (48)	70 (38)	50 (31)	110 (61)	108 (68)
Lincz, 2003	8 (8.)	21 (7.)	44 (48)	133 (44)	38 (42)	145 (48)	60 (33)	175 (29)	120 (66)	423 (70)
Chiusolo, 2006	25 (25)	19 (19)	44 (44)	45 (45)	31 (31)	36 (36)	94 (47)	83 (41)	106 (53)	117 (58)
Moon, 2007	36 (18)	94 (21)	103 (52)	196 (45)	57 (29)	144 (33)	175 (44)	384 (44)	217 (55)	484 (55)
Kim, 2007	35 (20)	297 (17)	80 (46)	863 (50)	58 (33)	540 (31)	150 (43)	1457 (42)	196 (56)	1943 (57)
Lima, 2007	14 (11)	17 (9)	57 (46)	79 (42)	52 (42)	92 (48)	85 (34)	113 (30)	161 (65)	263 (69)
Total	129 (16)	457 (15)	397 (49)	1,456 (48)	272 (34)	1,087 (36)	634 (41)	2,262 (40)	910 (58)	3,338 (59)

(b)

All studies	Distribution of *MTHFR A1298C* genotype						Frequency of *MTHFR A1298C* alleles			
First author, year	*CC*		*AC*		*AA*		*C*		*A*	
	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls
Gonzalez-Fraile, 2002	13 (12)	10 (12)	5 (51)	35 (44)	39 (36)	34 (43)	81 (37)	5 5 (34)	133 (62)	103 (65)
Lincz, 2003	9 (11)	31 (10)	43 (53)	139 (47)	29 (35)	124 (42)	61 (37)	201 (34)	101 (62)	387 (65)
Chiusolo, 2006	8 (8)	13 (13)	44 (44)	50 (50)	48 (48)	37 (37)	60 (30)	76 (38)	140 (70)	124 (62)
Moon, 2007	8 (4)	7 (1)	52 (26)	120 (27)	136 (69)	307 (70)	68 (17)	134 (15)	324 (82)	734 (84)
Kim, 2007	6 (3)	53 (3)	51 (29)	500 (29)	116 (67)	1147 (67)	63 (18)	606 (17)	283 (81)	2,794 (82)
Lima, 2007	11 (8)	12 (6)	33 (26)	49 (26)	79 (64)	127 (67)	55 (22)	73 (19)	191 (77)	303 (80)
Total	55 (7)	126 (4)	278 (35)	893 (31)	447 (57)	1,776 (63)	388 (24)	1,145 (20)	1,172 (75)	4,445 (79)

[a] Data concerned TT + CT

CC was the least associated. The genotype distributions and the allele frequencies are shown in Table 2. One study provided data only for T-carriers (Gonzalez Ordonez et al. 2000). In all studies the distribution of the C677T genotype in the control group was in Hardy–Weinberg equilibrium ($P > 0.05$), indicating a lack of genotyping errors and/or population stratification (Zintzaras and Sakelaridis 2007). For A1298C genotype, in one study (Lima et al. 2007) the control group was not in HWE ($P = 0.02$), and a sensitivity analysis was performed for this study. However, testing for HWE was not applicable in one study (Gonzalez Ordonez et al. 2000). One study reported significant linkage disequilibrium between the two polymorphisms (Moon et al. 2007).

Main results, subgroup and sensitivity analyses

The results of the meta-analysis are shown in Table 3 and Fig. 1. The overall analysis investigating the association between *677T* allele and the risk of developing MM relative to the *C* allele revealed non-significant heterogeneity between the studies ($P_q = 0.73$, $I^2 = 0\%$). The FE OR was also not significant: OR = 1.11 (0.96–1.25). However, in subgroup analysis, according to ethnicity, the FE OR was marginally significant in Caucasians [FE OR = 1.26 (1.01–1.59)], whilst non-significant in East Asians [FE OR = 1.02 (0.87–1.20)]. The overall analysis of the recessive model for *T* allele showed non-significant heterogeneity ($P_q = 0.71$, $I^2 = 0\%$) and produced non-significant associations in both Caucasians and East Asians. However, the dominant model for the effect of *T* allele produced significant association overall [FE OR = 1.23 (1.04–1.47); $P_q = 0.20$, $I^2 = 30\%$] and in Caucasians [FE OR = 1.54 (1.14–2.08); $P_q = 0.28$, $I^2 = 22\%$], but not in East Asians [FE OR = 1.05 (0.82–1.34); $P_Q = 0.28$].

