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EPIDEMIOLOGY

Methylenetetrahydrofolate reductase polymorphisms and breast cancer risk: a meta-analysis from 41 studies with 16,480 cases and 22,388 controls

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Abstract The association between methylenetetrahydrofolate reductase (MTHFR) gene polymorphisms and breast cancer risk has been widely reported, but results were inconsistent and underpowered. To clarify the effects of MTHFR polymorphisms on the risk of breast cancer, an updated meta-analysis of all available studies relating C677T and/or A1298C polymorphisms of MTHFR gene to the risk of breast cancer was conducted. Eligible articles were identified by search of databases including MEDLINE, PubMed, Web of Science, EMBASE and Chinese Biomedical Literature database (CBM) for the period up to January 2010. Finally, a total of 41 studies with 16,480 cases and 22,388 controls were included, all for C677T polymorphism and 20 with 12,170 cases and 15,865 controls for A1298C polymorphism. The pooled ORs were performed for the allele contrasts, additive genetic model, dominant genetic model, and recessive genetic model, respectively. Subgroup analyses were also performed by ethnicity and menopausal status. With respect to C677T polymorphism, significantly elevated breast cancer risk was found in overall analysis (T vs. C: OR = 1.041, 95% CI = 1.009–1.073; TT vs. CC: OR = 1.132, 95% CI = 1.019–1.259; TT vs. CC + CT: OR = 1.119, 95% CI = 1.014–1.236); in the subgroup analysis by ethnicity, significantly increased risk was found in East Asian population (T vs. C: OR = 1.121, 95% CI = 1.016–1.237; TT vs. CC: OR = 1.331, 95% CI = 1.073–1.650; TT vs. CC + CT: OR = 1.265, 95% CI = 1.058–1.513) but not in Caucasian population; in the subgroup analysis by menopausal status, no statistically significant association was found. With respect to A1298C polymorphism, no significant association with breast cancer risk was demonstrated in overall, ethnicity- and menopausal status-based population. It can be concluded that potentially functional MTHFR C677T polymorphism may play a low penetrance role in the development of breast cancer.

Keywords Methylenetetrahydrofolate reductase · Folate · One carbon metabolism · Polymorphism · Breast cancer · Meta-analysis

Introduction

It has been confirmed that folate plays an important role in metabolic processes, such as RNA and DNA synthesis, DNA repair, and DNA methylation [1]. Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme for intracellular folate homeostasis and metabolism. It catalyses the irreversible conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which is the primary circulating form of folate and provides methyl groups for the methylation of homocysteine to methionine [2]. Altered MTHFR enzyme activity has been linked to the development of cancer [3–5].

MTHFR gene locates on chromosome 1p36.3 and contains 11 exons [6]. Two common polymorphisms of MTHFR gene are C677T (rs1801133, Ala222Val), in exon

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4 at nucleotide 677, which is associated with a decreased activity of MTHFR, an increased level of homocysteine, and an altered distribution of folate and A1298C (rs1801131, Glu429Ala), in exon 7 at nucleotide 1298, which is also related to a reduced MTHFR activity, but at a lower degree compared to C677T [3, 4, 7]. It is biologically reasonable to hypothesize a potential relationship between MTHFR polymorphisms and breast cancer risk.

A series of studies have investigated the association between the two MTHFR polymorphisms and breast cancer susceptivity, but provided controversial or inconclusive results. Meanwhile, 21 case–control articles could not be included in the most recent meta-analysis [8] examining the association of the above-mentioned MTHFR polymorphisms with breast cancer risk, which was published in February 2007. As a result, the need for an updated meta-analysis has become evident. In light of the above, we conducted a meta-analysis of all available studies relating the C677T and/or A1298C polymorphisms of the MTHFR gene to the risk of developing breast cancer.

