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ORIGINAL RESEARCH

Plausible relationship between homocysteine and obesity risk via *MTHFR* gene: a meta-analysis of 38,317 individuals implementing Mendelian randomization

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Objective: Numerous studies have explored the role of methylenetetrahydrofolate reductase gene (*MTHFR*) C677T polymorphism and homocysteine (Hcy) concentration in obesity, but the results are inconsistent. Hence, we performed a meta-analysis implementing Mendelian randomization approach to test the assumption that the increased Hcy concentration is plausibly related to the elevated risk of obesity.

Methods: Eligible studies were selected based on several inclusion and exclusion criteria. Correlations between *MTHFR* C677T and obesity risk, *MTHFR* C677T and Hcy concentration in obesity, Hcy concentration, and obesity were estimated by ORs, effect size and standard mean difference with their corresponding 95% CIs, respectively. Furthermore, Mendelian randomization analysis was performed to estimate the relationship between Hcy level and obesity.

Results: Consequently, this meta-analysis implemented with Mendelian randomization approach was conducted among 8,622 cases and 29,695 controls. The results indicated that *MTHFR* C677T is associated with an increased risk of obesity (for T vs C: OR=1.06, 95% CI=1.02–1.10; for TT vs CC: OR=1.13, 95% CI=1.03–1.24). Moreover, in obese subjects, the pooled Hcy concentration in individuals of TT genotype was 2.91 mmol/L (95% CI: 0.27–5.55) higher than that in individuals of CC genotype. Furthermore, the pooled Hcy concentration in subjects with obesity was 0.74 mmol/L (95% CI: 0.36–1.12) higher than that in controls. The evaluated plausible OR associated with obesity was 1.23 for 5 μmol/L Hcy level increase.

Conclusions: Through this meta-analysis, we emphasize a strong relationship between Hcy level and obesity by virtue of *MTHFR* C677T polymorphism.

Keywords: homocysteine, *MTHFR*, obesity, polymorphism

Introduction

Nowadays, many chronic diseases including cardiovascular diseases, hypertension and diabetes mellitus are closely related to obesity and being overweight/obese could strongly elevate the likelihood of these chronic diseases, which is becoming a serious public health issue globally.[1] Twin, family, and adoption studies indicated that the rate of heritability of body mass index (BMI) is high, accounting for 40–70%,[2] indicating that genetic factors have a pivotal role in the pathophysiology of obesity. Thus, detecting genetic factors which caused overweight/obesity could

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be of great significance not only in comprehending the developmental pathogenesis of this disease but also in providing more effective intervention programs to reduce the incidence of obesity.

Methylenetetrahydrofolate reductase (*MTHFR*) is a key rate-limiting enzyme accounting for the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which serves as a crucial enzymatic process in the remethylation of homocysteine (Hcy) to methionine.[3] For the *MTHFR* C677T polymorphism, a single base pair C to T substitution causes an alanine into valine change. As a result, the homozygous *MTHFR* 677TT genotype expresses a heat-sensitive enzyme with reduced activity, which leads to reduced folate level and elevated plasma Hcy level.[3,4] Previous epidemiological studies have indicated that Hcy concentration and folate level were associated with an enhanced risk of overweight/obesity.[5–7] The mechanisms with respect to these observations remain unclear. Nevertheless, some researchers have speculated that enhanced Hcy concentrations might influence the development of obesity by means of controlling body fat storage in the epigenetic regulation of gene expression because the Hcy metabolism pathway is strongly related to methylation of DNA and amino acid residues on histones.[8–11] Moreover, recent researches from genetic studies and animal experiments could stand by this hypothesis.[12–14]

In recent years, there exist numerous studies exploring the association between *MTHFR* C677T polymorphism and obesity. However, it is difficult to draw a definitive conclusion to date because of the controversial results. Additionally, the associations of *MTHFR* C677T polymorphism, Hcy level and obesity are still equivocal. For providing more evidence of the underlying association, we carried out a meta-analysis of the published articles with regard to the risk of obesity associated with an enhanced Hcy concentration and the *MTHFR* C677T polymorphism to obtain pooled estimates of these associations. Moreover, a Mendelian randomization approach, which is acknowledged as an epidemiological method based on the random assignment of an individual's genotype from his/her parental genotypes, was performed to test the assumption that enhanced Hcy concentration is plausibly associated with the elevated risk of obesity.

Materials and methods

Selection of studies

Studies that evaluated the association between the *MTHFR* gene 677 C > T polymorphism and Hcy level with the development of obesity were included in this meta-analysis. A detailed literature retrieval was conducted independently by two investigators for publication from PubMed, Embase and Web of Science by using the following terms: "*MTHFR*", "rs1801133", "*MTHFR* C677T", "homocysteine", "Hcy", "obesity" and "obese", up to 21 September 2018.

The following criteria were used to select the eligible studies: 1) case-control, cross-sectional or case-cohort designed studies; 2) providing the distributions of the *MTHFR* C677T genotypes in obesity and in controls free of obesity, respectively. Reviews or letters, abstract and editorials were excluded. The language was restricted to English.

Data extraction

Data were carefully drawn by two independent investigators, and any disagreements were resolved after discussion with a third investigator. Following information was extracted from each study: 1) the surname of the first author, the publication year, the country and ethnicity of subjects; 2) number of cases, number of controls, the diagnostic standard of cases and controls, genotype distribution in all groups. For articles including different study populations, data were extracted, respectively. Extracted data were analyzed by using the Stata, version 12.0 (StataCorp LP, College Station, TX, USA).