The analysis of the *A1298C* polymorphism and its association with MM revealed that the *C* versus *A* allele showed no heterogeneity among studies ($P_q = 0.43$, $I^2 = 0\%$), the FE pooled OR being non-significant: OR = 1.06 (0.91–1.22). Overall, the dominant and recessive models for *C* allele produced no significant

Table 3 Odds ratios and heterogeneity results for the genetic contrasts of (a) *C677T* and (b) *A1298C MTHFR* polymorphisms for multiple myeloma

(a)

C677T contrast	Population	Fixed effects OR (95% CI)	Random effects OR (95% CI)	I^2 (%)	*P* value *Q* test
T versus *C*	All	1.11 (0.96–1.25)	1.11 (0.98–1.26)	0	0.73
	Caucasians	1.26 (1.01–1.59)	1.26 (1.01–1.59)	0	0.91
	East Asians	1.02 (0.87–1.20)	1.02 (0.87–1.20)	na	0.98
Recessive model	All	1.09 (0.87–1.38)	1.10 (0.87–1.39)	0	0.71
	Caucasians	1.29 (0.82–2.05)	1.29 (0.82–2.05)	0	0.90
	East Asians	1.00 (0.75–1.33)	1.00 (0.68–1.46)	na	0.19
Dominant model	All	1.23 (1.04–1.47)	1.27 (1.02–1.58)	30	0.20
	Caucasians	1.54 (1.14–2.08)	1.55 (1.09–2.20)	22	0.28
	East Asians	1.05 (0.82–1.34)	1.05 (0.80–1.36)	na	0.28

(b)

A1298C contrast	Population	Fixed effects OR (95%ci)	Random effects OR (95%ci)	I^2 (%)	*P* value *Q* test
C versus *A*	All	1.06 (0.91–1.22)	1.06 (0.91–1.22)	0	0.43
	All in HWE	1.04 (0.89–1.21)	1.04 (0.88–1.22)	10	0.35
	Caucasians	0.99 (0.79–1.24)	0.98 (0.71–1.35)	48	0.15
	East Asians	1.08 (0.87–1.34)	1.08 (0.87–1.34)	na	0.61
Recessive model	All	1.12 (0.76–1.60)	1.13 (0.79–1.62)	0	0.42
	All in HWE	1.06 (0.72–1.58)	1.08 (0.71–1.65)	13	0.33
	Caucasians	0.86 (0.52–1.41)	0.86 (0.53–1.42)	0	0.61
	East Asians	1.57 (0.84–2.95)	1.62 (0.71–3.68)	na	0.22
Dominant model	All	1.06 (0.88–1.27)	1.06 (0.88–1.27)	0	0.48
	All in HWE	1.04 (0.86–1.26)	1.04 (0.85–1.28)	8	0.36
	Caucasians	1.04 (0.76–1.43)	1.04 (0.65–1.66)	53	0.12
	East Asians	1.04 (0.81–1.33)	1.04 (0.81–1.33)	na	0.86

associations in either Caucasians or East Asians. The sensitivity analysis (exclusion of study with the controls not in HWE) did not alter the pattern of results.