Methods

Search strategy

This study was performed according to the proposal of Meta-analysis of Observational Studies in Epidemiology group (MOOSE) [9]. A comprehensive search strategy was conducted towards the electronic databases including MEDLINE, PubMed, Web of Science, EMBASE, and Chinese Biomedical Literature database (CBM) using terms "breast cancer," "breast neoplasm," "methylene-tetrahydrofolate reductase," "MTHFR," "polymorphism," "variant," "folate," and "one-carbon metabolism," and the last search updated on January 19, 2010. Reference lists of the identified articles were also examined and the literature retrieval was performed in duplication by two independent reviewers (X. Qi and X. Ma).

Inclusion criteria

We reviewed titles and abstracts of all citations and retrieved literatures. The following inclusion criteria were used for literature selection: (1) the publication was a case–control study referring to the association between MTHFR polymorphisms and breast cancer in females; (2) all cases were first diagnosed as invasive or in situ breast cancer; (3) the articles must offer the sample size, distribution of alleles, genotypes, or other information that can help us infer the results; (4) when multiple publications reported on the same or overlapping data, we used the most recent or largest population as recommended by Little et al. [10]; and (5) publication language was confined to English and Chinese.

Data extraction

Data was extracted from each study by two reviewers (X. Qi and X. Ma) independently according to the pre-specified selection criteria. Decisions were compared and disagreements about study selection were resolved by consensus or by involving a third reviewer (J. Jiang). The following information was extracted from the studies: first author, reference year, sample size, studied polymorphisms, ethnicity of subjects, menopausal status, source of controls, and distribution of alleles and genotypes in case and control groups.

Statistical analysis

Meta-analysis was performed as described previously [11, 12]. Crude ORs with their 95% CIs were used to assess the strength of association between the MTHFR gene polymorphisms and breast cancer risk. The pooled ORs were performed for the allele contrasts, additive genetic model, dominant genetic model, and recessive genetic model, respectively. Subgroup analyses were also performed by ethnicity and menopausal status. Heterogeneity assumption was assessed by Chi-square-based Q-test and I-squared test. The heterogeneity was considered statistically significant if $P < 0.10$. With lacking of heterogeneity among studies, the pooled OR estimate of the each study was calculated by the fixed effects model (Mantel–Haenszel) [13]. Otherwise, the random effects model (DerSimonian and Laird) was used [14, 15]. The departure of frequencies of MTHFR polymorphisms from expectation under Hardy–Weinberg equilibrium (HWE) was assessed by Chi-square test in controls. Possible publication bias was tested by Begg's funnel plot and Egger's test.

All statistical tests were conducted with STATA software package (version 10.0, College Station, TX). A P value of 0.05 for any test or model was considered to be statistically significant.

Results

Eligible studies

After examined carefully according to the inclusion criteria, four publications [16–19] were excluded because their study subjects were overlapped in other articles. Our final pool of eligible studies included 41 studies [8, 20–59] with 16,480 cases and 22,388 controls, all for C677T polymorphism (Table 1) and 20 with 12,170 cases and 15,865

Table 1 Characteristics of case–control studies included in MTHFR C667T (rs1801133, Ala222Val) polymorphism and breast cancer risk