Statistical analysis

For controls in each study, the Chi-squared test was employed to evaluate whether the Hardy–Weinberg equilibrium (HWE) was violated. Sensitivity analysis by removing one study with controls not in HWE was performed to assess the stability of the results.

Four genetic models including homozygous codominant model (TT vs CC), allelic model (T vs C), dominant model (TT+TC vs CC) and recessive model (TT vs TC +CC) are considered, and associations were represented as ORs with their matching 95% CIs for each study. Based on the individual ORs, a pooled OR was concluded. For each of those four models, Metan command in Stata was performed to evaluate the mean difference between *MTHFR* 677TT group and *MTHFR* 677CC group in obese subjects. Hcy level was pooled to compute the standardized mean difference with its corresponding 95% CI for comparing the obese subjects with the healthy ones. Cochrane's Q test[15,16] was carried out to test the between-study heterogeneity (significance at I^2>50.0% and P<0.10). If there is no heterogeneity, we fitted the fixed-effects model to the

data; otherwise, we employed the random-effects model.[17] For the meta-analysis of the association between *MTHFR* C677T and obesity, subgroup analyses by ethnicity and age range (defined age ≥18 as adults, <18 as children) were also performed. We used Begg's funnel plot and Egger's regression test (P<0.05 was considered statistically significant) to estimate publication bias.[18]

Mendelian randomization analysis integrates the information of genotype-intermediate phenotype and genotype-disease association into an analytical framework, which can provide an unbiased estimate of the intermediate phenotype-disease association. For the genetic variant *MTHFR* C677T to be a valid instrumental variable in Mendelian randomization, three conditions are to be satisfied: 1) the *MTHFR* C677T has to be associated with Hcy level robustly; 2) confounding factors, which could bias the association of Hcy level and obesity, should not be associated with the genotype in the *MTHFR*; 3) variant of *MTHFR* 677C > T has an influence on the obesity only through the specific intermediate Hcy level.[19] *MTHFR* C677T may meet all these conditions well based on the available evidence.[9,14,20,21] Therefore, Mendelian randomization coefficient evaluated by utilizing *MTHFR* C677T as an instrument should make a plausible reference to Hcy level and obesity. Compared to *MTHFR* 677CC, the genotype of *MTHFR* 677TT is associated with the increased risk of obesity, and its effect is gauged by the $OR_{TT\ vs\ CC}$. Further, compared with CC, TT is associated with the mean difference (Δ) of Hcy level. $OR_1=(OR_{TT\ vs\ CC})^{1/\Delta}$ would be an unconfounded estimate of the effect of obesity due to one unit change on the Hcy level. In this analysis, we adopted $OR_k=(OR_{TT\ vs\ CC})^{k/\Delta}$ for an increase of k units,[22] and we thus analyzed 5 μmol/L increment in Hcy level to assess the OR.[23]

Results

Characteristics of the studies

The detailed information of screening various studies in the meta-analysis is described in Figure 1. The 20 studies provided 8,622 cases and 29,695 controls,[9,11,24–38] which supplied the genotypes to estimate the association of *MTHFR* C677T and obesity. In these studies, the frequencies of the TT genotype were the lowest in cases and in controls, while that for genotype CC was the highest. Five studies only described the association between *MTHFR* C677T and Hcy level in obese patients.[39–42] In two studies,[9,31] the genotype distribution in the control subjects was not in accordance with HWE (P<0.05) (Table 1).

The mean Hcy concentration difference between *MTHFR* genotypes in obese patients

According to the inclusion criteria, nine studies (8 references, 420 obese patients)[24,26,28,38–42] were selected, and they reported the Hcy concentration in different genotypic groups by means of the arithmetic mean and the corresponding SD in obese patients. In all these studies, none of them was not in HWE, and the mean Hcy level was higher in *MTHFR* 677TT subjects than that in the other genotypes. The pooled mean Hcy level in *MTHFR* 677TT subjects was 2.91 μmol/L (95% CI: 0.27–5.55) higher than that in *MTHFR* 677CC subjects (P=0.031) (Figure 2). Meanwhile, the *MTHFR* 677TT subjects had 3.09 μmol/L (95% CI: (−0.23)-6.42) greater Hcy level than *MTHFR* 677CT subjects (P=0.068) (Figure 3).

The association between *MTHFR* C677T and the risk of obesity

No significant heterogeneity (I^2=6.4%, P=0.377) among 19 studies was shown in the primary outcome for revealing the association of *MTHFR* 677TT with the risk of obesity, compared to *MTHFR* 677CC. The fixed effect (FE) pooled OR was significant: FE OR=1.13 (95% CI: 1.03–1.24) (P=0.007) (Figure 4). Above all, the T allele in the *MTHFR* C677T conferred a higher risk of obesity (FE OR=1.06 [95% CI: 1.02–1.10] [P=0.003], I^2=21.8%, P=0.185) (Figure 5). *MTHFR* 677TT revealed a significantly greater risk for obesity compared with CC+CT genotype (FE OR=1.12 [95% CI: 1.04–1.22] [P=0.003], I^2=0, P=0.558). *MTHFR* 677 TT+CT also revealed a significantly greater risk for obesity compared with CC genotype (FE OR=1.06 [95% CI: 1.00–1.12] [P=0.045], I^2=21.0%, P=0.194). Subgroup analysis by the ethnicity demonstrated its correlations under recessive, homozygous codominant, and allelic models in Asian (TT vs CC+CT: OR=1.20, 95% CI=1.09–1.33, P<0.001; TT vs CC: OR=1.24, 95% CI=1.09–1.41, P=0.001; T vs C: OR=1.11, 95% CI=1.04–1.17, P=0.001). Moreover, subgroup analysis by the age showed the associations under recessive, homozygous codominant, and allelic models in adults (TT vs CC+CT: OR=1.11, 95% CI=1.02–1.22, P=0.022; TT vs CC: OR=1.12, 95% CI=1.01–1.24,

