Methylenetetrahydrofolate reductase gene rs1801133 polymorphism and essential hypertension risk from a comprehensive analysis

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Abstract

Background

Essential hypertension (EH) is common and multifactorial disorders likely to be influenced by multiple genes. The methylenetetrahydrofolate reductase (MTHFR) gene rs1801133 polymorphism is related to MTHFR enzyme activity and to plasma homocysteine concentration. In addition, variations in MTHFR functions likely play roles in the etiology of EH. So far, larger number of studies between *MTHFR* rs1801133 polymorphism and EH have provided controversial or inconclusive results. To better assess the purported relationship, we performed a comprehensive analysis of 50 publications.

Methods

Eligible studies were identified by searching the PubMed, Wanfang and CNKI databases. Odds ratios (ORs) with 95% confidence intervals (CIs) were estimated to assess this association.

Results

Overall, increased significant associations were detected between *MTHFR* rs1801133 polymorphism and EH risk (such as T vs. C: OR = 1.37, 95%CI = 1.24–1.52, P = 0.000), the same as in race subgroup (Asian: T vs. C: OR = 1.46, 95%CI = 1.29–1.67, P = 0.000; China: T vs. C: OR = 1.51, 95%CI = 1.30–1.74, P = 0.000). Similar associations were also found in source of control and genotype methods subgroups.

Conclusions

Our study showed evidence that *MTHFR* rs1801133 null genotype may increase EH risk. Future studies with larger sample size are warranted to further evaluate this association in more detail.

Background

Essential hypertension (EH) has a high prevalence rate worldwide, which is considered as a complex interaction of diverse gene-gene or environmental conditions[1, 2]. The present evidence-based treatment of EH is a critical intervention in reducing cardiovascular (CV) morbidity and mortality[3]. A contemporary meta-analysis of 123 studies with 613,815 hypertensive participants showed for every 10-mm Hg reduction in systolic blood pressure, there is a significant decreasing of the risk of major CV disease events (relative risk 0.80, 95% confidence interval [CI] 0.77–0.83), coronary heart disease (0.83, 0.78–0.88), stroke (0.73, 0.68–0.77), and heart failure (0.72, 0.67–0.78)[4]. EH accounts for 90%-95% of all patients with hypertension, and about 20%-60% of its etiology is related to genetic factors[5].

Because a high plasma concentration of homocysteine (Hcy) may predispose to atherosclerosis by injuring the vascular endothelium, which results in hypertension. Elevated Hcy has been identified as an independent risk factor for hypertension[6–9]. Methylenetetrahydrofolate reductase (MTHFR) plays an important role in the metabolism of Hcy. The C/T mutation of C667T site (rs1801133, a C to T transition at nucleotide position 677 in exon 4 generates an alanine to valine change at amino acid 222) of MTHFR gene may lead to the decrease of MTHFR activity and heat tolerance, which may lead to the metabolic damage of Hcy, and then to moderate increase of plasma Hcy level[10, 11]. Above information indicated that *MTHFR* C677T or rs1801133 may related to EH development and susceptibility. It makes sense to demonstrate the association between this polymorphism and EH risk, which may provide guidance for the prevention and diagnosis of EH in clinic.

So far, numerous studies have reported the association between *MTHFR* rs1801133 polymorphism and EH risk, however, this relationship remains ambiguous and controversial. We performed a comprehensive analysis including 50 different case-control studies to achieve a convinced conclusion.

Materials And Methods

Identification of eligible studies

The PubMed, Wanfang and CNKI databases (updated on Dec 30, 2020) were applied using following relative keywords: polymorphism/variant/mutation, hypertension/essential hypertension, and MTHFR/methylenetetrahydrofolate reductase. We included all studies that described the relationship between *MTHFR* rs1801133 polymorphism and EH susceptibility. All included studies should meet all of the following criteria: (1) association between *MTHFR* rs1801133 polymorphism and EH risk; (2) case-control study; (3) each genotype frequency is shown in Tables; (4) genotype distributions of control consistent with Hardy-Weinberg equilibrium (HWE) about control were more than 0.05.

Data extraction

We collected following information in our analysis: first author's last name, year of publication, origin for ethnicity, sample size (cases/controls), study design, HWE of controls and genotype methods, number of genotype frequency in cases/controls.

Quality assessment

In our current meta-analysis, the quality was assessed using the Newcastle-Ottawa Scale (NOS) for cross-sectional study quality assessment tool. The methodological quality of each study (sampling strategy, response rate, and representativeness of the study), comparability, and outcome were checked using the NOS tool. Studies with a score of more than 7 out of 10 were considered as achieving good quality. This cut-off point was declared after reviewing relevant kinds of literature[12].

Statistical analysis

The extracted data were imported to Stata software (version 10.0, Corporation, College Station, Texas) for analysis. Odds ratios (ORs) with 95% confidence intervals (CIs) were used to measure the strength of the association[13, 14]. Subgroup analysis stratified by race was performed first. Race was categorized as European, Asian, Mixed, China and non-China five types. Source of control subgroups were performed on two classifications: hospital-based (HB) and population-based (PB). For *MTHFR* rs1801133, we investigated the relationship between genetic variants and LC risk in five different models (T-allele vs. C-allele, TT vs. CC, TC vs. CC, TT+TC vs. CC and TT vs. TC+CC).

Heterogeneity evaluation within the included studies was assessed using Cochrane's *Q* test (Chi-square) and I^2 (%) statistics. A fixed effect model will be applied when the effects are assumed to be homogenous (P>0.05, $I^2 \leq 50\%$); otherwise, the random effect model will be adopted (P<0.05, $I^2 \geq 50\%$).[15, 16] If heterogeneity is existed, to explore the source of heterogeneity, subgroup analysis will be performed through the ethnicity, publication year, study design and genotype methods.

The presence of potential publication bias is determined through the Egger/Begg's test and presented graphically by a funnel plot[17]. In addition, the departure of frequencies of *MTHFR* polymorphism from expectation under HWE is assessed by χ^2 test in controls using the Pearson chi-square test[18]. Another, sensitivity analysis is conducted to assess the stability of the results. Finally, the power and sample size analysis of our meta-analysis was calculated by a program called PS: Power and Sample Size Calculation

(http://biostat.mc.vanderbilt.edu/wiki/Main/PowerSampleSize#Windows)[19].

Genotyping methods

Genotyping for SNP of *MTHFR* gene polymorphisms was conducted using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), TaqMan, sequencing, PCR, amplification refractory mutation system-PCR (ARMS-PCR) and high-resolution melt (HRM) genotyping facility.

Meta-regression

Considering the subgroup of publication year, ethnicity, source of control, genotype method as independent variables and the log as dependent variable, the random-effect meta-regression results were presented to definite the source of publication bias[20].

Gene interaction network of MTHFR gene

In order to fully understand the role and potential and functional partners of MTHFR in EH, String online server (http://string-db.org/) uses the gene-gene interaction network of MTHFR gene [21, 22].