Potential bias

Whether genotyping was blinded to clinical status was not disclosed in any of the studies. The cumulative meta-analysis test of the dominant model of *MTHFR 677T* allele showed a downward trend of RE OR for the period 2000–2007, although the association remained significant. The dominant model for *MTHFR 1298C* allele also showed a downward trend, but the association remained non-significant for the whole period (Fig. 2). In the recursive cumulative meta-analysis of the dominant model of *MTHFR 677T* allele, the relative change in RE OR did not stabilize in a specific OR and persistently approached the OR = 1 (relative change in 2002/2000: 0.62, in 2003/2002: 0.78, in 2006/2003: 0.81 and in 2007/2006: 0.91), indicating the need for more evidence for supporting an association. The analysis of the dominant model of *1298C* showed a relative change in RE OR fluctuating around the value of one (relative change in 2003/2002: 0.99, in 2006/2003: 0.80 and in 2007/2006: 1.01), indicating the permanent lack of association over time.

The Egger test and the Begg–Mazumdar test for the dominant model of *MTHFR 677T* allele revealed a differential magnitude of effect in large versus small studies ($P < 0.01$ and $P = 0.03$, respectively) since the study with the smallest number of cases (Gonzalez Ordonez et al. 2000) showed significant association in contrast to the two larger studies (Kim et al. 2007; Moon et al. 2007). However, there was no statistical difference between the OR of the first study versus the pooled OR of the subsequent studies ($P = 0.09$). The RE OR, without including the first study, was OR = 1.18 (0.99, 1.41), and the between-study heterogeneity was not significant ($P = 0.53$, $I^2 = 0\%$). Generally, in the meta-analyses, there is an indication of potential bias, since a differential magnitude of effect in large versus small studies emerged.