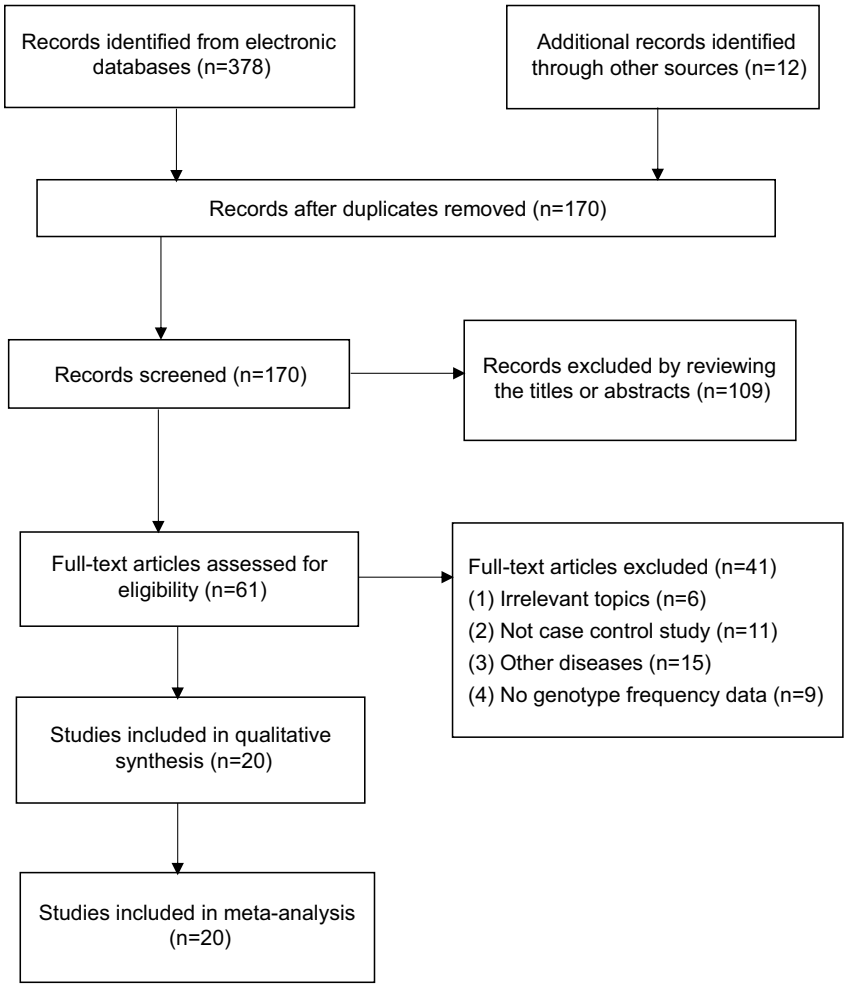

Figure 1 PRISMA flow diagram for selection of studies in the meta-analysis.

P=0.036; T vs C: OR=1.05, 95% CI=1.01–1.10, *P*=0.023) and under recessive and allelic genetic models in children (TT vs CC+CT: OR=1.16, 95% CI=1.00–1.34, *P*=0.05; T vs C: OR=1.09, 95% CI=1.00–1.19, *P*=0.047) with the risk of developing obesity (Table 2).

The associations of plasma Hcy level with obesity

The standard mean difference (SMD) of Hcy level between the subjects with and without obesity indicated the effect on obesity. A forest plot is displayed in Figure 6. In this meta-analysis, there was significant heterogeneity (I^2=95.9%, *P*<0.001) among the included studies. In 19 of these studies,[7,9,24,28,38,43–56] the mean Hcy level was higher in the obese group than that in the control group (Figure 6). The pooled mean Hcy level in the obese group was 0.74 μmol/L (95% CI: 0.36–1.12) higher than that in the control group for the random-effects model (*P*<0.001). The subgroup analysis by ethnicity was carried out, and all the corresponding results from White, Asian, and others showed significant differences in Hcy concentration between obese subjects and control ones. Moreover, both Begg's and Egger's tests were performed to see whether there is potential publication bias. No evidence of substantial publication bias was found for the Hcy–obesity association (data not shown).