Results

Study selection and characteristics in our meta-analysis

We established databases according to the extracted information from each article. Using the keywords, we identified 353 articles from PubMed, 56 from CNKI and 362 from Wanfang databases. 547 articles were excluded after reading abstract section and 224 articles were left for full article evaluation. Among them, 38 article were about systematic analysis/meta-analysis/review; 24 just only were offered case group; 16 articles were duplicated with including other papers; 34 had no original numbers for case/control groups, only showed total numbers; 27 article were about H-type hypertension; 4 were related to aortic hypertension and 26 were hypertension-in-pregnancy (Figure 1). After above review, 54 articles about 55 case-control studies were left, in which 5 case-control studies were not consistent with HWE and were excluded, finally, 50 case-control studies from 54 different literatures were included in our current analysis. All essential information was listed in Table 1. Table 1 showed first author, publishing year, race, the numbers of cases and controls, HWE, genotype numbers in cases/controls, study design and genotype methods. So in our study, there are 10 European case-control studies, 36 Asian case-control studies and 4 Mixed. The T frequency in Asians was 45.55%, in Europeans was (47.88%), and in Mixed was (47.38), which did not exist statistically significant (P > 0.05). The distribution of genotypes in all the controls was agreement with HWE. In addition, we checked the Minor Allele Frequency (MAF) reported for the seven main worldwide populations in the 1000 Genomes Browser[23] (https://www.ncbi.nlm.nih.gov/snp/rs1801133): Global (0.335); European (0.345); East Asian (0.328); South Asian (0.167); African (0.123); African American (0.125); Asian (0.265) (Figure 2A). In order to observe the frequency of T-allele and C-allele both in case and control groups, we analyzed and found the frequency between case and control was pretty much the same (Figure 2B). Finally, we analyzed the trend of rs1801133 polymorphism from TCGA database, TT (AA) frequency was relatively low than other genotypes (Figure 2C). This polymorphism is associated with coronary artery, rather than aorta artery left ventricle and tibial artery (https://www.gtexportal.org/home/) (Figure 2D).

Quantitative data synthesis

Table 2 showed that the summary odds ratios of *MTHFR* based on10533 EH cases and 11743 matched controls, we observed increased association between the *MTHFR* rs1801133 polymorphism and EH in total population (for example: T-allele vs. C-allele: OR=1.37, 95%CI=1.24-1.52, P_h<0.001, p<0.001, I-squared=82.1%, Figure 3A). Then, subgroup by ethnicity analysis, similar trend was also observed (T-allele vs. C-allele: OR=1.46, 95%CI=1.29-1.67, P_h <0.001,

p<0.001, I-squared=84.8% for Asian; OR=1.27, 95%CI=1.07-1.51, P_h =0.008, p=0.007, I-squared=59.7% for European; OR=1.51, 95%CI=1.30-1.74, P_h <0.001, p<0.001, I-squared=86.9% for China and OR=1.21, 95%CI=1.06-1.37, P_h <0.001, p=0.003, I-squared=66.4% for non-China, Figure 3B). In order to analyze the source of control and find the source of heterogeneity, HB and PB subgroups were calculated, significant increased relationships were shown (T-allele vs. C-allele: OR=1.49, 95%CI=1.28-1.75, P_h <0.001, p<0.001, I-squared=85.4% for HB; T-allele vs. C-allele: OR=1.27, 95%CI=1.12-1.44, P_h <0.001, p<0.001, I-squared=77.2% for PB, Figure 3C). Different methods for detecting this polymorphism were applied in all including studies, we tried to find whether positive results may be observed, finally, some significant findings were found, such as PCR (T-allele vs. C-allele: OR=1.48, 95%CI=1.14-1.91, P_h <0.001, p=0.003, I-squared=85.1%), PCR-RFLP (T-allele vs. C-allele: OR=1.49, 95%CI=1.30-1.72, P_h <0.001, p<0.001, I-squared=65.1%) and HRM(T-allele vs. C-allele: OR=1.32, 95%CI=1.15-1.51, P_h <0.001, p<0.001, I-squared=47.5%) (Figure 3D, E).

Publication bias and sensitive analysis

The Begg's funnel plot and Egger's test were performed to access the publication bias of literature. Significantly obvious evidence of publication bias was detected in five genetic model analysis (such as Figure 4 A, B about T-allele vs. C-allele) (Table 3).

To delete studies which may influence the power and stability of the whole study, the sensitive analysis was applied, finally, no sensitive case-control studies were found for this SNP in five models (such as Figure 4C about T-allele vs. C-allele).

Meta-regression

The analysis showed significant relationship for allele model (T-allele vs. C-allele) for the ethnicity, source of control and genotype methods with a regression coefficient of 0.001, 0.004, 0.010 and 0.002, respectively, rather than publication year, which means the heterogeneity from rs1801133 polymorphism in EH may be from the ethnicity, source of control and genotype methods subgroups (Figure 5A-E).

Gene-gene network diagram and interaction of online website

String online server indicated that MTHFR gene interacts with numerous genes. The most ten significant genes from network of gene-gene interaction has been listed in Figure 6A,B. The ten genes are: methionine (MTR); thymidylate synthase (TYMS); C-1-tetrahydrofolate synthase (MTHFD1); serine hydroxymethyltransferase 1 (SHMT1); serine hydroxymethyltransferase 2 (SHMT2); bifunctional methylenetetrahydrofolate dehydrogenase (MTHFD2); probable bifunctional methylenetetrahydrofolate dehydrogenase (MTHFD2L); aminomethyltransferase (AMT) and methionine synthase reductase (MTRR).

Discussion

The cause of hypertension is unknown, the risk factors include genetic factors, age and unhealthy lifestyle, among which 70–80% of hypertension is related to unhealthy lifestyle. As the risk factors for high blood pressure accumulate, the risk of high blood pressure increases[24, 25].

Most people with hypertension do not have typical symptoms, it is easy to be ignored and can't go to see a doctor in time. Therefore, how to identify high-risk patients is a very meaningful work, which can predict the high-risk patients with high blood pressure as early as possible, timely monitor blood pressure, follow-up regularly, pay attention to improve unhealthy lifestyles. Once the blood pressure is rising, it can be immediate for detection, gain treatment timely, avoid complications, and ultimately reduce the incidence of hypertension and improve the quality of life of patients[3, 26, 27].

The detection of significant polymorphisms may be the suitable method to forecast the possible of individuals to the risk of hypertension. Our current study focused on EH, a common type of hypertensions, and included 10533 EH patients and 11743 health individuals. In the overall analysis, we found individuals carried TT or T-allele may increase EH risk than CC or C-allele (between 37% and 89%). Significant heterogeneity was indicated in all genetic models, so to search the source, we analyzed the associations in other subgroups, such as ethnicity, source of control and genotype method. In the same time, significant relationships were also observed from ethnicity, source of control and genotype method subgroups, which were proved that rs1801133 polymorphism was a risk factor for EH in further. In addition, we used the meta-regression to evaluate the source of heterogeneity, finally, which came from above three subgroups. The power of our study was 1, which suggested our conclusions was convince and persuasive.

