

Association between polymorphisms in folate metabolism genes and maternal risk for Down syndrome: A meta-analysis

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Abstract. Previous studies have focused on the association between polymorphisms of the genes involved in folate metabolism and Down syndrome (DS); however, the results remain inconclusive. The present meta-analysis was conducted to assess the association between RFC-1 A80G/MTR A2756G/CBS 844ins68 polymorphisms and the maternal risk of DS. Published studies were retrieved from PubMed, Embase, China National Knowledge Infrastructure and Chinese Biomedicine databases. Pooled odds ratios (ORs) with 95% confidence interval (CIs) were calculated using the fixed- or random-effects model. Additionally, test of heterogeneity, cumulative meta-analysis, sensitivity analysis and assessment of bias were also performed. Finally, 11, 11 and 6 studies were deemed eligible for meta-analyses of RFC-1 A80G, MTR A2756G and CBS 844ins68, respectively. A significant association between RFC-1 A80G polymorphism and DS risk was observed for G vs. A (OR=1.19, 95% CI: 1.004-1.40, P=0.04) and the recessive model (OR=1.28, 95% CI: 1.05-1.56, P=0.01). In the stratified analysis by source of control or sample size, a significantly increased risk was observed among hospital-based studies and large-sample groups (>200 subjects), respectively. In addition, the cumulative meta-analysis of the RFC-1 A80G variant revealed a trend toward an association as the amount of data increased. However, for the MTR A2756G and CBS 844ins68 polymorphisms, no obvious association was found for all genetic models. In summary, the present meta-analysis demonstrated that RFC-1 A80G, but not MTR A2756G or CBS 844ins68, was considered as a maternal risk factor for DS in the offspring.

Introduction

Down syndrome (DS) or trisomy 21 is the most common genetic cause of mental retardation. In 95% of the cases, DS is caused by an error occurring during meiosis, mainly of maternal origin (1-3). Despite extensive research, the molecular mechanisms underlying the meiotic non-disjunction are poorly understood. However, several studies suggested that genomic DNA hypomethylation may be associated with chromosomal instability and abnormal segregation (4,5). Moreover, impairment of folate metabolism has been causally associated with DNA hypomethylation (6-8). Thus, polymorphisms involved in folate metabolism have been investigated as maternal risk factors for DS. Several key enzymes, including methylenetetrahydrofolate reductase (MTHFR), methionine synthase (MTR) and cystathionine b-synthase (CBS) are involved in the folate metabolic pathway. MTHFR converts tetrahydrofolate to 5,10-methylenetetrahydrofolate, which is vital for nucleic acid metabolism (9,10). C677T and A1298C, two common variants in the MTHFR gene, have been investigated in two previous meta-analyses for their role as maternal risk factors for DS (11,12).

The MTR enzyme, encoded by the MTR gene, catalyzes the remethylation of homocysteine to methionine, which is required for the production of the universal methyl donor adenosylmethionine (13,14). CBS acts in the trans-sulfuration of homocysteine to cystathionine, playing a critical role in linking the folate and methionine cycles in regulating homocysteine levels (15,16). In addition to the metabolism, folate-transporting proteins, including reduced folate carrier-1 (RFC-1), are also crucial for the maintenance of DNA methylation. RFC-1 is responsible for folate uptake from the jejunum and its subsequent translocation across biological membranes in a variety of cells (17). The A80G polymorphism of the RFC-1 gene has been recently demonstrated to affect plasma folate and homocysteine levels (18,19).

To date, a number of studies have investigated the association between RFC-1 A80G/MTR A2756G/CBS 844ins68 polymorphisms and the risk of DS in the offspring (20-34); however, it remains inconclusive whether these polymorphisms in the mother are causal in determining DS susceptibility in the offspring. Therefore, a meta-analysis of all relevant studies was conducted to quantitatively assess the effect of these three polymorphisms on the risk of DS.

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Key words: down syndrome, RFC-1 A80G, MTR A2756G, CBS 844ins68, folate metabolism gene polymorphisms

Data collection methods

Search strategy. Literature databases, including PubMed, Embase, China National Knowledge Infrastructure (CNKI) and Chinese Biomedicine, were searched for relevant studies (the last search was updated in April 2013). The following search terms were used: ('cystathionine-beta-synthase', 'CBS', 'methionine synthase', 'MTR', 'rs1805087', 'reduced folate carrier 1', or 'RFC 1', 'rs1051266'), ('Down syndrome' or 'trisomy 21'), and (polymorphism OR variant). The search was limited to studies published in English or Chinese language. In addition, a snowball search was conducted to identify additional potentially relevant studies in the references of reviews and retrieved articles.

Inclusion and exclusion criteria. The studies included in the present meta-analysis were required to meet the following criteria: i) RFC-1 A80G or MTR A2756G or CBS 844ins68 polymorphisms and maternal risk for DS; ii) case-control design iii) sufficient maternal genotype data for calculation of odds ratio (OR) with 95% confidence interval (CI); iv) published in English or Chinese. Studies were excluded for the following reasons: i) Data duplication; ii) no usable maternal genotype data provided; and iii) abstracts, comments and reviews.