The plausible relationship between Hcy level and obesity via Mendelian randomization

By means of *MTHFR* C677T as an instrument variable for Hcy level, the Hcy level per unit increment associated with the predicted OR of obesity by indirect or direct measurement is shown in Figure 7. The Hcy concentrations were

Table 1 The genotypic and allelic distributions of *MTHFR* C677T for cases and controls

First author	Year	Country	Ethnicity	Age range	Genotype distribution						Allele frequency				*P*-value HWE	Number of cases/ controls
					Cases			Controls			Cases		Controls			
					CC	CT	TT	CC	CT	TT	C	T	C	T		
Glueck[24]	2003	America	White	Adults	13	12	3	5	5	0	38	18	15	5	0.292	28/10
Terruzzi[9]	2007	Italy	White	Adults	18	54	12	14	33	5	90	78	61	43	0.026	84/52
Lewis(BWHHS)[11]	2008	UK	White	Adults	360	410	112	1165	1086	283	1130	634	3416	1652	0.214	882/2543
Lewis(ALSPAC for women)[11]	2008	UK	White	Adults	163	155	38	2707	2713	715	481	231	8127	4143	0.375	356/6135
Lewis(ALSPAC for children)[11]	2008	UK	White	Children	115	93	25	2155	2190	552	323	143	6500	3294	0.902	233/4897
Lewis(CCHS)[11]	2008	Denmark	White	Adults	588	574	107	3812	3356	736	1750	788	10,980	4828	0.946	1269/7904
Settin[25]	2009	Saudi	Asian	Adults	89	34	5	69	36	5	212	44	174	46	0.912	128/110
Tavakkoly Bazzaz[26]	2010	Iran	Asian	Adults	44	21	9	113	80	14	109	39	306	108	0.975	74/207
Gara[28]	2011	Tunisian	African	Children	15	14	2	9	12	1	44	18	30	14	0.228	31/22
Bokor[27]	2011	France	White	Children	97	99	17	130	154	33	293	133	414	220	0.2	213/317
Tabassum[30]	2012	Indian	Asian	Children	290	144	20	581	218	31	724	184	1380	280	0.068	454/830
Yin[31]	2012	China	Asian	Adults	354	341	56	471	441	66	1049	453	1383	573	0.006	751/978
Chauhan[29]	2012	Indian	Asian	Adults	348	185	29	272	148	16	881	243	692	180	0.451	562/436
Hernandez-Guerrero[32]	2013	Mexico	Mestizo	Adults	18	38	19	15	28	10	74	76	58	48	0.63	75/53
Fan[33]	2015	China	Asian	Adults	115	244	158	160	375	206	474	560	695	787	0.662	517/741
Chedraui[34]	2016	Ecuador	White	Adults	51	43	17	35	39	7	145	77	109	53	0.399	111/81
Kupcinskiene[35]	2016	Lithuania	White	Adults	156	135	28	159	129	15	447	191	447	159	0.082	319/303
Shen[36]	2016	Canada	White	Adults	190	182	51	181	212	53	562	284	574	318	0.447	423/446
Zhi[37]	2016	China	Asian	Adults	287	633	434	198	438	249	1207	1501	834	936	0.838	1354/885
Fu[38]	2018	China	Asian	Children	121	364	273	531	1353	861	606	910	2415	3075	0.99	758/2745

Abbreviations: *MTHFR*, methylenetetrahydrofolate reductase; HWE, Hardy-Weinberg equilibrium test; The *P*-value for HWE testing for controls is shown; BWHHS, British Women's Heart and Health Study; ALSPAC, Avon Longitudinal Study of Parents and Children women cohort study; CCHS, Copenhagen City Heart Study.

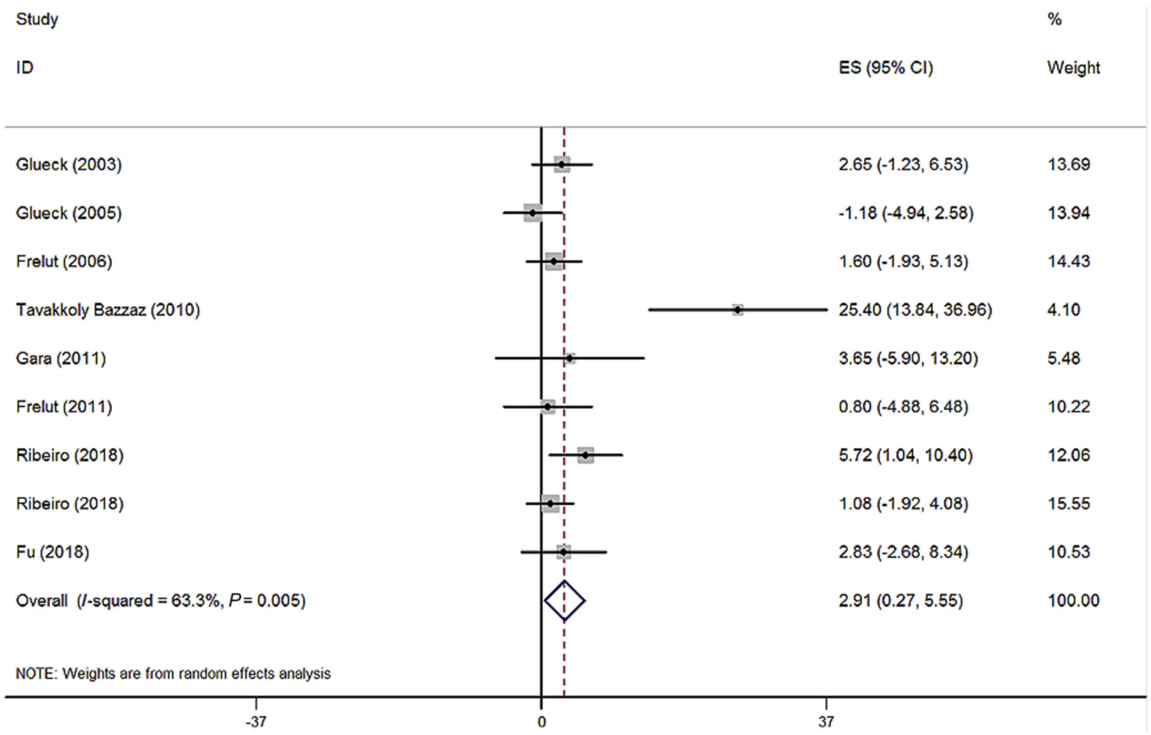

Figure 2 Forest plot of the evaluation for the effect size (ES) in Hcy level between the MTHFR genotypes (TT vs CC) in obese patients.