Previous, several meta-analyses about rs1801133 polymorphism and hypertension have been published. For example, Wu et al. included 30 case-control studies and supported rs1801133 polymorphism played a risk role in developing EH[28]. Kosmas et al. identified 23 comparisons related hypertensive disease of pregnancy and gained a conclusion that T-allele of rs1801133 polymorphism could increase the hypertensive in pregnancy by 1.21-fold[29]. Third, Qian et al. just combined 26 published studies both for hypertension and hypertension-in-pregnancy, and suggested rs1801133 polymorphism could be one independent risk factor[30]. Fourth, Yang et al. performed a meta-analysis of 27 studies including 5418 EH and 4997 controls and supported the evidence the carriers of the rs180113T allele were susceptible to EH susceptibility[31]. Above studies have some disadvantages: first, the HWE in several studies were not consistent with more than 0.05, which may increase the heterogeneity and reduce the power of conclusions; second, each study omitted other related case-control studies from, our present study is a relative comprehensive analysis; third, some articles did not distinguish the types of hypertension, because different kinds of hypertension have different etiology, pathogenesis and genetic background, so it is better to focus on one kind; fourth, our analysis increased genotype subgroup and evaluation of power; fifth, we analyzed gene-gene interactions between the related genes and MTHFR to make clear the potential function. However, the conclusions from our current study was similar with previous meta-analysis.

The bright spot from our study was the gene-gene interaction. We showed the significant ten genes. The average score is more than 0.9, the top three genes are MTR (0.995), TYMS (0.992) and MTHFD1 (0.989). MTHFR and MTR both participate the homocysteine metabolism, which regular different stages, MTHFR converts 5,10-methylene-THF into 5-methyl-THF, however, MTR catalyzes the demethylation of 5-methyl-THF to THF and the re-methylation of homocysteine to methionine[32, 33]. MTR 2756 A/G polymorphism also was associated with hypertension risk[34].

Some limitations in our meta-analysis should be considered. Beginning, the heterogeneity was found in our study, which came from ethnicity, source of control and genotype methods, further studies should optimize the design of retrospective and prospective studies to overcome this deficiency. Second, EH is a complex disease including genetic and other factors (such as environment, diet, concomitant disease, etc.)[35], studies should analyze the gene-gene or gene environment interactions with larger sample studies. Third, further meta may include all kinds of hypertension, and analyze association for each kind and find the different genetic background. Fourth, the specific mechanism of rs1801133 polymorphism should be explored.

In present, our meta-analysis showed the evidence that *MTHFR* 677T null genotype was associated with increased EH risk. Therefore, further well-designed large studies are necessary. Also, we should focus on the mechanism of rs1801133T-allele in animal model to explain the complete chain of evidence for prevention of EH in the future.

Abbreviations

EH: essential hypertension; MTHFR: methylenetetrahydrofolate reductase; SNP: single nucleotide polymorphism; HB: hospital-based; PB: population-based; SOC: source of control; PCR-RFLP: ARMS-PCR: amplification refractory mutation system-PCR; HRM: high-resolution melt.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Links for database

1000 Genomes Browse: https://www.ncbi.nlm.nih.gov/snp/rs1801133

PubMed: https://pubmed.ncbi.nlm.nih.gov/

CNKI: https://kns.cnki.net/kns/brief/default_result.aspx

Wanfang: https://pubmed.ncbi.nlm.nih.gov/

Power: http://biostat.mc.vanderbilt.edu/wiki/Main/PowerSampleSize%5C043Windows

String: http://string-db.org/

Competing interests

The authors declare that they have no competing interests.

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Author's contributions

YF and LW designed the study and drafted the manuscript; LW extracted, analyzed, interpreted the data, and collected the clinical data; LW and WZ performed the targeted sequencing, analyzed and interpreted the data; LW and WZ participated in the study coordination and revised the manuscript. All authors read and approved the final version of the manuscript.

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Tables

Table 1 Characteristics of studies of *MTHFR* gene rs1801133 polymorphism and essential hypertension risk included in our meta-analysis