Data extraction. The following information was extracted from each study by two investigators independently: Name of the first author, year of publication, source of control subjects, country of origin, ethnicities of the individuals involved, number of cases and controls, and number of genotypes for the three polymorphisms in cases and controls. Disagreements were resolved by discussion between the two investigators.

Statistical analysis. STATA software, version 12 (StataCorp LP, College Station, TX, USA) was used to perform all the statistical analyses. P-values <0.05 were considered to indicate statistically significant differences. The distribution of genotypes in the control group of each study was assessed for Hardy-Weinberg equilibrium (HWE) and P<0.05 was considered as significant disequilibrium.

The association between the RFC-1 A80G polymorphism and maternal risk for DS was evaluated using OR and 95% CI (35) under the codominant, dominant and recessive genetic models, as well as the allele model. For the MTR A2756G and CBS 844ins68 polymorphisms, the four genetic models were also used. In addition, subgroup analysis for the RFC-1 A80G and MTR A2756G polymorphisms was performed based on ethnicity, sample size and source of controls.

All the meta-analyses were evaluated for heterogeneity using the Chi-squared-based Q test and the I² test (36). A random-effects model was used when the heterogeneity test result was P<0.10; otherwise, the fixed-effects model was used (37). Moreover, sensitivity analysis was performed to evaluate the stability of the results following sequential removal of each study. Cumulative meta-analyses of associations for each polymorphism were also performed through assortment of studies with publication time. Finally, publication bias was assessed via Egger's test and funnel plots: P<0.05 was considered to indicate statistical significance and the

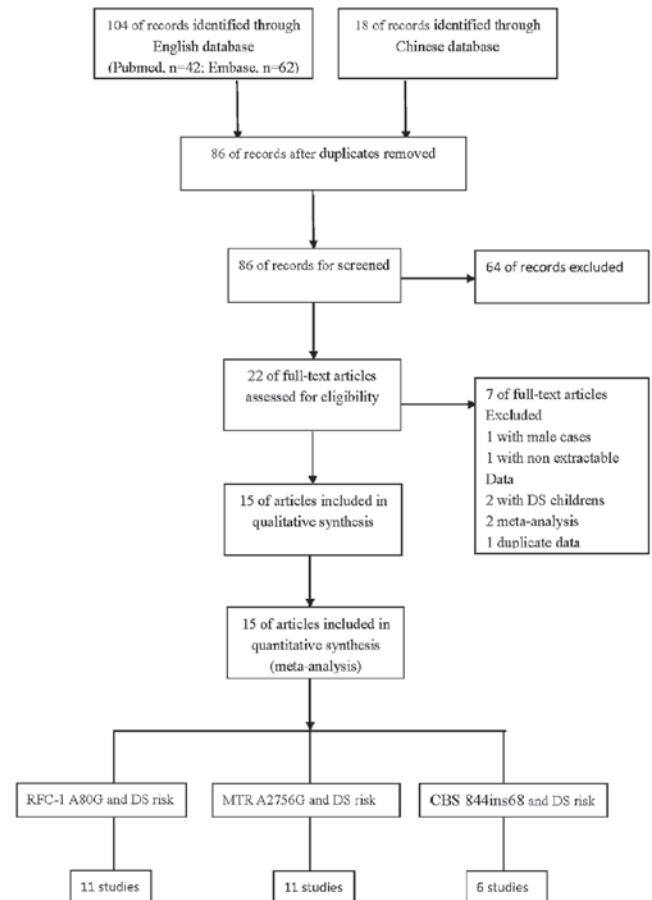


Figure 1. Flow chart of retrieved studies and studies excluded based on specified criteria. DS, Down syndrome.

funnel plot should be asymmetric when there was publication bias (38-40).

Results

Characteristics of the included studies. A total of 122 titles in the PubMed, Embase, CNKI and Chinese Biomedicine databases were found to be relevant to the search terms. Subsequently, the abstracts and full articles of the retrieved studies were read to assess their eligibility for inclusion in the meta-analysis. Finally, 15 studies investigating the association between any of the RFC-1 A80G, MTR A2756G and CBS 844ins68 polymorphisms and DS were included in the meta-analysis. Among these studies, 2 and 4 studies investigated only MTR A2756G and RFC-1 A80G polymorphisms, respectively; 3 studies included both RFC-1 A80G and MTR A2756G polymorphisms; 2 studies included both MTR A2756G and CBS 844ins68 polymorphisms; and 4 studies investigated RFC-1 A80G, MTR A2756G and CBS 844ins68 polymorphisms. The flow chart of the study selection process is shown in Fig. 1.

There were 11 studies with 2,389 mothers (997 cases and 1,392 controls) for the RFC-1 A80G polymorphism, 11 studies with 2,717 mothers (1,162 cases and 1,555 controls) for the MTR A2756G polymorphism and 6 studies with 1,859 mothers (825 cases and 1,034 controls) for the CBS

Table I. Distribution of the RFC-1 A80G and MTR A2756G genotypes among DS mothers (cases and controls) included in the meta-analysis.