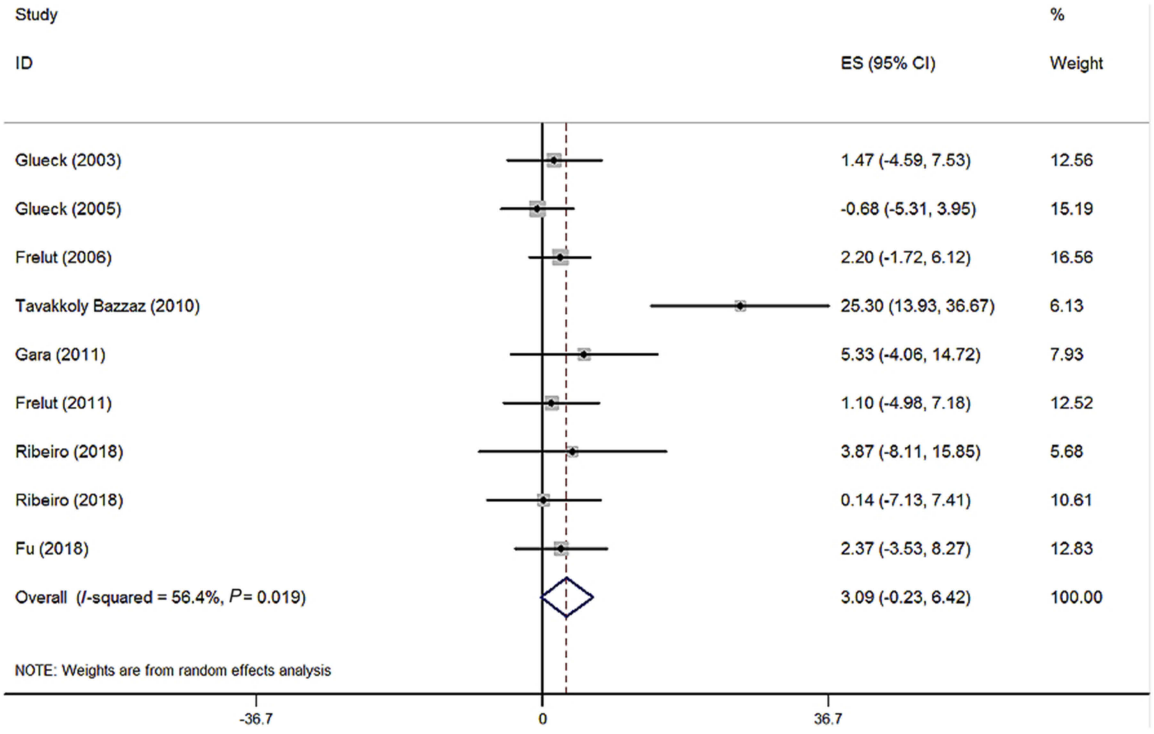

Figure 3 Forest plot of the evaluation for the effect size (ES) in Hcy level between the MTHFR genotypes (TT vs CT) in obese patients.

positively associated with the risk of developing obesity. The evaluated plausible OR was 1.23 (95% CI: 1.05–1.45) for 5 μmol/L Hcy concentration increase.

Discussion

This study indicated that *MTHFR* 677T allele was significantly associated with the increased plasma Hcy level.