No.	First author	Year	Country	Ethnicity	Source of controls	Cases			Controls		
						CC	CT	TT	CC	CT	TT
1	Sharp [20]	2002	UK	Caucasian	Population-based	30	19	5	25	21	11
2	Campbell [21]	2002	UK	Caucasian	Hospital-based	140	162	33	118	92	23
3	Semenza [22]	2003	USA	Caucasian	Hospital-based	42	58	5	112	111	24
4	Langsenlehner [23]	2003	Austria	Caucasian	Population-based	208	222	64	215	215	65
5	Ergul [24]	2003	Turkey	Caucasian	Hospital-based	60	41	17	94	87	12
6	Shrubsole [25]	2004	China	East Asian	Population-based	374	555	183	387	577	196
7	Försti [26]	2004	Finland	Caucasian	Not stated	134	81	8	181	104	13
8	Lee [27]	2004	Korea	East Asian	Hospital-based	58	96	32	50	80	17
9	Grieu [28]	2004	Australia	Caucasian	Population-based	166	141	27	242	259	50
10	Lin [29]	2004	China	East Asian	Population-based	43	38	7	173	145	24
11	Le Marchand [30][a]	2004	USA	Mixed	Population-based	573	479	137	1211	920	283
12	Qi [31]	2004	China	East Asian	Population-based	42	104	71	59	105	54
13	Kalemi [32]	2005	Greece	Caucasian	Not stated	19	16	7	23	20	8
14	Deligezer [33]	2005	Turkey	Caucasian	Not stated	98	68	23	128	83	12
15	Justenhoven [34]	2005	Germany	Caucasian	Population-based	249	274	61	261	279	93
16	Chou [35]	2006	China	East Asian	Hospital-based	73	51	18	132	120	33
17	Xu [36]	2007	USA	Mixed	Population-based	398	476	189	440	509	155
18	Hekim [37]	2007	Turkey	Caucasian	Not stated	22	16	2	38	26	4
19	Macis [38]	2007	Italy	Caucasian	Population-based	14	20	12	28	41	11
20	Lissowska [8][a]	2007	Poland	Caucasian	Population-based	982	815	177	1132	915	235
21	Yu [39]	2007	China	East Asian	Population-based	56	54	9	225	170	25
22	Kan [40][a]	2007	China	East Asian	Population-based	74	29	22	65	29	9
23	Stevens [41][a]	2007	USA	Mixed	Population-based	208	224	62	236	193	65
24	Reljic [42]	2007	Croatia	Caucasian	Population-based	40	44	9	27	34	4
25	Inoue [43]	2008	Singapore	East Asian	Population-based	239	120	21	393	226	43
26	Kotsopoulos [44]	2008	Canada	Caucasian	Hospital-based	383	421	140	252	341	87
27	Suzuki [45]	2008	Japan	East Asian	Hospital-based	150	220	84	338	425	146
28	Cheng [46]	2008	China	East Asian	Hospital-based	185	133	31	268	221	41
29	Langsenlehner [47]	2008	Austria	Caucasian	Not stated	51	43	11	40	48	17
30	Ericson [48]	2009	Sweden	Caucasian	Population-based	255	235	50	531	452	91
31	Gao [49]	2009	China	East Asian	Population-based	202	305	117	235	301	88
32	Ma [50]	2009	Japan	East Asian	Hospital-based	124	183	81	115	188	84
33	Platek [51]	2009	USA	Mixed	Population-based	429	446	119	788	795	219
34	Henríquez-Hernández [52]	2009	Spain	Caucasian	Population-based	52	65	18	107	138	47
35	Cam [53]	2009	Turkey	Caucasian	Not stated	48	49	13	47	42	6
36	Maruti [54]	2009	USA	Mixed	Population-based	133	139	46	301	284	62
37	Ma [55]	2009	Brazil	Mixed	Hospital-based	225	188	45	222	187	49
38	Li [56]	2009	China	East Asian	Population-based	38	17	10	90	50	3
39	Yuan [57]	2009	China	East Asian	Hospital-based	16	35	29	32	35	13
40	Jin [58]	2009	China	East Asian	Not stated	18	20	3	49	41	10
41	Bentley [59]	2010	USA	Caucasian	Hospital-based	346	402	191	429	592	205

[a] P_{HWE} in controls <0.05

controls for A1298C polymorphism (Table 2). The genotype distribution in the controls of all studies was consistent with HWE expect four for C677T polymorphism [8, 30, 40, 41] and two for A1298C polymorphism [8, 46].