Authors	Year	Source of controls	Country	Ethnicity	Genotype distribution								P _{HWE}	(Refs.)
					Cases				Controls					
					GG	AG	AA	GG	AG	AA	AG	AA		
RFC-1 A80G polymorphism														
Wang <i>et al</i>	2013	HB	China	Asian	16	41	47	13	71	100	0.93	(20)		
Neagos <i>et al</i>	2010	PB	Romania	Caucasian	9	16	1	11	30	5	0.03	(22)		
Brandalize <i>et al</i>	2010	HB	Brazil	Mixed	65	101	73	42	91	64	0.37	(21)		
Fintelman-Rodrigues <i>et al</i>	2009	PB	Brazil	Mixed	25	64	25	26	55	29	0.99	(26)		
Coppedè <i>et al</i>	2007	HB	Italy	Caucasian	15	17	1	7	14	9	0.73	(23)		
Coppedè <i>et al</i>	2006	PB	Italy	Caucasian	27	29	13	31	42	20	0.42	(24)		
Chango <i>et al</i>	2005	PB	France	Caucasian	29	66	24	26	52	16	0.25	(25)		
Biselli <i>et al</i>	2008	HB	Brazil	Mixed	20	33	14	34	49	30	0.16	(28)		
Scala <i>et al</i>	2006	HB	Italy	Caucasian	26	41	27	48	113	102	0.10	(27)		
Liao <i>et al</i>	2010	PB	China	Asian	14	22	24	16	40	12	0.14	(31)		
Biselli <i>et al</i>	2008	HB	Brazil	Mixed	21	36	15	50	94	50	0.67	(28)		
MTR A2756G polymorphism														
Brandalize <i>et al</i>	2010	HB	Brazil	Mixed	9	71	159	6	61	130	0.72	(21)		
Fintelman-Rodrigues <i>et al</i>	2009	PB	Brazil	Mixed	7	28	79	2	37	71	0.25	(26)		
Coppedè <i>et al</i>	2007	HB	Italy	Caucasian	0	8	23	1	8	24	0.74	(23)		
Chango <i>et al</i>	2005	PB	France	Caucasian	2	33	84	1	32	87	0.29	(25)		
Scala <i>et al</i>	2006	HB	Italy	Caucasian	1	21	72	3	73	183	0.15	(27)		
Liao <i>et al</i>	2010	PB	China	Asian	1	6	53	1	10	57	0.48	(31)		
Biselli <i>et al</i>	2008	HB	Brazil	Mixed	4	21	47	8	57	129	0.59	(28)		
Zampieri <i>et al</i>	2012	HB	Brazil	Mixed	5	38	62	9	49	127	0.15	(33)		
da Silva <i>et al</i>	2005	HB	Brazil	Mixed	6	51	97	4	46	108	0.73	(32)		
Coppedè <i>et al</i>	2009	PB	Italy	Caucasian	2	22	66	3	30	78	0.95	(30)		
Wang <i>et al</i>	2012	HB	China	Asian	0	12	72	0	14	106	0.50	(49)		

DS, Down syndrome; PB, population-based; HB, hospital-based; HWE, HardyWeinberg equilibrium in controls.

Table II. Distribution of the CBS 844ins68 genotype among DS mothers (cases and controls) included in the meta-analysis.

Authors	Year	Source of controls	Country	Ethnicity	Genotype distribution						P _{HWE}	(Refs.)
					Cases			Controls				
					ii	iw	22	ii	iw	22		
Brandalize <i>et al</i>	2010	HB	Brazil	Mixed	4	28	207	3	25	169	0.08	(21)
Fintelman-Rodrigues <i>et al</i>	2009	PB	Brazil	Mixed	3	31	80	4	21	85	0.08	(26)
Chango <i>et al</i>	2005	HB	France	Caucasian	0	10	109	1	13	106	0.41	(25)
Scala <i>et al</i>	2006	HB	Italy	Caucasian	0	11	83	0	35	229	0.25	(27)
Zampieri <i>et al</i>	2012	HB	Brazil	Mixed	4	18	83	2	38	145	0.78	(33)
da Silva <i>et al</i>	2005	HB	Brazil	Mixed	2	42	110	3	38	117	0.97	(32)

DS, Down syndrome; PB, population-based; HB, hospital-based; HWE, HardyWeinberg equilibrium in controls; w, wide-type allele; i, allele with the 68bp insertion.

844ins68 polymorphism. The studies included in the present meta-analysis were conducted on different ethnic populations: 6 studies included a Caucasian population, 3 studies included an Asian population, and 6 studies included a mixed population. The studies were published between 2005 and 2013. Additionally, the distribution of genotypes in the control groups deviated from HWE in the study of Neagos *et al* (22). The characteristics of all the included studies are summarized in Tables I and II.

RFC-1 A80G polymorphism. A total of 11 case-control studies on the association between RFC-1 A80G polymorphism and maternal risk for DS were included (Table III). The results of the combined analyses revealed a significantly increased maternal DS risk for the RFC-1 A80G polymorphism: G vs. A: OR=1.19, P=0.04; and recessive model: OR=1.28, P=0.01 (Fig. 2A). When stratified by ethnicity, a significantly elevated maternal risk for DS was observed among Caucasians in the recessive model (OR=1.38, P=0.046), but not among Asians or in the mixed population (Table III).