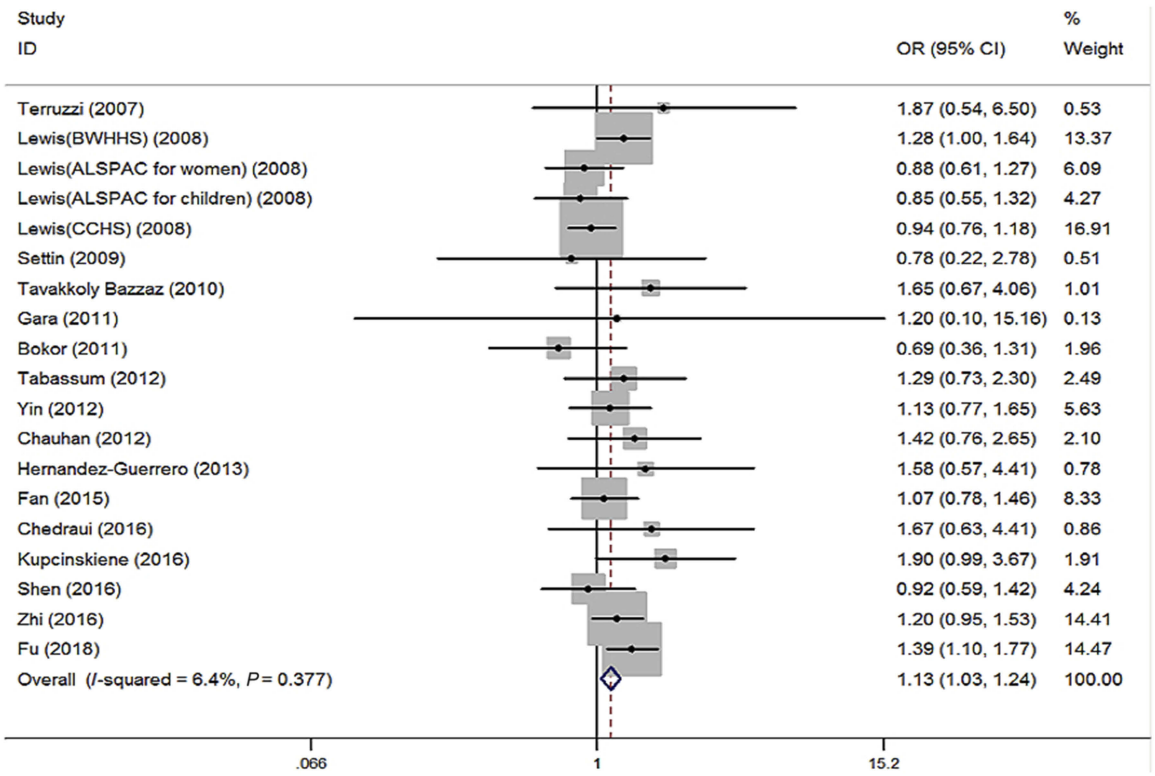

Figure 4 Forest plot of the *MTHFR* C677T associated with obesity risk (under homozygous codominant model: TT vs CC).
Abbreviations: BWHHS, british women's heart and health study; ALSPAC, avon longitudinal study of parents and children women cohort study; CCHS, copenhagen city heart study.

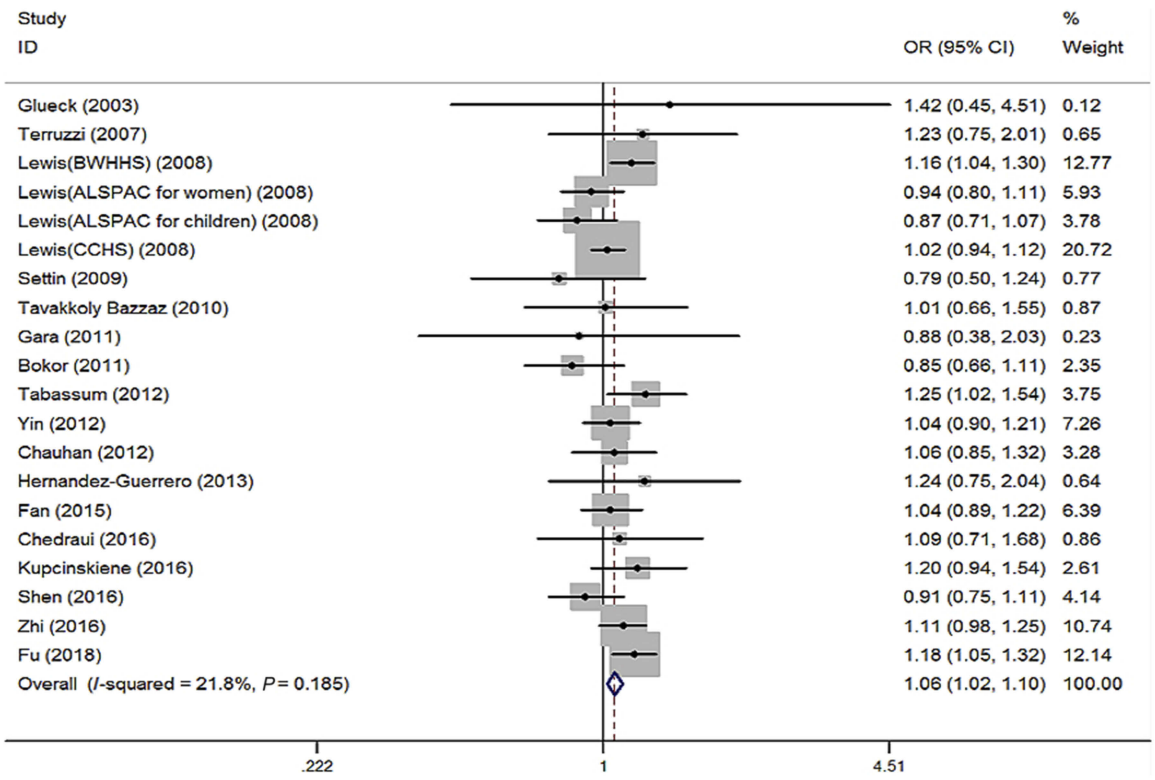

Figure 5 Forest plot of the *MTHFR* C677T associated with obesity risk (under allelic model: T vs C).
Abbreviations: BWHHS, british women's heart and health study; ALSPAC, avon longitudinal study of parents and children women cohort study; CCHS, copenhagen city heart study.

