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Research Paper

The association between *RFC1* G80A polymorphism and cancer susceptibility: Evidence from 33 studies

Xiaoyi Huang[1#], Yisha Gao[1#], Jing He[2#], Jiao Cai[3], Na Ta[1], Hui Jiang[1], Jinhong Zhu[4✉], Jianming Zheng[1✉]

1. Department of Pathology, Changhai Hospital, Second Military Medical University, Shanghai 200433, China
2. Department of Pediatric Surgery, Guangzhou Women and Children's Medical Center, Guangzhou Medical University, Guangzhou 510623, Guangdong, China
3. Department of Pathophysiology, Second Military Medical University, Shanghai 200433, China
4. Molecular Epidemiology Laboratory and Department of Laboratory Medicine, Harbin Medical University Cancer Hospital, Harbin 150040, Heilongjiang, China

# Xiaoyi Huang, Yisha Gao and Jing He contributed equally to this work.

✉ Corresponding authors: Jianming Zheng, Department of Pathology, Changhai Hospital, Second Military Medical University, 168 Changhai Road, Shanghai 200438, China, Tel /Fax: (+86-021) 81873689, E-mail: jmzheng1962@smmu.edu.cn; Jinhong Zhu, Molecular Epidemiology Laboratory and Department of Laboratory Medicine, Harbin Medical University Cancer Hospital, 150 Haping Road, Harbin 150040, Heilongjiang, China, Tel: (+86-0451) 86298786, Fax: (+86-0451) 86298398, E-mail: jinhongzhu625@gmail.com

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Abstract

Aberrant folate metabolism is closely related to tumorigenesis. Genetic variations in the *Reduced folate carrier 1* (*RFC1*) may alter the progress of folate metabolism, and thereby cause the initiation and progress of the cancer. Considerable studies have performed to investigate the association between *RFC1* G80A (rs1051266) polymorphism and cancer susceptibility, but the conclusions were conflicting. Therefore, we conducted a meta-analysis to reevaluate the association of *RFC1* G80A polymorphism with cancer risk. PubMed and EMBASE were searched for eligible studies. The association of *RFC1* G80A polymorphism and cancer risk was evaluated by the pooled odds ratios (ORs) and corresponding 95% confidence intervals (CIs). The significant association was found between *RFC1* G80A polymorphism and hematological malignance susceptibility (A vs. G: OR=1.11, 95%CI=1.003-1.23, *P*=0.045; GA vs. GG: OR=1.18, 95%CI=1.06-1.31, *P*=0.002; AA+GA vs. GG: OR=1.18, 95%CI=1.07-1.29, *P*=0.001). Stratified analysis by ethnicity indicated that the association became more prominent among Caucasians (GA vs. GG: OR=1.28, 95%CI=1.12-1.45, *P*＜0.001; AA+GA vs. GG: OR=1.21, 95%CI=1.08-1.36, *P*=0.001). In term of the cancer type, this polymorphism significantly increased the risk of acute lymphoblast leukemia (GA vs. GG: OR=1.13, 95%CI=1.001-1.28, *P*=0.048; AA+GA vs. GG: OR=1.28, 95%CI=1.13-1.46, *P*＜0.001) and acute myeloid leukemia (GA vs. GG: OR=2.57, 95%CI=1.37-4.85, *P*=0.003). No significant association between *RFC1* G80A polymorphism and overall solid cancer risk was observed, but a protective association with digestive cancer risk was found (GA vs. GG: OR=0.89, 95%CI= 0.81-0.99, *P*=0.030). The comprehensive meta-analysis encouraged the notion that *RFC1* G80A polymorphism may play an important role in hematopoietic system malignance. These findings need further validation in the large multicenter investigations.

Key words: *reduced folate carrier 1* gene; polymorphism; cancer susceptibility; meta-analysis

Introduction

Cancer, one of the leading causes of death all around the world, is a result of multiple environmental and genetic risk factors, as well as gene–environment interactions. According to the cancer statistics, approximately1658370 new cancer may occur in USA in 2015 [1]. Although substantial resources have been dedicated to cancer research, cancer is still a huge threat to human. It is well known