In the stratified analyses by sample size, a significantly increased risk was observed among large-sample studies (>200 subjects) (G vs. A: OR=1.20, P=0.02; GG vs. AA: OR=1.45, P=0.02; dominant model: OR=1.25, P=0.04; and recessive model: OR=1.29, P=0.03); however, an increased risk was not observed in small-sample studies (≤200 subjects) (Table III). Moreover, when subgroup analysis was performed by source of controls, a significantly increased DS risk was found among hospital-based (HB) controls (G vs. A: OR=1.34, P=0.001; GG vs. AA: OR=1.76, P<0.001; dominant model: OR=1.37, P=0.02; recessive model: OR=1.47, P<0.001), but not among population-based (PB) controls. Interestingly, a statistical correlation between homozygotes and the dominant model, observed in large-sample and HB control groups, was not found in the overall comparison.

MTR A2756G and CBS 844ins68 polymorphisms. The analysis of the MTR A2756G polymorphism and its association with maternal DS risk revealed that the fixed-effects pooled OR for the recessive model: GG vs. GA+AA was non-significant: OR=1.24, P=0.37. (Fig. 2B) Additionally, the allele, dominant and codominant models revealed no significant association. Finally, for the CBS 844ins68 polymorphism, no statistically significant association with maternal DS risk was observed in any of the comparisons (Table IV). Fig. 2C shows the recessive model comparison for the CBS 844ins68 polymorphism. Additionally, a subgroup analysis by ethnicity, source of controls and sample size was performed for the two polymorphisms; however, no significant associations were observed.

Heterogeneity analysis. There was significant heterogeneity in the three genetic models for the RFC-1 A80G polymorphism: G vs. A: P=0.04, GG vs. AA: P=0.03 and GG+GA vs. AA: P=0.01 (Table III). The source of heterogeneity was assessed by ethnicity (Caucasian/Asian/mixed), publication year (prior to or during 2009/after 2009), source of controls (HB/PB) and sample size (≤200/>200 subjects). The subgroup analyses revealed removed heterogeneities in several subgroups, including the Caucasian and mixed population, and the

Table III. Effect of the RFC-1 A80G polymorphism on DS risk.

Variables	G vs. A		GG vs. AA		GA vs. AA		GG+GA vs. AA		GG vs. GA+AA	
	OR (95% CI)	P-value ^a	OR (95% CI)	P-value ^a	OR (95% CI)	P-value ^a	OR (95% CI)	P-value ^a	OR (95% CI)	P-value ^a
Overall	1.19 (1.004-1.40)	0.05	1.34 (0.98-1.96)	0.06	1.10 (0.89-1.29)	0.50	1.18 (0.89-1.58)	0.04	1.28 (1.05-1.56)	0.46
All in HWE	1.18 (0.99-1.40)	0.04	1.36 (0.96-1.93)	0.05	1.06 (0.88-1.28)	0.46	1.16 (0.87-1.56)	0.03	1.27 (1.04-1.55)	0.39
Ethnicity										
Caucasian	1.35 (0.97-1.86)	0.07	1.80 (0.85-3.79)	0.05	1.20 (0.84-1.69)	0.43	1.45 (0.82-2.57)	0.11	1.38 (1.005-1.88)	0.31
Mixed	1.14 (0.96-1.35)	0.97	1.30 (0.92-1.82)	0.97	1.10 (0.84-1.45)	0.89	1.21 (0.92-1.59)	0.93	1.16 (0.87-1.53)	0.71
Asian	1.00 (0.43-2.33)	0.01	1.10 (0.19-6.34)	0.01	0.74 (0.31-1.78)	0.05	0.71 (0.16-3.08)	0.02	1.56 (0.89-2.73)	0.13
Sample size										
>200	1.20 (1.03-1.40)	0.297	1.45 (1.05-1.99)	0.29	1.08 (0.88-1.33)	0.97	1.25 (1.01-1.55)	0.70	1.29 (1.02-1.63)	0.25
≤200	1.21 (0.80-1.83)	0.02	1.52 (0.61-3.78)	0.03	1.02 (0.68-1.53)	0.08	1.32 (0.51-3.40)	0.004	1.27 (0.89-1.81)	0.54
Source of controls										
HB	1.34 (1.12-1.61)	0.24	1.76 (1.12-2.60)	0.19	1.14 (0.91-1.42)	0.63	1.37 (1.06-1.79)	0.31	1.47 (1.14-1.88)	0.41
PB	0.97 (0.77-1.23)	0.25	0.93 (0.58-1.50)	0.29	0.91 (0.64-1.29)	0.29	0.88 (0.50-1.55)	0.06	1.03 (0.75-1.42)	0.76

^aThe random-effects model was used when the P-value for heterogeneity test equalled 0.10; otherwise, the fixed-effects model was used. Bold print indicates statistical significance. DS, Down syndrome; OR, odds ratio; CI, confidence interval; PB, population-based; HWE, HardyWeinberg equilibrium in controls.