Table 2 Stratified analysis of associations of *MTHFR* C677T polymorphisms with obesity

Subgroup	Dominant		Recessive		Homozygous Codominant		Allelic Model	
	OR (95% CI)	P_h	OR (95% CI)	P_h	OR (95% CI)	P_h	OR (95% CI)	P_h
Overall	1.06 (1.00–1.12)	0.194	1.13 (1.04–1.22)	0.558	1.13 (1.03–1.24)	0.377	1.06 (1.02–1.10)	0.185
Ethnicity								
Asian	1.09 (0.99–1.19)	0.282	1.20 (1.09–1.33)	0.954	1.24 (1.09–1.41)	0.872	1.11 (1.04–1.17)	0.509
White	1.04 (0.97–1.12)	0.111	1.02 (0.90–1.15)	0.283	1.03 (0.91–1.18)	0.152	1.03 (0.97–1.08)	0.117
Others	1.04 (0.55–1.98)	0.448	1.46 (0.65–3.28)	0.996	1.52 (0.59–3.92)	0.842	1.13 (0.74–1.74)	0.491
Age								
Adults	1.05 (0.99–1.12)	0.605	1.11 (1.02–1.22)	0.45	1.12 (1.01–1.24)	0.511	1.05 (1.01–1.10)	0.616
Children	1.08 (0.95–1.23)	0.018	1.16 (1.00–1.34)	0.504	1.19 (0.98–1.43)	0.151	1.09 (1.00–1.19)	0.018

Abbreviations: *MTHFR*, methylenetetrahydrofolate reductase; OR, odds ratio; CI, confidence interval; P_h, *P*-value for heterogeneity test.

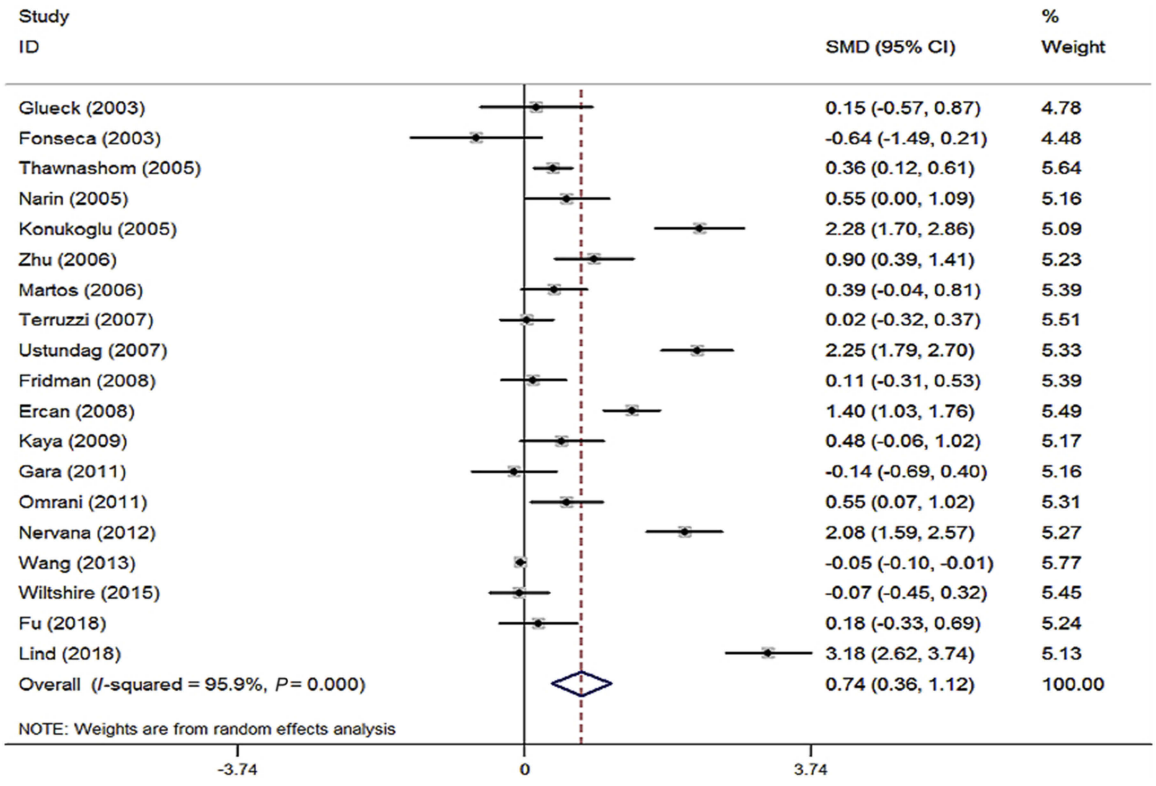

Figure 6 Forest plot of standardized mean difference (SMD) in Hcy levels between obese patients and control subjects in included studies.

Moreover, the mean Hcy level in obese patients was higher than that in those without obesity. The findings by means of Mendelian randomization method reinforced the hypothesis that the increased Hcy concentration plausibly influenced the elevated risk of obesity.

MTHFR C677T is a point mutation that changes cysteine into thymine nucleotide, which results in the substitution of alanine to valine in the *MTHFR* enzyme.[57] Because of the reduced activity of the enzyme, the variant in the MTHFR gene decreases the thermostability of the enzyme, especially at 37°C or greater. Compared to the normal nonmutated controls, the activity of *MTHFR* enzyme in homozygous subjects is lower close to 50–60% at 37°C and 65% at 46°C.[58,59] The deactivation of this enzyme leads to the increased Hcy level in the homozygous subjects. Thus, the Hcy concentration of homozygous subjects is higher than those of heterozygous mutated subjects, and the heterozygous subjects have