Author	Year	Country	Ethnicity	Case	Control	Case			Control			SOC	HWE	Genotype	NOS
						TT	TC	CC	TT	TC	CC				
Gao	1999	China	Asian	127	170	15	68	44	24	84	62	PB	0.600	PCR-RFLP	7
Wang	2002	China	Asian	105	46	37	51	17	9	23	14	HB	0.935	PCR-RFLP	6
Sun	2003	China	Asian	55	173	27	22	6	18	69	86	HB	0.456	ARMS-PCR	6
Liu	2005	China	Asian	100	100	26	45	29	19	50	31	HB	0.883	PCR	7
Li	2006	China	Asian	26	30	2	6	18	2	7	21	HB	0.226	PCR-RFLP	6
Hu	2006	China	Asian	110	115	16	39	55	12	42	61	PB	0.249	PCR	7
Tang	2007	China	Asian	252	195	20	93	139	6	51	138	HB	0.629	PCR-RFLP	6
Lin	2008	China	Asian	50	123	4	27	19	6	44	73	HB	0.847	PCR	6
Zhang	2012	China	Asian	189	165	8	53	128	7	41	117	PB	0.175	PCR-RFLP	8
Yin	2012	China	Asian	670	682	68	358	244	51	309	322	PB	0.096	PCR-RFLP	8
Yao	2013	China	Asian	150	150	49	69	32	22	67	61	HB	0.607	PCR-RFLP	7
Cai	2014	China	Asian	200	200	62	99	39	50	89	61	PB	0.129	PCR	7
Liu	2019	China	Asian	934	1075	295	439	200	356	505	214	HB	0.151	Taqman	7
Ghogomu	2016	China	Asian	41	50	14	24	3	0	5	45	HB	0.709	PCR	6
Dai	2015	China	Asian	114	104	32	57	25	26	49	29	PB	0.562	PCR-RFLP	8
Wen	2015	China	Asian	174	634	76	53	45	85	291	258	PB	0.837	PCR	7
Wu	2016	China	Asian	123	120	11	39	73	10	40	70	PB	0.223	Gene Chip	7
Fan	2016	China	Asian	214	494	75	102	37	141	234	119	HB	0.493	TaqMan	6
Zhao	2017	China	Asian	200	200	47	99	54	29	91	80	HB	0.705	PCR-RFLP	7
Sui	2020	China	Asian	102	109	31	52	19	22	49	38	HB	0.397	HRM	6
Yu	2020	China	Asian	137	128	5	47	85	5	42	81	HB	0.877	TaqMan	6
Yu	2020	China	Asian	163	160	31	79	53	27	76	57	HB	0.845	TaqMan	7
Nong	2019	China	Asian	122	110	49	58	15	16	59	35	PB	0.267	PCR-RFLP	8
Zhao	2016	China	Asian	100	100	23	50	27	15	45	40	HB	0.689	PCR-RFLP	7
Tian	2018	China	Asian	743	718	203	373	167	148	370	200	HB	0.333	HRM	7
Sui	2019	China	Asian	113	73	44	50	19	10	41	22	HB	0.186	PCR-RFLP	7
Liu	2020	China	Asian	934	1075	295	439	200	356	505	214	PB	0.151	TaqMan	7
Zhang	2020	China	Asian	741	538	164	313	264	92	268	178	PB	0.603	TaqMan	8
Nishio	1996	Japan	Asian	47	82	5	26	16	9	44	29	HB	0.201	PCR	7
Nakata	1998	Japan	Asian	173	184	19	91	63	36	83	65	HB	0.309	PCR	7
Lwin	2006	Japan	Asian	116	219	19	58	39	38	117	64	PB	0.215	PCR	8
Hui	2007	Japan	Asian	261	271	49	129	83	44	123	104	HB	0.454	PCR-SSCP	6
Markan	2007	India	Asian	153	133	8	40	105	0	28	105	PB	0.174	PCR-RFLP	8
Nassereddine	2015	Morocco	Asian	101	102	14	40	47	3	45	54	PB	0.074	PCR-RFLP	7
Candrasatria	2020	Indonesia	Asian	213	202	6	73	134	3	42	157	PB	0.920	TaqMan	7
Arina	2019	Indonesia	Asian	53	53	5	16	32	0	10	43	HB	0.448	PCR-RFLP	7
Benes	2001	Czech	European	193	209	27	93	73	17	106	86	PB	0.045	PCR	7
Kahleova	2002	Czech	European	164	173	27	55	82	18	69	86	PB	0.457	ARMS-PCR	8
Rodriguez-Esparragon	2003	Spain	European	232	215	34	115	83	20	100	95	PB	0.386	PCR	8
Heux	2004	New Zealand	European	247	249	35	125	87	25	119	105	HB	0.298	PCR-RFLP	7
Tylicki	2005	Austria/Poland	European	90	90	11	39	40	10	38	42	PB	0.752	PCR	7
Ilhan	2008	Turkey	European	78	100	10	32	36	2	26	72	HB	0.844	real-time PCR	7
Deshmukh	2009	USA	European	42	118	4	16	22	18	48	52	PB	0.221	sequencing	8
Ng	2009	Australia	European	38	80	10	14	14	8	32	40	PB	0.670	PCR	8
Fowdar	2012	Australia	European	377	393	33	174	170	35	183	175	PB	0.186	PCR-RFLP	7
Bayramoglu	2013	Turkey	European	125	99	22	38	65	5	38	56	HB	0.654	PCR	7
Fridman	2013	Argentina	Mixed	75	150	6	40	29	15	64	71	PB	0.917	PCR-RFLP	8
Perez-Razo	2015	Mexico	Mixed	373	391	87	174	112	101	200	90	HB	0.637	TaqMan	6
Perez-Razo	2015	Mexico	Mixed	199	199	67	98	34	56	108	35	HB	0.168	TaqMan	7
Vazquez-Alaniz	2014	Mexico	Mixed	194	194	39	93	62	43	97	54	HB	0.964	Taqman	7

HB: hospital-based; PB: population-based; SOC; source of control; PCR-RFLP: polymerase chain reaction followed by restriction fragment length polymorphism; HRM: High-Resolution Melt; ARMS-PCR: amplification refractory mutation system-PCR; HWE: Hardy-Weinberg equilibrium of control group.

Table 2 Stratified analyses of *MTHFR* gene rs1801133 polymorphism and essential hypertension risk

Variables	N	Case/ Control	T vs, C	TC vs. CC	TT vs. CC	TT+TC vs. CC	TT vs. TC+CC
			OR(95%CI) P_h P I-squared	OR(95%CI) P_h P I-squared	OR(95%CI) P_h P I-squared	OR(95%CI) P_h P I-squared	OR(95%CI) P_h P I-squared
Total	50	10533/11743	1.37(1.24-1.52)0.000 0.000 82.1%	1.25(1.12-1.39)0.000 0.000 59.4%	1.89(1.50-2.38)0.000 0.000 78.6%	1.45(1.21-1.75)0.000 0.000 86.2%	1.55(1.32-1.83)0.000 0.000 74.5%
Ethnicity							
Asian	36	8106/9083	1.46(1.29-1.67)0.000 0.000 84.8%	1.35(1.18-1.54)0.000 0.000 63.4%	2.09(1.57-2.79)0.000 0.000 77.2%	1.55(1.22-1.97)0.000 0.000 87.0%	1.74(1.41-2.15)0.000 0.000 70.6%
European	10	1586/1726	1.27(1.07-1.51)0.008 0.007 59.7%	1.09(0.95-1.27)0.297 0.219 15.8%	1.49(0.90-2.46)0.000 0.119 83.6%	1.22(0.84-1.78)0.000 0.299 85.7%	1.24(0.94-1.63)0.001 0.127 69.1%
Mixed	4	841/934	0.93(0.82-1.06)0.219 0.313 32.2%	0.86(0.69-1.08)0.156 0.205 42.5%	1.50(0.88-2.56)0.042 0.133 63.4%	1.37(0.90-2.09)0.017 0.139 70.6%	1.06(0.92-1.24)0.118 0.397 48.9%
China	28	6989/7837	1.51(1.30-1.74)0.000 0.000 86.9&	1.37(1.17-1.67)0.000 0.000 68.4%	2.07(1.59-2.71)0.000 0.000 82.4%	1.58(1.32-1.89)0.000 0.000 78.7%	1.71(1.38-2.13)0.000 0.000 810%
Not-China	22	3544/3906	1.21(1.06-1.37)0.000 0.003 66.4%	1.11(0.98-1.27)0.047 0.109 36.2%	1.39(1.06-1.82)0.000 0.018 60.3%	1.19(1.03-1.38)0.002 0.018 52.6%	1.32(1.04-1.66)0.001 0.020 55.2%
SOC							
HB	27	5235/5746	1.49(1.28-1.75)0.000 0.000 85.4%	1.38(1.16-1.64)0.000 0.000 67.1%	1.96(1.47-2.67)0.000 0.000 79.5%	1.57(1.28-1.91)0.000 0.000 78.1%	1.60(1.29-1.99)0.000 0.000 72.5%
PB	23	5298/5997	1.27(1.12-1.44)0.000 0.000 77.2%	1.14(1.01-1.29)0.011 0.036 44.7%	1.61(1.22-2.11)0.000 0.001 74.5%	1.25(1.09-1.44)0.000 0.001 59.8%	1.49(1.16-1.92)0.000 0.002 76.5%
Genotype methods							
PCR	14	1689/2400	1.48(1.14-1.91)0.000 0.003 85.1%	1.27(0.97-1.66)0.000 0.079 67.2%	1.91(1.22-3.00)0.000 0.005 77.2%	1.46(1.10-1.95)0.000 0.009 75.0%	1.71(1.11-2.63)0.000 0.014 79.5%
PCR-RFLP	18	3174/3105	1.49(1.30-1.72)0.000 0.000 65.1%	1.40(1.26-1.57)0.538 0.000 0.0%	2.22(1.62-3.06)0.000 0.000 61.3%	1.57(1.35-1.83)0.030 0.000 42.3%	1.78(1.36-2.33)0.002 0.000 55.8%
ARMS-PCR	2	219/346	2.39(0.55-10.45)0.000 0.244 96.3%	1.85(0.87-2.47)0.002 0.468 89.9%	5.64(0.43-73.32)0.000 0.186 94.3%	2.72(0.34-21.96)0.000 0.348 94.3%	3.72(0.78-17.71)0.001 0.099 90.5%
TaqMan	10	4102/4456	1.04(0.90-1.16)0.007 0.492 60.6%	0.99(0.83-1.18)0.015 0.916 56.3%	0.98(0.87-1.11)0.108 0.802 37.7%	1.75(0.79-3.88)0.007 0.843 60.1%	1.03(0.93-1.14)0.212 0.552 25.2%
HRM	2	845/827	1.32(1.15-1.51)0.167 0.000 47.5%	1.29(1.02-1.64)0.125 0.031 57.6%	1.76(1.34-2.32)0.202 0.000 38.5%	1.43(1.15-1.78)0.104 0.002 62.2%	1.48(1.18-1.86)0.609 0.001 0.0%
Other	4	504/609	1.25(0.79-1.97)0.002 0.346 80.0%	1.25(0.82-1.91)0.082 0.291 55.2%	1.45(0.63-3.32)0.030 0.386 66.6%	1.29(0.78-2.15)0.021 0.319 72.8%	1.29(0.65-2.56)0.081 0.468 55.5%