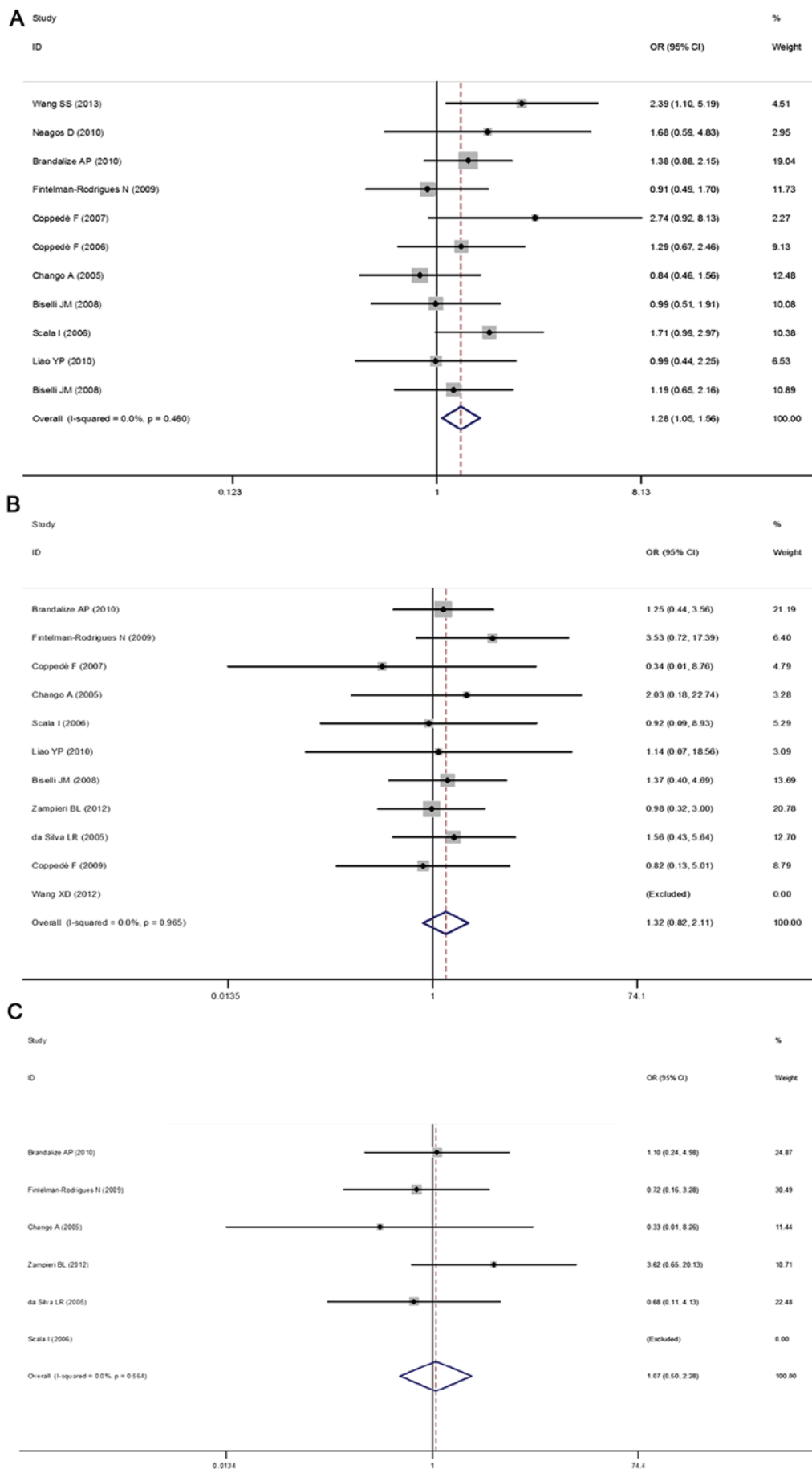


Figure 2. Forest plot of the association between the risk of Down syndrome and the three maternal polymorphisms. (A) RFC-1 A80G polymorphism (GG vs. GA+AA), (B) MTR A2756G polymorphism (GG vs. GA+AA) and (C) CBS 844ins68 polymorphism (ii vs. iw+ww). OR, odds ratio; CI, confidence interval; w, wide-type allele; i, allele with the 68 bp insertion.

Table IV. Association of the MTR A2756G polymorphism with DS risk.