that both environmental and hereditary factors play critical roles in the initiation and progression of cancer [2]. Among genetic factors, genetic mutation and epigenetic change such as DNA methylation can lead to carcinogenesis [3]. One-carbon metabolism is closely related to DNA methylation, in which folate, a member of water-soluble B vitamins family [4], plays an important role [5, 6]. Folate is an essential material in cell proliferation and tissue regeneration [7, 8], which cannot be synthesized in the mammalian cell, and must be obtained from extracellular environment to support one-carbon transfer biosynthetic reactions [9]. Folate deficiency can result in DNA hypomethylation and gene instability and subsequently induce disease by changing gene expression and increasing DNA impairment. Studies have shown that folate metabolism dysfunction are implicated in a variety of diseases, such as neuroblastoma [10], breast cancer [11], non-Hodgkin lymphoma [12, 13], and neural tube defect [14]. Given the crucial role of folate, its transporters including folate receptors (FRs), proton-coupled folate transporter (PCFT) and reduced folate carrier (RFC) are of great importance to the maintenance of hemostasis humans [15].

Reduced folate carrier 1 (*RFC1*)/*solute carrier family 19 members 1* (*SLC19A1*) gene, located on chromosome 21, encodes a folate transport protein that has been considered as one of the major components of folate transport system [15, 16]. Besides, RFC1 also plays a part in antifolate transportation during chemotherapy [17], and *RFC1* gene variation can affect the outcome and toxicity of methotrexate (MTX) therapy in leukemia [18]. *RFC1* G80A polymorphism located in exon 2 leads to an amino acid substitution of histidine for the arginine at codon 27 (H27R) of *RFC1*.This polymorphism may influence the function of RFC and one-carbon metabolism, thereby inducing tumorigenesis [19]. Lack of folate leads to DNA synthesis disorders and therefore causes genomic instability. Thus, *RFC1* G80A polymorphism can affect DNA synthesis in the pathogenesis of cancer. Previously, *RFC1* G80A polymorphism had been proven to be a risk factor of acute lymphoblastic leukemia in some studies [20], but other studies showed that *RFC1* G80A polymorphism was not related to the risk of acute lymphoblastic leukemia [21]. Moreover, the impact of *RFC1* G80A polymorphism on solid cancer risk was not definitely known. Recently, several studies have explored the association between *RFC1* G80A polymorphism and solid tumor, including colorectal cancer [22-25], gastroesophageal cancer [26], bladder cancer [27], breast cancer [28-31] and nervous system cancer [32]. Naushad et al. [30] found that *RFC1* G80A polymorphism conferred increased susceptibility to breast cancer, while De Cassia Carvalho Barbosa et al. [31] indicated that the same polymorphism had the protective effect on breast cancer. The association between *RFC1* G80A polymorphism and cancer risk was controversy. Therefore, it is necessary to conduct a comprehensive analysis to clarify the association.

Materials and Methods

Literature search strategy

The PubMed and EMBASE were searched thoroughly without any language restriction to seek potential studies. The following keywords were used for literature search: "RFC, *reduced folate carrier* gene, rs1051266, *RFC1* G80A, or *SLC19A1*", "polymorphism, variant, or variation", and "cancer, neoplasm, or carcinoma". Additionally, literature was searched by manually screening the reference lists of the eligible studies and reviews. The last search was performed on March 31, 2015.

Inclusion and exclusion criteria

The articles included met the following criteria: (a) studies focused on the association between *RFC1* G80A polymorphism and cancer risk; (b) case-control studies or other observational studies; (c) studies providing the genotype distribution data or other original data that can be used to calculate genotype distribution. Exclusion criteria were as follows: (a) duplicate studies; (b) lack of enough data of genotype distribution or data for deducing genotype distribution; (c) not case-control studies.

Study selection and Data extraction

Two investigators extracted data from eligible articles separately. The following data were extracted from the original articles: (1) authors, (2) year of publication, (3) country in which study was conducted, (4) ethnicity, (5) type of cancer, (6) sample size, and (7) alleles and genotypes distribution. When argument occurred between the two investigators, the disagreements were solved by the third investigator. The disagreements were fully discussed by all the three investigators and then vote by ballot among all the researchers.

Statistical analysis

All genotypes frequency of *RFC1* G80A polymorphism was calculated and chi-square test was employed to assess the Hardy-Weinberg Equilibrium (HWE) in control subjects for every study. The association between *RFC1* G80A polymorphism and cancer risk was assessed by calculating pooled odds ratio (OR) and 95% confidence interval (CI) under the five genetic models: comparisons of allele frequencies, homozygous, heterozygous, dominant, and recessive

models. All the statistical tests were two-sided and the result with $P<0.05$ was regarded as a statistically significant. Q value and I^2 were used to evaluate the between-study heterogeneity in the heterogeneity test. The random effects model were hired to calculate the pooled OR and 95% CI when $I^2>50\%$. Otherwise, the fixed effect model was adopted. Moreover, subgroup analyses were performed according to ethnicity and cancer type. Subsequently, the sensitivity analysis was performed by successively removing one study at a time to recalculate OR and 95% CI. Publication bias was assessed by funnel plots and Egger's linear regression test. If the funnel plots were asymmetry or $P<0.1$, publication bias was considered significant. All the results above were performed by STATA 12.0 (STATA Corporation, College Station, TX).