P_h: value of Q-test for heterogeneity test; P: Z-test for the statistical significance of the OR

Table 3 Publication bias tests (Begg's funnel plot and Egger's test for publication bias test) for *MTHFR* gene rs1801133 polymorphism and essential hypertension risk

Egger's test						Begg's test	
Genetic type	Coefficient	Standard error	t	P value	95%CI of intercept	z	P value
T vs, C	4.232	1.139	3.71	0.001	(1.940- 6.524)	2.67	0.008
TC vs. CC	1.735	0.515	3.37	0.002	(0.699- 2.771)	2.47	0.014
TT vs. CC	1.005	0.273	3.68	0.001	(0.455- 1.554)	1.83	0.067
TT+TC vs. CC	1,882	0.534	3.52	0.001	(0.808- 2.956)	2.6	0.009
TT vs. TC+CC	1.502	0.475	3.16	0.003	(0.546- 2.457)	2	0.046

Figures

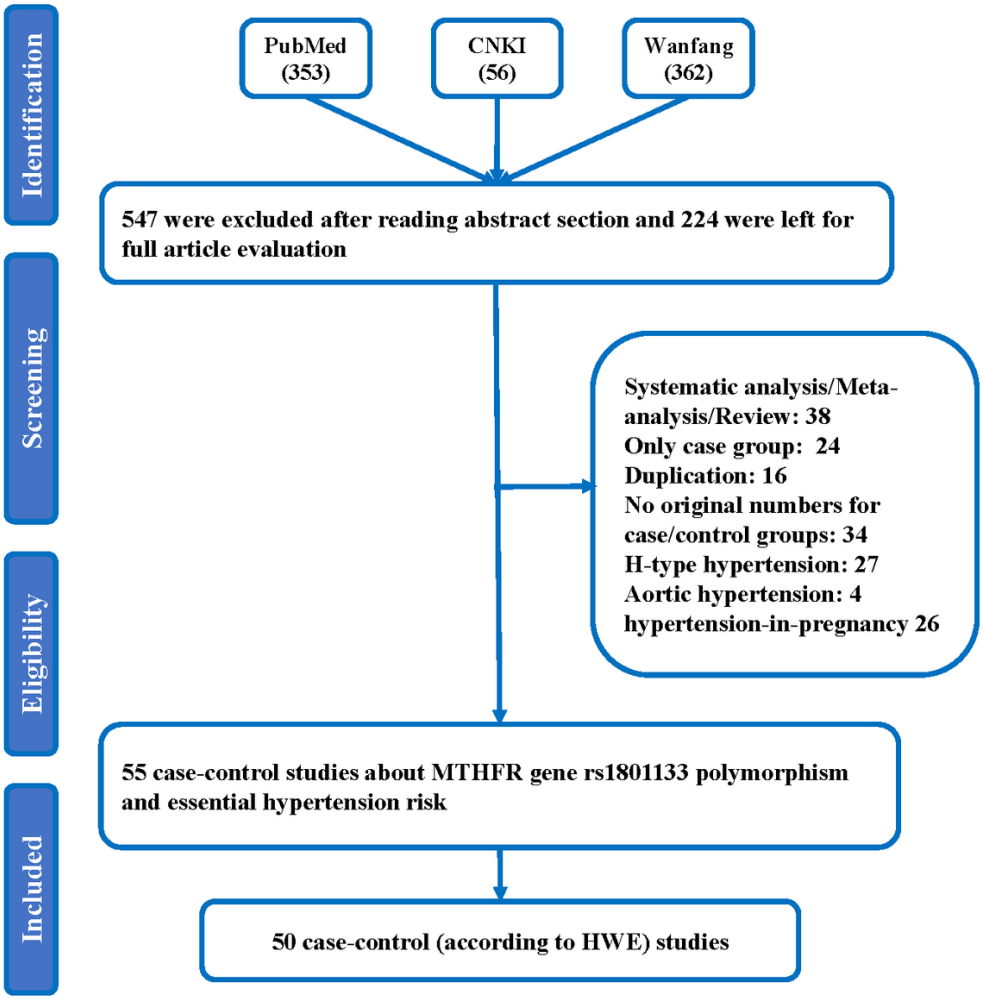


Figure 1

Flowchart illustrating the search strategy used to identify association studies for MTHFR gene rs1801133 polymorphisms and EH risk.