Variables	G vs. A		GG vs. AA		GA vs. AA		GG+GA vs. AA		GG vs. GA+AA	
	OR (95% CI)	P-value ^a	OR (95% CI)	P-value ^a	OR (95% CI)	P-value ^a	OR (95% CI)	P-value ^a	OR (95% CI)	P-value ^a
Overall	1.05 (0.9-1.22)	0.89	1.33 (0.83-2.15)	0.98	1.00 (0.89-1.13)	1.00	1.02 (0.86-1.22)	0.76	1.32 (0.82-2.11)	0.96
Ethnicity										
Caucasian	0.90 (0.68-1.20)	0.78	0.89 (0.30-2.70)	0.85	0.97 (0.78-1.20)	0.99	0.89 (0.65-1.21)	0.80	0.92 (0.30-2.77)	0.85
Mixed	1.12 (0.93-1.35)	0.80	1.48 (0.86-2.55)	0.87	1.02 (0.86-1.20)	0.91	1.10 (0.89-1.37)	0.47	1.44 (0.85-2.46)	0.78
Asian	0.99 (0.54-1.81)	0.41	1.08 (0.66-17.63)	-	1.00 (0.72-1.37)	0.80	0.98 (0.52-1.87)	0.36	1.14 (0.07-18.56)	-
Sample size										
>250	1.08 (0.9-1.31)	0.49	1.28 (0.73-2.25)	0.99	1.02 (0.87-1.20)	0.93	1.08 (0.87-1.34)	0.35	1.22 (0.70-2.14)	0.98
≤250	0.98 (0.76-1.26)	0.94	1.49 (0.61-3.65)	0.7	0.97 (0.81-1.13)	1.00	0.93 (0.70-1.24)	0.91	1.58 (0.65-3.84)	0.66
Source of controls										
HB	1.08 (0.91-1.29)	0.71	1.22 (0.70-2.12)	0.97	1.02 (0.88-1.18)	0.99	1.09 (0.89-1.33)	0.60	1.17 (0.68-2.02)	0.97
PB	0.96 (0.73-1.28)	0.86	1.73 (0.66-4.52)	0.71	0.96 (0.77-1.19)	0.98	0.89 (0.65-1.22)	0.82	1.85 (0.72-4.80)	0.67

^aThe random-effects model was used when the P-value for heterogeneity test equalled 0.10; otherwise, the fixed-effects model was used. PB, population-based; HB, hospital-based; OR, odds ratio; CI, confidence interval.

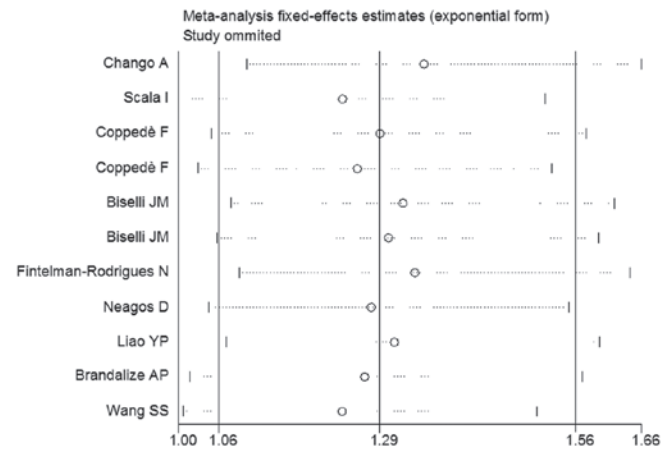


Figure 3. Sensitivity analysis on the association between RFC-1 A80G and the risk of Down syndrome in the recessive model. The results were computed by omitting each study.

large-sample group. However, meta-regression analyses did not reveal any sources contributing to the substantial heterogeneity. For the MTRR A2756G and CBS 844ins68 polymorphisms and their association with DS risk, no statistically significant heterogeneity was observed in any of the genetic models.

Sensitivity analysis. Sensitivity analysis was performed following sequential removal of each eligible study. As regards the association of the RFC-1 A80G polymorphism with maternal DS risk, no single study qualitatively affected the pooled ORs, indicating that the results of the meta-analysis were highly stable (Fig. 3). There were 2 studies that departed from HWE for the CBS 844ins68 polymorphism but, when they were excluded, the pooled OR remained unaffected (Table V).

Cumulative meta-analysis. Cumulative meta-analyses of the 3 associations were also conducted via the assortment of studies by publication time. The results of the association of the RFC-1A80G polymorphism with DS risk for cumulative meta-analysis in chronological order are shown in Fig. 4. The pooled ORs tended to be stable, and the associations exhibited a trend towards significance with the accumulation of more data over time.

Publication bias. Egger's test and funnel plots were applied to evaluate potential publication bias for the RFC-1 A80G, MTR A2756G and CBS 844ins68 polymorphisms (Table VI). The results revealed no evidence of publication bias. The funnel plot investigating the maternal RFC-1 A80G/MTR A2756G/CBS 844ins68 polymorphisms and DS risk is shown in Fig. 5.

Discussion

The folate metabolism pathway plays an important role in DNA methylation, DNA synthesis and cell division (41). Recent meta-analyses suggested that the MTHFR C667T polymorphism of the folate metabolism gene may be associated with an increased occurrence of congenital heart defects or neural tube defects (42,43). Abnormal folate metabolism

Table V. Effect of the CBS 844ins68 polymorphism on DS risk.