Quantitative synthesis

The main results of this meta-analysis and the heterogeneity test were shown in Tables 3 and 4. With respect to

Table 2 Characteristics of case–control studies included in MTHFR A1298C (rs1801131, Glu429Ala) polymorphisms and breast cancer risk

No.	First author	Year	Country	Ethnicity	Source of controls	Cases			Controls		
						AA	AC	CC	AA	AC	CC
1	Sharp [20]	2002	UK	Caucasian	Population-based	27	25	3	24	25	11
2	Ergul [24]	2003	Turkey	Caucasian	Hospital-based	50	48	20	90	85	18
3	Shrubsole [25]	2004	China	East Asian	Population-based	768	311	42	824	344	40
4	Försti [26]	2004	Finland	Caucasian	Not stated	94	102	27	133	127	38
5	Le Marchand [30]	2004	USA	Mixed	Population-based	741	372	77	1493	801	120
6	Qi [31]	2004	China	East Asian	Population-based	155	58	4	144	71	3
7	Justenhoven [34]	2005	Germany	Caucasian	Population-based	273	256	53	295	266	73
8	Chou [35]	2006	China	East Asian	Hospital-based	104	30	8	172	95	18
9	Xu [36]	2007	USA	Mixed	Population-based	558	417	87	536	457	110
10	Lissowska [8][a]	2007	Poland	Caucasian	Population-based	892	874	220	1086	941	251
11	Kan [40]	2007	China	East Asian	Population-based	70	41	14	61	32	8
12	Stevens [41]	2007	USA	Mixed	Population-based	224	228	42	252	201	40
13	Inoue [43]	2008	Singapore	East Asian	Population-based	225	139	16	387	234	41
14	Kotsopoulos [44]	2008	Canada	Caucasian	Hospital-based	466	390	85	398	309	73
15	Cheng [46][a]	2008	China	East Asian	Hospital-based	207	125	19	310	207	17
16	Ericson [48]	2009	Sweden	Caucasian	Population-based	242	242	57	487	480	105
17	Gao [49]	2009	China	East Asian	Population-based	446	169	9	425	188	11
18	Ma [50]	2009	Japan	East Asian	Hospital-based	254	119	15	256	116	15
19	Platek [51]	2009	USA	Mixed	Population-based	443	402	83	842	758	181
20	Ma [55]	2009	Brazil	Mixed	Hospital-based	269	168	21	279	157	22

[a] P_{HWE} in controls <0.05

C677T polymorphism, significantly elevated breast cancer risk was found in overall analysis (T vs. C: OR = 1.041, 95% CI = 1.009–1.073; TT vs. CC: OR = 1.132, 95% CI = 1.019–1.259; TT vs. CC + CT: OR = 1.119, 95% CI = 1.014–1.236). In the subgroup analysis by ethnicity, significantly increased risk was found in East Asian population (T vs. C: OR = 1.121, 95% CI = 1.016–1.237; TT vs. CC: OR = 1.331, 95% CI = 1.073–1.650; TT vs. CC + CT: OR = 1.265, 95% CI = 1.058–1.513) but not in Caucasian (T vs. C: OR = 0.991, 95% CI = 0.946–1.038). In the subgroup analysis by menopausal status, no statistically significant association was found (T vs. C for premenopausal: OR = 0.994, 95% CI = 0.902–1.094; for postmenopausal: OR = 1.033, 95% CI = 0.964–1.107).

With respect to A1298C polymorphism, no significant association with breast cancer risk was demonstrated in overall population (C vs. A: OR = 0.986, 95% CI = 0.938–1.037), ethnicity-based population (C vs. A for Caucasian: OR = 1.036, 95% CI = 0.975–1.101; for East Asian: OR = 0.953, 95% CI = 0.877–1.036), and menopausal status-based population (C vs. A for premenopausal: OR = 1.027, 95% CI = 0.944–1.117; for postmenopausal: OR = 0.902, 95% CI = 0.800–1.017).

Sensitive analysis

Sensitivity analyses were conducted to determine whether modification of the inclusion criteria of the meta-analysis affected the final results. These were carried out by limiting the meta-analysis to studies conforming to HWE and altering corresponding statistic variables and analysis models. All the results were not materially altered (data not shown).

Bias diagnosis

The Begg's funnel plot and Egger's test were performed to access the publication bias of literatures. As showed in Fig. 1, the shape of the funnel plot did not reveal obvious asymmetry. Then, the Egger's test was used to provide statistical evidence of funnel plot symmetry. The results still did not suggest any evidence of publication bias (data not shown).