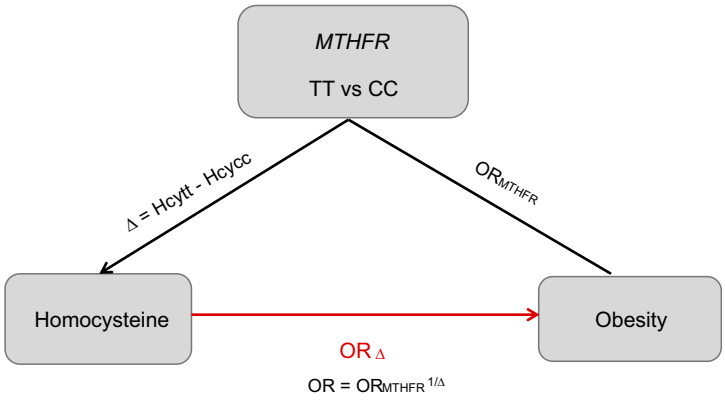

Figure 7 Forest plot of standardized mean difference (SMD) in Hcy levels between obese patients and control subjects in included studies.

mildly elevated the Hcy concentration compared to the nonmutated controls.[59] The findings of our meta-analysis supported the hypothesis that the *MTHFR* C677T was strongly linked with the Hcy level in obese subjects. The homozygous subjects have significantly greater Hcy concentration than that of the heterozygous subjects in obese patients, as previously reported in the literature.[26]

Previous studies have reported inconsistent results with regard to the altered Hcy concentrations in obese patients. Recently, a research on 3,833 obese patients and 3,367 normal controls found that the level of Hcy in obese patients was lower than that in normal controls.[54] However, other studies indicated that the Hcy concentrations were significantly greater in obese patients than that in subjects without obesity.[52,53,56] The present meta-analysis mainly analyzed the weighted mean difference of Hcy levels between obese cases and normal controls, which suggested that the absolute pooled mean Hcy level in obesity was significantly greater than that in controls. Due to the heterogeneity of subjects encompassed in these studies concerning the ethnicity of different regions and the coexistence of obesity-related disease,[52–54,56] we applied a random-effects model to reduce the heterogeneity. Hcy level has a crucial influence on the process of regulating the correlation between methylation of DNA and amino acid residues on histones. This process has been recognized as one of the epigenetic mechanisms that regulate the gene expression.[9–11,13,14] The improved Hcy concentrations might affect the progress of developing obesity by means of regulating gene expression in body fat accumulation. Recently, research on genetics and animal experiments seem to elucidate this hypothesis.[12–14] Overall, the homocysteine metabolism pathway might have a substantial role in leading to obesity.

MTHFR C677T was first identified as a significant variant associated with obesity in a Thai population.[46] Subsequently, another study suggested that *MTHFR* 677T allele had an elevated obesity risk with a 1.24-fold compared with *MTHFR* 677C allele in Indian children.[30] Although a large number of studies have assessed the associations between *MTHFR* C677T and overweight/obesity, the results are controversial in different populations.[9,11,25,26,28,30,33,46,60] Our previous study attempted to investigate the relationship between *MTHFR* C677T and obesity-related traits in a Chinese children population. As a result, we demonstrated that *MTHFR* 677T had an effect on elevating obese risk in Chinese children.[38] The reasons why contradicted results exist in studies concerning *MTHFR* C677T and obesity are still unclear, but a vital reason might be the racial heterogeneity in the included studies. The distribution frequency of the 677TT genotype was greatest in the Italian and Hispanic.[61] However, the homozygous frequency was very low for Blacks in Brazil and American.[62–64] Furthermore, the study design flaws, small sample size or other biases seem to be more common factors for the discrepancies comprising in different studies concerning genetic factors.[65,66] On the basis of case–control, cross-sectional or case–cohort designed studies, the overall results of the present meta-analysis suggested that *MTHFR* C677T is associated with obesity and *MTHFR* TT genotype has an influence on increasing the risk of obesity. In addition, sensitivity analysis suggested that an omission of studies that depart from HWE did not change the magnitude of the observed effect, indicating that the results were generally reliable and robust.

To the best of our knowledge, this is the first meta-analysis aggregating all the data available for evaluating the association between *MTHFR*-linked Hcy level and obesity. Thus, it has provided the most substantial data

on this issue. However, several limitations should be addressed. First, findings from this meta-analysis pooled individual unadjusted results, while potential confounders including age and gender should be taken into account for a more accurate estimate. Second, Hcy measurement via different HPLC and immunoassay approaches showed discrepancies among different laboratories,[67] and they may differ in their sensitivity and cutoff values. Therefore, different methods of Hcy measurement seem to result in variation among studies, and this cannot be ignored as an obvious flaw. Third, the genotyping of this SNP was performed by different analytic methods, which could influence the results. Note that both studies,[9,31] where the genotype distribution in the control groups was not in accordance with HWE employed the same genotyping method. However, by removing the two studies that depart from HWE, we confirmed the result did not change a lot. Finally, only English articles were included, which might ignore the publication bias, although both Begg's and Egger's tests showed no evidence for the existence of substantial publication bias concerning the small effect size.

In conclusion, our results provided sufficient evidence that the TT genotype in *MTHFR* C677T plausibly leads to the susceptibility of obesity. Through Mendelian randomization, the findings supported the assumption that increased Hcy concentration is strongly linked to elevated risk of obesity. To some degree, the presence of gene–environment interactions may contribute to the discordance of results encompassed in the genetic association studies. Therefore, future prospective studies that explore the gene–environment interaction with larger sample size are expected to help further illuminate the genetics of obesity.

Acknowledgments

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Author contributions

Study concept and design: LF and YQH; acquisition of data: LF, YQH, and YL; analysis and interpretation of data: LF and YL; drafting of the manuscript: LF and YL; critical revision of the manuscript for important intellectual content: LF, YL, DL, SD and YQH. All authors contributed to data analysis, drafting and revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interests in this work.

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