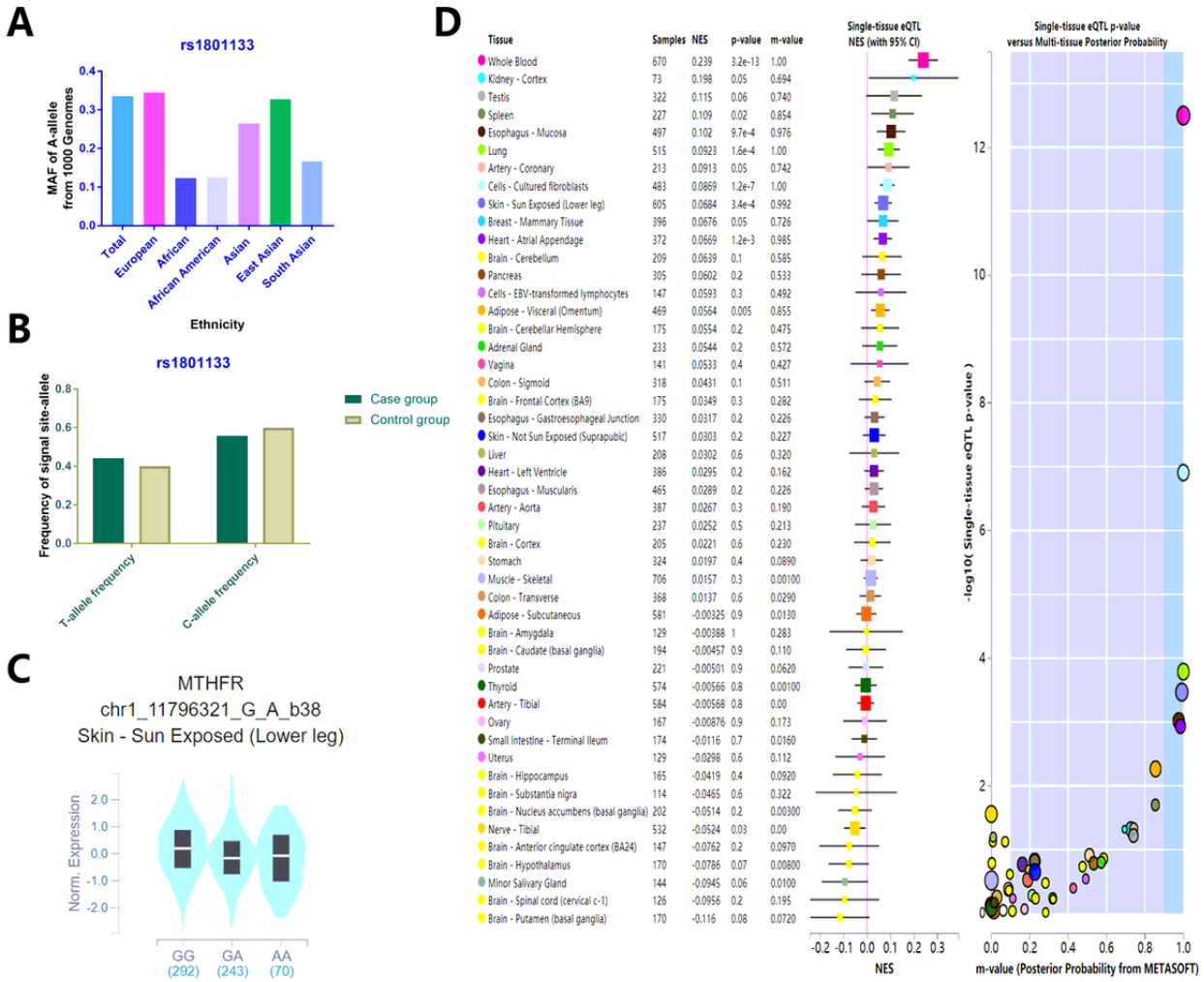


Figure 2

A: The MAF of minor-allele (mutant-allele) for MTHFR gene rs1801133 polymorphism from the 1000 Genomes online database. B: The frequency about T-allele or C-allele both in case and control groups. C: The distribution of each genotype from on online GTEx Portal (https://www.gtexportal.org/home/). D: The risk frequency of rs1801133 polymorphism to several disease from TCGA database.

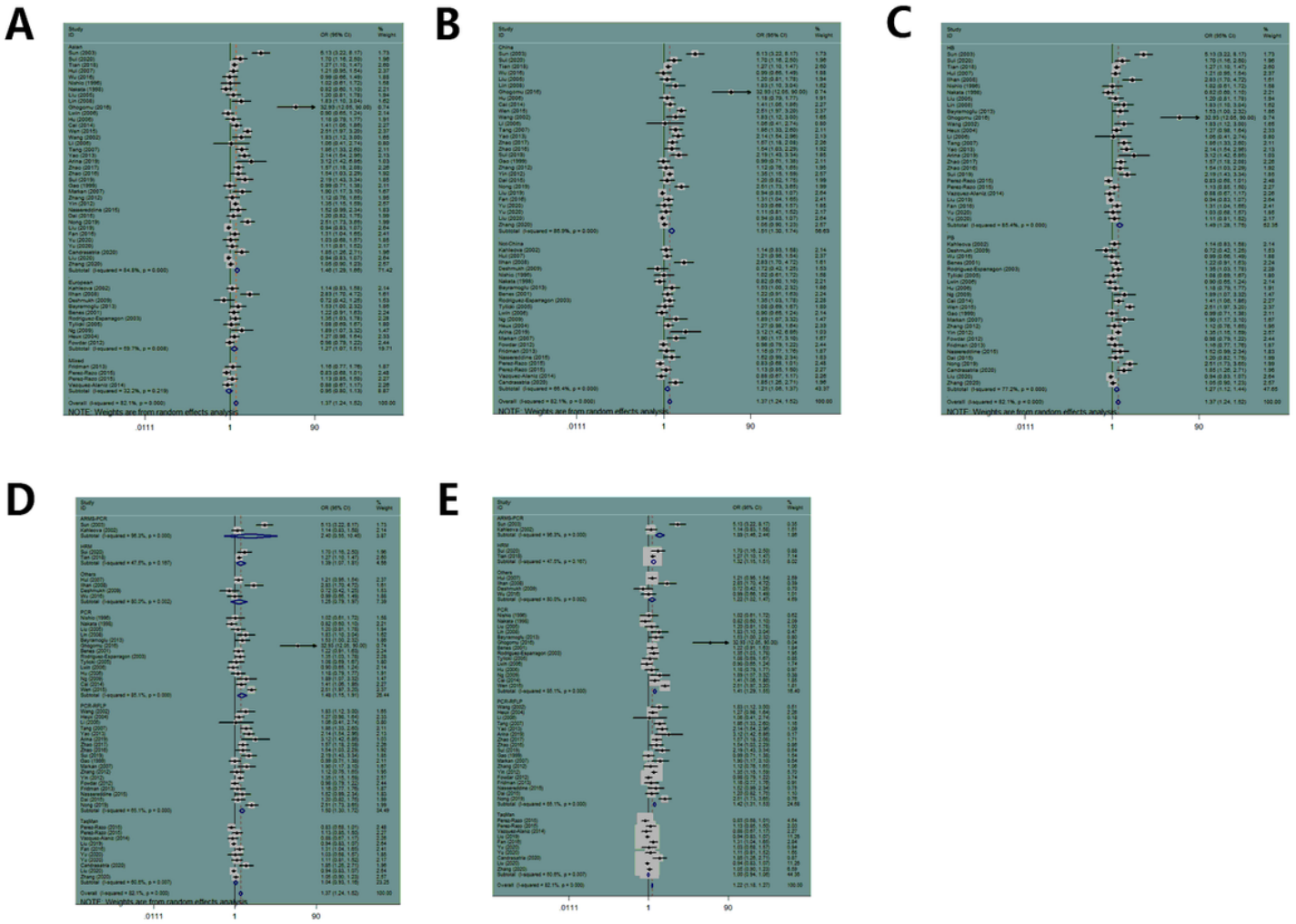
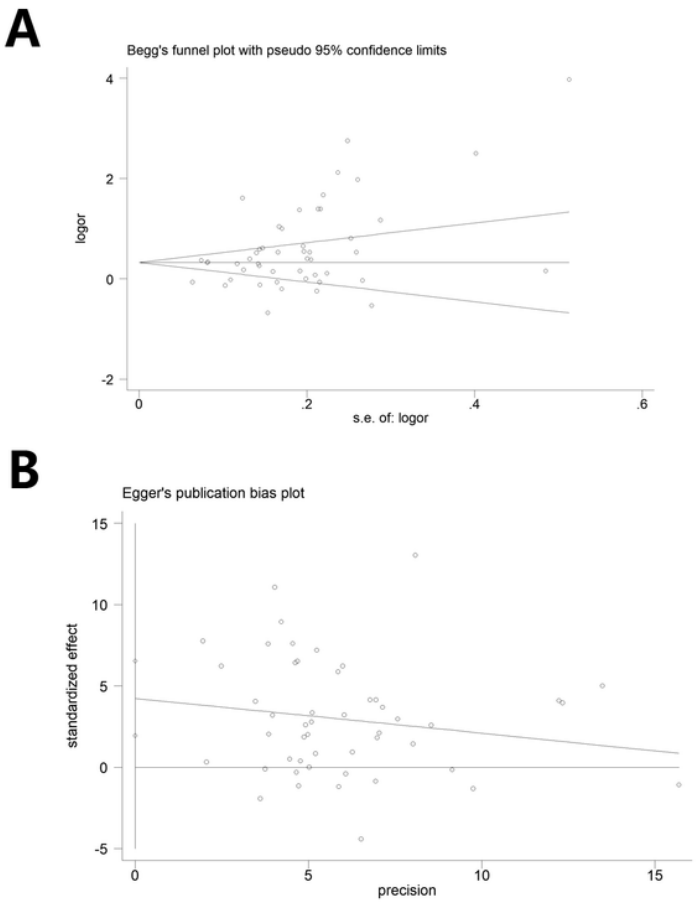