Discussion

Worldwide, breast cancer is the most common malignancy in women, with over 1 million cases reported and 410,712

Table 3 Summary ORs and 95% CI of MTHFR C667T (rs1801133, Ala222Val) polymorphism and breast cancer risk

Analysis model	Ethnicity	OR	95% CI	P^a
Overall effects (41 studies)				
T vs. C	Caucasian	0.991	0.946–1.038	0.259
	East Asian	**1.121**[b]	**1.016–1.237**	**0.005**
	Total	**1.041**[b]	**1.009–1.073**	**0.006**
TT vs. CC	Caucasian	0.990[b]	0.854–1.148	0.061
	East Asian	**1.331**[b]	**1.073–1.650**	**0.005**
	Total	**1.132**[b]	**1.019–1.259**	**0.001**
TT + CT vs. CC	Caucasian	0.980	0.921–1.042	0.502
	East Asian	1.066	0.981–1.158	0.101
	Total	1.035	0.992–1.079	0.210
TT vs. CC + CT	Caucasian	1.013[b]	0.864–1.187	0.009
	East Asian	**1.265**[b]	**1.058–1.513**	**0.023**
	Total	**1.119**[b]	**1.014–1.236**	**0.001**
Menopausal status				
Premenopausal (11 studies)				
T vs. C	–	0.994	0.902–1.094	0.621
TT vs. CC	–	1.050	0.850–1.296	0.263
TT + CT vs. CC	–	0.951	0.834–1.084	0.801
TT vs. CC + CT	–	1.084	0.890–1.321	0.189
Postmenopausal (11 studies)				
T vs. C	–	1.033	0.964–1.107	0.321
TT vs. CC	–	1.014[b]	0.837–1.228	0.098
TT + CT vs. CC	–	1.069	0.984–1.162	0.562
TT vs. CC + CT	–	0.994[b]	0.827–1.193	0.084

[a] *P* value for heterogeneity

[b] Estimates for random effects model

For East Asian, significance of T vs. C, TT vs. CC and TT vs. CC + CT is 0.023, 0.009 and 0.010, respectively

For total, significance of T vs. C, TT vs. CC and TT vs. CC + CT is 0.023, 0.021 and 0.025, respectively

Table 4 Summary ORs and 95% CI of MTHFR A1298C (rs1801131, Glu429Ala) polymorphism and breast cancer risk

Analysis model	Ethnicity	OR	95% CI	P^a
Overall effects (20 studies)				
C vs. A	Caucasian	1.036	0.975–1.101	0.324
	East Asian	0.953	0.877–1.036	0.330
	Total	0.986[b]	0.938–1.037	0.092
CC vs. AA	Caucasian	1.012	0.831–1.233	0.147
	East Asian	1.031	0.803–1.324	0.566
	Total	1.006	0.915–1.107	0.247
CC + AC vs. AA	Caucasian	1.071	0.988–1.161	0.842
	East Asian	0.929	0.842–1.024	0.300
	Total	1.002	0.954–1.052	0.223
CC vs. AA + AC	Caucasian	0.985	0.864–1.123	0.124
	East Asian	1.056	0.825–1.353	0.576
	Total	0.991	0.904–1.087	0.259
Menopausal status				
Premenopausal (5 studies)				
C vs. A	–	1.027	0.944–1.117	0.251
CC vs. AA	–	1.294	0.937–1.788	0.168
CC + AC vs. AA	–	1.083	0.907–1.292	0.722
CC vs. AA + AC	–	1.300	0.954–1.772	0.160
Postmenopausal (6 studies)				
C vs. A	–	0.902	0.800–1.017	0.016
CC vs. AA	–	0.999[b]	0.775–1.287	0.099
CC + AC vs. AA	–	1.030	0.938–1.132	0.471
CC vs. AA + AC	–	0.966[b]	0.745–1.252	0.066

[a] *P* value for heterogeneity

[b] Estimates for random effects model

deaths in 2002 [60]. It is estimated that 192,370 women will be diagnosed with and 40,170 women will die of breast cancer in the United States in 2009 [61]. Many candidate genes have been reported to be involved in breast cancer susceptibility, including MTHFR, CYP19 [11], SOD2 [12], CASP8 [62], SULT1A1 [63], GSM1 [64], COX2 [65], hOGG1 [66] etc. MTHFR is one of the primary candidate genes concerning that altered MTHFR enzyme activity may influence the general balance between DNA synthesis, repair, and methylation processes [3–5, 38].