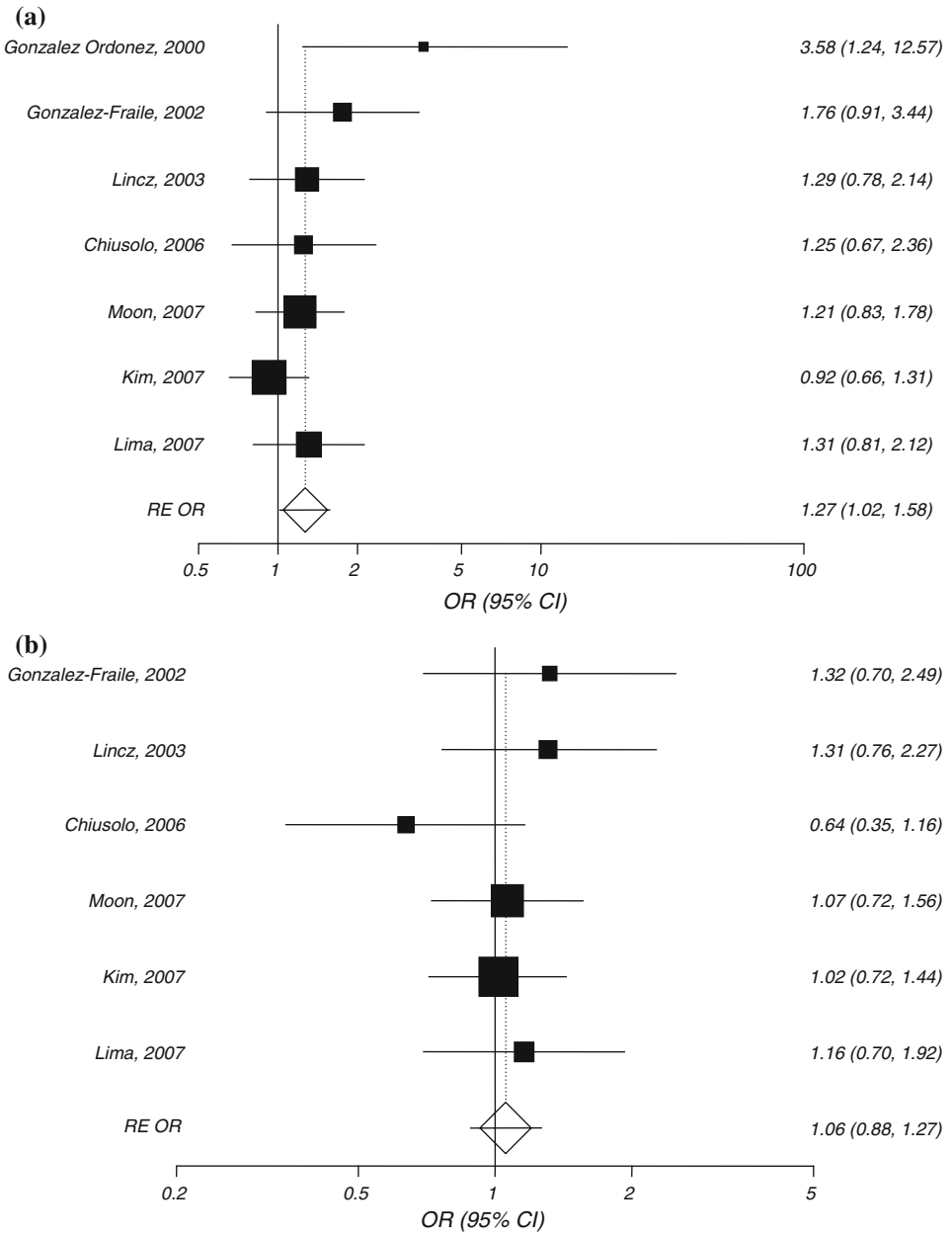

Fig. 1 Random effects (*RE*) odds ratio (*OR*) estimates with the corresponding 95% confidence interval (*CI*) for the dominant model of (**a**) allele *MTHFR 677T* and (**b**) allele *MTHFR 1298 C* and the risk of multiple myeloma. The OR estimate of each study is marked with a *solid black square*. The size of the square represents the weight that the corresponding study exerts in the meta-analysis. The confidence intervals of pooled estimates are displayed as a horizontal line through the diamond; this line might be contained within the diamond if the confidence interval is narrow. The horizontal axis is plotted on a log scale

Discussion

Herein, we report the results of a meta-analysis of gene-canditate studies regarding *C677T* and *A1298C* polymorphisms and their associations with MM. The strength of the present analysis lies in the aggregation of published case-control studies providing more information than the individual studies (Munafo and Flint 2004). Overall, the meta-analysis showed a lack of heterogeneity and non-significant association. However, sub-group analysis of the dominant model of *MTHFR 677T* allele in Caucasians produced statistically significant results and no heterogeneity. There was no consistency in genetic effects between Caucasians and East Asians since the effect of East Asians deviated from the overall effect. Finally, it emerged that the *A1298C* polymorphism was not associated with MM. In *C677T*, the smallest and first published study revealed an association, whereas the large ones did not. Hence, the lack of strong association may be due to the non-replicated genetic associations suggested by earlier small studies (Zintzaras and Lau 2008a, b). Studies may often give very different results, and exploratory, hypothesis-generating findings may not be replicated across several studies, though, variability in the results of different studies does not necessarily represent bias. Furthermore, the results of cumulative and recursive cumulative meta-analyses for C677T polymorphism indicated that more information is required to draw safer conclusions on the magnitude of the risk effect

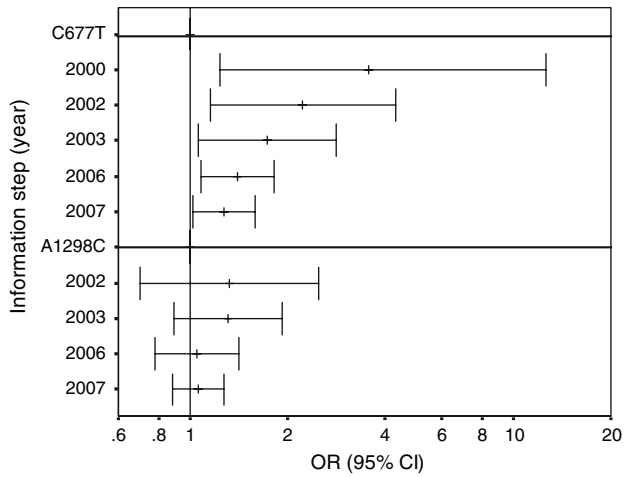

Fig. 2 Cumulative meta-analysis for the dominant models of allele *MTHFR 677T* and allele *MTHFR 1298C*. The random effects odds ratio (*OR*) with the corresponding 95% confidence interval (*CI*) at the end of each year-information step is shown