Figure 3

T-allele frequencies for the MTHFR gene rs1801133 polymorphism among cases/controls stratified by subgroups in T-allele vs. C-allele model. A: regular ethnicity subgroup; B: China/Non-China subgroup; C: source of control subgroup; D: genotype method subgroup by random effect model; E: genotype method subgroup by fixed effect model.



C

Study omitted	Estimate	[95% Conf.	Interval]
Gao (1999)	1.3347487	1.2132684	1.4683925
Wang (2002)	1.3783938	1.2440199	1.5272821
Sun (2003)	1.3662972	1.2340424	1.5127262
Liu (2005)	1.3779933	1.2403771	1.5308778
Li (2006)	1.3776544	1.242584	1.5274072
Hu (2006)	1.3545856	1.225548	1.4972095
Tang (2007)	1.3855388	1.252098	1.533201
Lin (2008)	1.3811601	1.2471545	1.5295646
Zhang (2012)	1.3789815	1.2454505	1.5268288
Yin (2012)	1.3879591	1.2539402	1.5363017
Yao (2013)	1.3763793	1.2425513	1.5246211
Cai (2014)	1.3656504	1.2338055	1.5115843
Liu (2019)	1.3697004	1.2368715	1.516794
Ghogomu (2016)	1.3355337	1.2158267	1.4670267
Dai (2015)	1.3767456	1.2422315	1.5258253
Wen (2015)	1.3736132	1.2393939	1.5223676
Wu (2016)	1.3786714	1.2448714	1.5268525
Fan (2016)	1.3851777	1.2508548	1.5339248
Zhao (2017)	1.37664	1.2428267	1.5248606
Sui (2020)	1.3655235	1.233814	1.5112928
Yu (2020)	1.3721656	1.2382339	1.5205836
Yu (2020)	1.3464417	1.2221434	1.4833819
Nong (2019)	1.3654729	1.2336394	1.511395
Zhao (2016)	1.3757955	1.2411017	1.5251073
Tian (2018)	1.3751805	1.2426533	1.5218416
Sui (2019)	1.3627801	1.2313105	1.5082871
Liu (2020)	1.3576223	1.2277486	1.5012343
Zhang (2020)	1.3598619	1.2297796	1.5037037
Nishio (1996)	1.3682694	1.2352147	1.5156564
Nakata (1998)	1.369385	1.2365472	1.516493
Lwin (2006)	1.3593818	1.2289131	1.5037018
Hui (2007)	1.3823161	1.2479085	1.5312004
Markan (2007)	1.3643563	1.2327449	1.5100186
Nassereddine (2015)	1.3783182	1.2443233	1.5267423
Candrasatria (2020)	1.3750025	1.2386785	1.5263298
Arina (2019)	1.3845069	1.2491508	1.5345302
Benes (2001)	1.3770018	1.2432172	1.5251832
Kahleova (2002)	1.3697938	1.236986	1.5168602
Rodriguez-Esparragon (2003)	1.3765301	1.2426056	1.5248885
Heux (2004)	1.3533893	1.2248304	1.4954417
Tylicki (2005)	1.3890699	1.2554309	1.5369345
Ilhan (2008)	1.379243	1.2444674	1.5286148
Deshmukh (2009)	1.3862811	1.2518748	1.5351177
Ng (2009)	1.3871406	1.2513579	1.5376569
Fowdar (2012)	1.3750471	1.2401118	1.5246645
Bayramoglu (2013)	1.3800006	1.2460688	1.5283278
Fridman (2013)	1.3792759	1.2447689	1.5283177
Perez-Razo (2015)	1.3637401	1.2320828	1.5094659
Perez-Razo (2015)	1.3871406	1.2513579	1.5376569
Vazquez-Alaniz (2014)	1.3838661	1.2469494	1.5358163
Combined	1.3720958	1.2406528	1.5174648

Figure 4

A: Begg's funnel plot for publication bias test (T-allele vs. C-allele). B: Egger's publication bias plot (T-allele vs. C-allele). C: Sensitive analysis (T-allele vs. C-allele).