Though series of case–control studies were performed to assess the importance of MTHFR gene polymorphisms in breast cancer risk, there has been no consistent conclusion. More importantly, 21 case–control articles could not be included in the most recent meta-analysis [8] examining the association of the above-mentioned

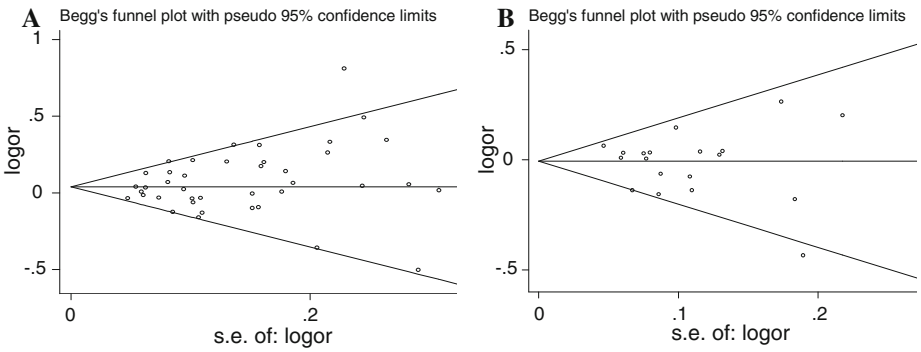

Fig. 1 Funnel plot analysis to detect publication bias. Each point represents a separate study for the indicated association. **a** Funnel plot for allele contrast (T vs. C) of C677T polymorphism in overall analysis; **b** Funnel plot for allele contrast (C vs. A) of A1298C polymorphism in overall analysis

MTHFR polymorphisms with breast cancer risk. As a result, the need for an up-to-date meta-analysis has become evident. Against this backdrop, we performed a systematic review and meta-analysis upon the two most focused polymorphisms of MTHFR gene (C677T and A1298C) in order to clarify the effect of MTHFR polymorphisms on the risk of breast cancer. A total of 41 studies with 16,480 cases and 22,388 controls were identified during this study. The results showed strong association between MTHFR C677T polymorphism and breast cancer risk in overall analysis, indicating that potentially functional MTHFR C677T polymorphism may play a low penetrance role in the development of breast cancer. Significant association was found in East Asian but not in Caucasian, suggesting a possible role of ethnic differences in genetic backgrounds and the environment they lived in.

To some extent, some limitations have affected the objectivity of the conclusions and should be considered when interpreting the results. Firstly, the sample sizes of several included studies are rather small and they do not have adequate power to detect the possible risk for MTHFR polymorphisms [20, 32, 37]. Secondly, the controls of several studies were not uniformly defined and some were hospital-based normal individuals or patients of other diseases which were not representative enough. Thirdly, owing to the limited evidence available on other MTHFR gene polymorphisms, this review was restricted to the two most investigated polymorphisms described above. Furthermore, data were not stratified by age, folate intake, smoking status, and other suspected factors. Therefore, a more precise analysis should be conducted if enough data were available.

In conclusion, during this meta-analysis with a large sample size, we found that MTHFR C677T polymorphism was significantly associated with breast cancer risk in overall and East Asian population while no association was shown in Caucasian and menopausal status-based population. Meanwhile, MTHFR A1298C polymorphism was not associated with breast cancer risk in overall, ethnicity- and menopausal status-based population. Large well-designed epidemiological studies will be necessary to validate the risk identified in the current meta-analysis.

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