Variables	i vs. w		ii vs. ww		iw vs. ww		ii+iw vs. ww		ii vs. iw+ww	
	OR (95% CI)	P-value ^a	OR (95% CI)	P-value ^a	OR (95% CI)	P-value ^a	OR (95% CI)	P-value ^a	OR (95% CI)	P-value ^a
Overall	1.03 (0.82-1.29)	0.82	1.10 (0.51-2.34)	0.61	1.02 (0.79-1.32)	0.65	1.03 (0.80-1.31)	0.76	1.07 (0.50-2.28)	0.56
All in HWE	0.99 (0.74-1.13)	0.71	1.29 (0.44-3.80)	0.30	0.95 (0.69-1.3)	0.75	0.97 (0.71-1.32)	0.78	1.30 (0.44-3.80)	0.28

^aThe random-effects model was used when the P-value for heterogeneity test equalled 0.10; otherwise, the fixed-effects model was used. DS, Down syndrome; HWE, HardyWeinberg equilibrium in controls; OR, odds ratio; CI, confidence interval; w, wide-type allele; i, allele with the 68 bp insertion.

and variants of key enzymes in the folate cycle have been described as possible risk factors for DS through impairing DNA methylation (44-46). A80G polymorphism in the RFC-1 gene may impair folate membrane transport. Recently, several studies were performed to evaluate the effects of the RFC-1 A80G/MTR A2756G/CBS 844ins68 variant on the risk of DS, but the results were inconclusive. For example, Brandalize *et al* (21) and Wang *et al* (20) reported opposite results on the correlation between the RFC-1 A80G polymorphism and DS risk.

In the present meta-analysis, a significant correlation was observed between the RFC-1 A80G polymorphism and maternal DS risk. A significantly increased DS risk was observed in G vs. A and in the recessive model (OR=1.19, P=0.04; and OR=1.28, P=0.01, respectively). However, for MTR A2756G/CBS 844ins68 variants, no significant association between the two polymorphisms and maternal DS risk was observed; the available evidence did not support these two polymorphisms as independent risk factors for DS, which may be attributed to the fact that the interaction of gene polymorphisms rather than any individual polymorphism may be a major determinant of disease risk (47).

In the subgroup analysis stratified by ethnicity, a significant increased DS risk was not observed among Caucasians, Asians and the mixed population for the MTR A2756G and CBS 844ins68 polymorphisms. Thus, the data of the present study suggest that the interactions of these polymorphisms with ethnic variations may not significantly affect DS risk. However, in the recessive model for the RFC-1 A80G variant, a statistically significant increased risk was observed (OR=1.38, P=0.046). Further large-scale studies are required to investigate the possible ethnic differences in the association of the RFC-1 A80G polymorphism with DS risk.

In the stratification analysis by study sample size for the RFC-1 A80G variant, a significantly increased DS risk was only observed in large-sample studies (>200 subjects). In addition, a statistically significant correlation was found between the RFC-1 A80G polymorphism and DS risk for the dominant model and homozygotes, which was not observed in the overall study sample. These results indicate that large-sample studies may offer quite different outcomes compared with small-sample studies, which is possibly due to the fact that small and underpowered studies may be unable to identify true genetic associations (48). Therefore, the use of a proper, large-sample study is crucial for reducing biases in such genotype association studies. In the subgroup analysis stratified by source of controls, a significantly increased DS risk was observed in the HB groups, but not among PB groups. However, HB controls may not always be truly representative of the general population, particularly when the polymorphisms under investigation are expected to affect disease conditions, which may be observed in the HB controls, indicating the presence of possible selection bias. Thus, further studies using proper controls with strict matching criteria are crucial for reducing such selection biases.

In the present meta-analysis, all the available relevant publications in English and Chinese were searched. Furthermore, the Q-test and I² statistics were applied to test the significance of heterogeneity. Heterogeneity was observed in three genetic models; subsequently, meta-regression analysis and subgroup

Table VI. Egger's publication bias test for RFC-1 A80G, MTR A2756G and CBS 844ins68 polymorphisms.

Comparisons	Coefficient	Standard error	t	P> t	95% CI
RFC-1 A80G polymorphism					
G vs. A	0.81	1.63	0.50	0.63	-2.88, 4.51
GG vs. AA	1.08	1.20	0.90	0.39	-1.63, 3.78
GA vs. AA	0.79	.74	1.07	0.314	-0.89, 2.47
GG+GA vs. AA	0.75	1.15	0.65	0.53	-1.84, 3.34
GG vs. GA+AA	1.05	1.26	0.83	0.43	-1.81, 3.92
MTR A2756G polymorphism					
G vs. A	-0.87	0.73	-1.19	0.27	-2.52, 0.79
GG vs. AA	-0.39	0.51	-0.77	0.46	-1.58, 0.79
GA vs. AA	-.122	0.59	-0.21	0.84	-1.45, 1.21
GG+GA vs. AA	-0.73	0.94	-0.78	0.46	-2.86, 1.40
GG vs. GA+AA	-0.27	0.56	-0.49	0.64	-1.56, 1.02
CBS 844ins68 polymorphism					
i vs. w	-2.37	1.04	-2.28	0.08	-5.26, 0.51
ii vs. ww	-1.25	1.74	-0.72	0.52	-6.78, 4.28
iw vs. ww	-2.13	2.07	-1.03	0.36	-7.88, 3.62
ii+iw vs. ww	-2.31	1.58	-1.46	0.22	-6.72, 2.09
ii vs. iw+ww	-1.16	1.85	-0.63	0.58	-7.06, 4.74

CI, confidence interval; w, wide-type allele; i, allele with the 68bp insertion.