overall (Zintzaras and Lau 2008a). Regarding the A1298C polymorphism, accumulation of evidence indicated lack of association, though more studies are required to corroborate these findings. However, positive associations were observed between the *MTHFR 677TT* genotypes and increased risk for MM in Caucasians. These findings could also suggest that low folate and elevated homocysteine levels may increase MM risk. Elevated homocysteine levels could result in heightened global DNA hypomethylation, potentially invoking chromosomal instability, reactivation of transposable elements and/or loss of imprinting factors that could contribute to increased MM risk (Ehrlich 2002).

Abnormal gene-specific demethylation and global hypomethylation (involving repeat sequences throughout the genome) could potentially lead to overexpression of protooncogene and activation of transposable elements contributing to neoplastic development (Jones and Baylin 2002). The resultant aberrant transcription and chromosomal instability is believed to contribute to disease onset or progression and increased tumor frequency and malignancy, a disease-specific factor. Global hypomethylation appears to be an early event for colon and breast cancer (Costa et al. 2006; Kondo and Issa 2004; Lin et al. 2001). With regard to hematological malignancies, hypomethylation has been reported in chronic lymphocytic leukemia (Wilson et al. 2007). However, in these cases, it is difficult to define the true "normal" methylation status for comparison. A number of studies indicate a significant overlap in the levels of methylation in neoplastic tissues and their normal counterparts. Consequently, while hypomethylation is habitually associated with malignant development or progression, the critical level of demethylation that fosters the neoplastic process at varying stages of disease has not yet been established. The studies included in our meta-analysis did not provide data according to the presence or absence of chromosomal abnormalities, though, functional MTHFR polymorphisms may be associated with MM cases displaying chromosomal instability by reducing the DNA repair capacity of the evolving clone (Debes-Marun et al. 2003). In other malignancies, such as colorectal cancer, studies have documented an association between the MTHFR and microsatellite instability (Eaton et al. 2005; Hubner et al. 2007). Microsatellite instability is considered a distinct form of chromosomal instability and a defining feature of a distinctive subset of colorectal carcinoma. However, a study of measures of DNA stability in human lymphocytes in vivo failed to detect any correlation between MTHFR polymorphisms and DNA strand breaks, misincorporated uracil and DNA methylation status (Narayanan et al. 2004). This indicates that any effect of MTHFR polymorphisms on human lymphocytes may not be dependent on the induction of chromosomal instability. However, the status of DNA methylation in MM may not influence the effect of *MTHFR C677T* gene polymorphism. In addition, given that the prevalence of genetic changes in Asian patients with MM is considerably different from that reported in Caucasians, the overall lack of association between the effect of *T* allele and susceptibility to MM in this population may indicate a different molecular pathogenesis (Bang et al. 2006). Finally, the overall lack of strong association and any discrepancy of results could be attributed to other loci affecting susceptibility to MM. In this regard, polymorphisms of two other folate-related genes (serine hydroxymethyltransferase and thymidylate synthase) have been associated with a lower risk of hematological malignancies (Skibola et al. 2002).

In summary, the accumulated evidence has indicated an association between *MTHFR C677T* gene polymorphism and risk of MM in Caucasians. However, the findings of the present meta-analysis, being based on a relatively small number of studies and participants, must be interpreted with caution. The relationship between *MTHFR* gene polymorphisms and MM yet remains to be clearly defined, warranting case-control studies that investigate gene–gene and gene–environment interactions to further elucidate the genetics of MM (Kitsios and Zintzaras 2007; Clayton and McKeigue 2001).

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