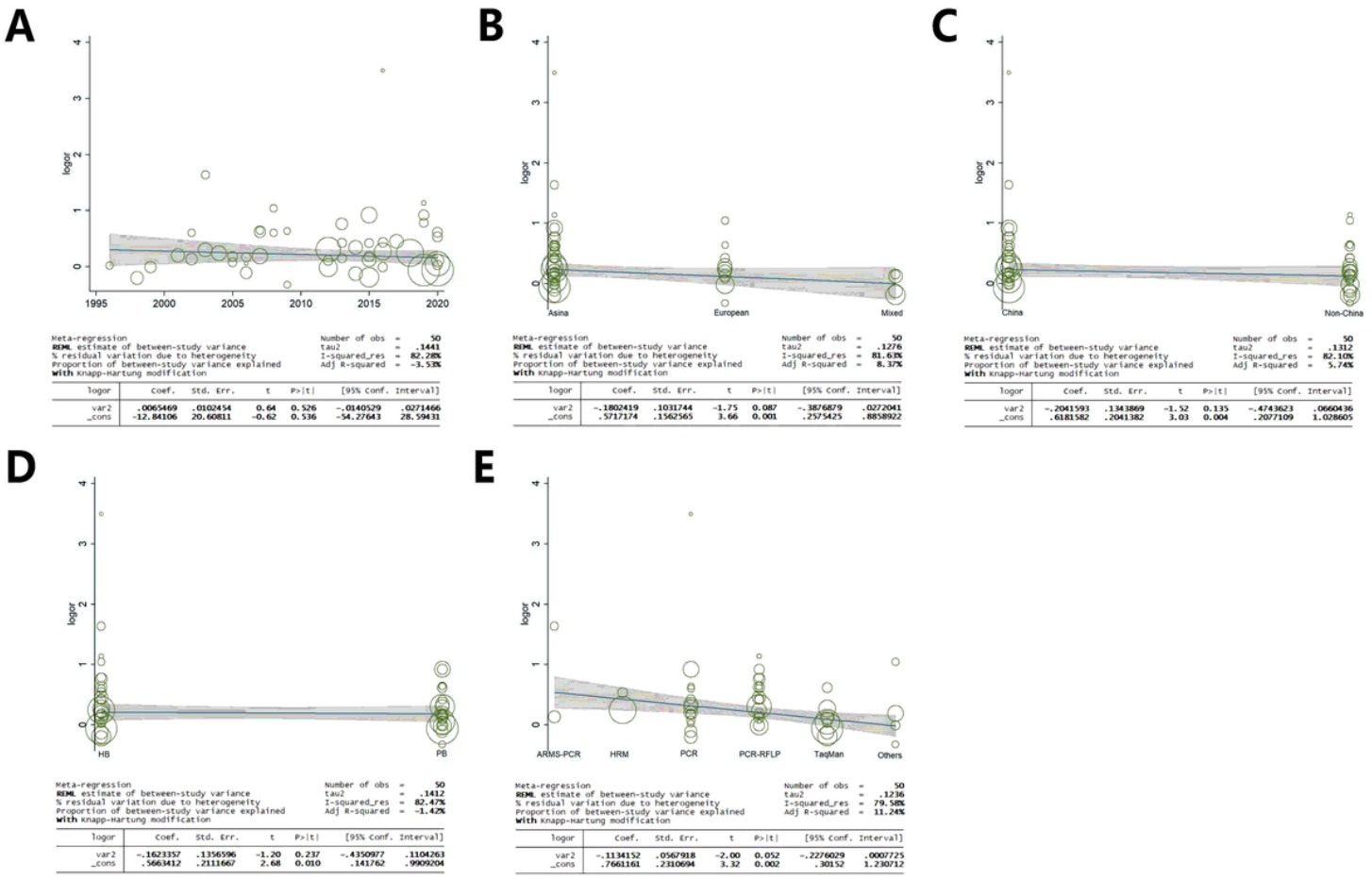


Figure 5

Random-effect meta-regression of log odds ratio versus publication year (A), regular ethnicity (B), China/Non-China (C), source of control (D), genotype methods (E) respectively in EH.

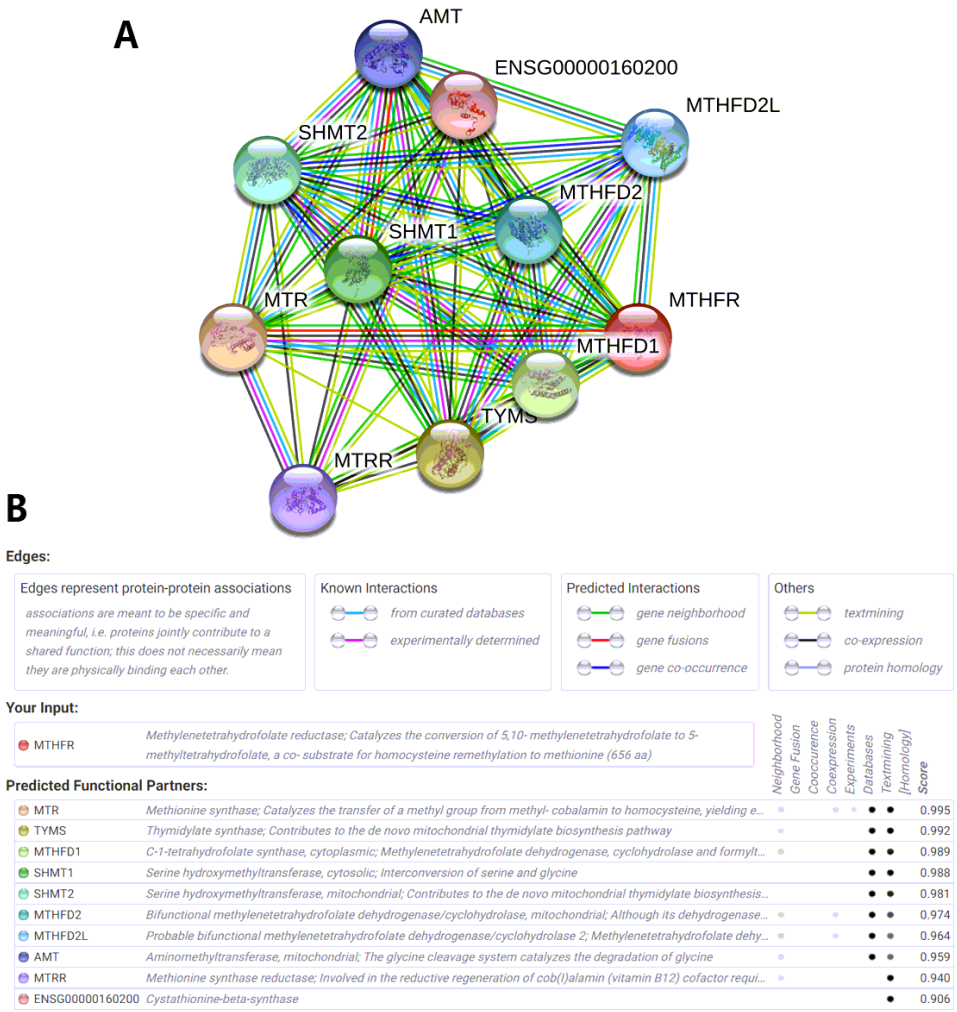


Figure 6

Human MTHFR interactions network with other genes obtained from String server. At least 10 genes have been indicated to correlate with MTHFR gene. A: the gene-gene interaction; B: the detail of relative ten core genes.