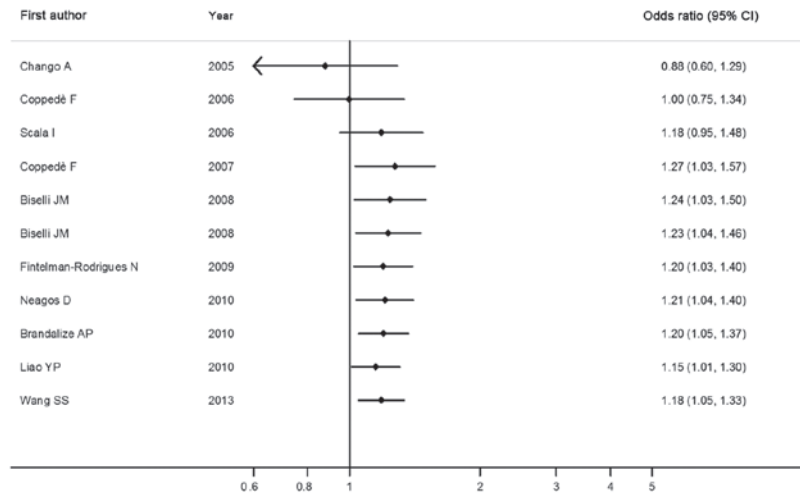


Figure 4. Cumulative meta-analysis: Pooled OR with the corresponding 95% CI at the end of each year; information is shown for RFC-1 A80G (G vs. A). OR, odds ratio; CI, confidence interval.

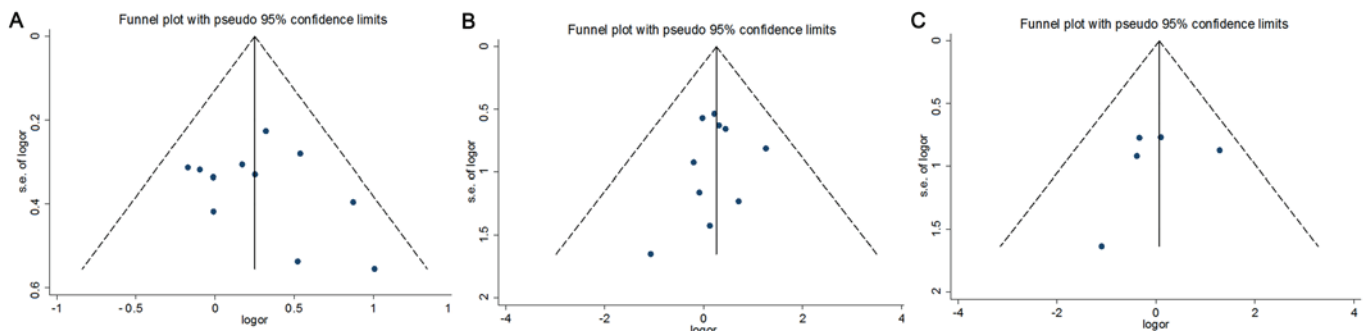


Figure 5. Funnel plot analysis for odds ratios of the dominant model for the three polymorphisms. (A) RFC-1 A80G, (B) MTR A2756G and (C) CBS 844ins68.

analyses were conducted. As a result, heterogeneities were removed in the subgroup analysis on the Caucasian and mixed populations, as well as on the large-sample group. Moreover, publication bias, an important factor considered in meta-analyses, was evaluated by funnel plots and Egger's tests. Neither the shape of the funnel plots nor the statistical results demonstrated publication bias, indicating the robustness and reliability of the results.

There were certain limitations to this meta-analysis: First, folate metabolism is complex and involves several regulatory mechanisms. Coppedè *et al* (24) indicated that the presence of both RFC-1 80GG and MTHFR 677GG was associated with a significantly increased risk of a DS offspring (OR=6, 95% CI: 1.00-35.9), which was also reported by other studies (26,27). However, such gene-gene interactions were not addressed in the present meta-analysis due to the lack of sufficient data. Second, a subgroup analysis by maternal age was not performed in the present meta-analysis. Maternal age is the major risk factor for DS, although several children with DS are born to mothers aged <35 years. Recently, Scala *et al* (27) demonstrated that the 80G allele may increase DS risk in mothers aged >34 years at conception. However, only two studies in the present meta-analysis reported the association between genetic polymorphisms and maternal age. Third, the primary articles included in the present meta-analysis only provided data on Caucasian, Asian and mixed ethnicities. The majority of the studies included Caucasians and mixed populations, whereas data on other ethnicities were not available. Finally, although there was no publication bias observed in the funnel plot and Egger's test, selection bias may have occurred, as only studies in English or Chinese were selected.

In summary, the present meta-analysis suggests that the RFC-1 A80G polymorphism may be a maternal genetic risk for DS, particularly in the large-sample and HB control groups. However, there was no evidence of an association between the MTR A2756G/CBS 844ins68 polymorphism and maternal DS risk. Further well-designed large studies are required to investigate gene-environment interactions, gene-maternal age interactions and combinations of gene polymorphisms.

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