Results

Search results and study characteristics

Using the keywords, 454 and 240 articles were retrieved from PubMed and EMBASE, respectively. Most of studies were excluded after carefully reading the titles and abstracts. The remaining articles were further evaluated by reading through the text. As a result, 28 eligible articles were included in the meta-analysis. Additionally, 2 articles were found through manually searching the reference lists of the eligible studies and reviews. In total, the meta-analysis contained 30 articles consisting of 33 studies with 12020 cases and 14343 controls, focusing on the relationship between *RFC1* G80A polymorphism and cancer risk (**Figure 1**). The characteristics of the studies included in the meta-analysis are shown in **Table 1**.

Table 1. The characteristics of the included studies on *RFC1* G80A polymorphism and cancer risk

Author	Year	Ethnicity	Country	Cancer type	Sample size	Cases			Controls		
					Cases/Controls	GG	GA	AA	GG	GA	AA
Whetstine[44]	2001	Caucasian	USA	ALL	54/51	10	24	20	9	25	17
Skibola[45]	2004	Caucasian	USA	non-Hodgkin lymphoma	334/729	109	158	67	266	331	132
Ulrich[22]	2005	Mixed	USA	colorectal cancer	1600/1962	513	788	299	585	976	401
Lightfoot[46]	2005	Caucasian	USA	non-Hodgkin lymphoma	589/755	199	277	113	263	369	123
Wang[26]	2006	Asian	China	oesophageal cancer	216/673	66	67	83	193	313	167
Wang[26]	2006	Asian	China	gastric cancer	633/673	177	242	214	193	313	167
Moore[27]	2007	Caucasian	Spain	bladder cancer	1084/1032	301	520	263	313	500	219
Xu[28]	2007	Caucasian	USA	breast cancer	1066/1108	247	532	287	237	561	310
Gast[47]	2007	Caucasian	Germany	ALL	542/542	125	251	79	178	256	108
Eklof[23]	2008	Caucasian	Sweden	colorectal cancer	219/410	70	93	56	116	190	104
Kotsopoulos[29]	2008	Caucasian	Canada	breast cancer	937/764	304	440	193	243	347	174
Liu[48]	2008	Asian	China	lung cancer	499/504	127	250	122	137	250	117
Sirachainan[32]	2008	Asian	Thailand	central nervous system tumors	73/205	18	38	17	44	104	57
Di[40]	2009	Asian	China	cervical cancer	107/107	31	48	28	18	46	43
de Jonge[49]	2009	Caucasian	Netherland	ALL	241/495	69	120	52	186	241	68
Kurzwelly[50]	2010	Caucasian	Germany	primary central nervous system lymphoma	185/212	68	87	30	76	91	45
Yeoh[51]	2010	Asian	Malaysia-Singapore	ALL	210/319	62	108	40	72	170	77
Curtin[24]	2011	Mixed	USA	colorectal cancer	724/922	226	351	147	280	459	183
Galbiatti[41]	2011	Caucasian	Brazil	head and neck aquamous cell carcinoma	322/531	92	137	93	126	221	184
Jokic[25]	2011	Caucasian	Croatia	colorectal cancer	300/300	85	160	55	90	155	55
Naushad[30]	2011	Asian	India	breast cancer	244/244	87	107	50	96	122	26
Chan[20]	2011	Asian	Singapore	ALL	184/177	43	98	43	61	75	41
Metayer[21]	2011	Caucasian	USA	ALL	348/422	106	188	54	132	205	85
Yang[52]	2011	Asian	China	ALL	361/367	93	172	96	105	191	71
Zhao[53]	2011	Asian	China	ALL	98/135	21	53	24	53	52	30
De Cassia[31]	2012	Caucasian	Brazil	breast cancer	156/156	58	71	27	30	89	37
Silva[38]	2013	Caucasian	Brazil	AML	21/137	4	10	7	49	56	32
Silva[38]	2013	Caucasian	Brazil	ALL	95/137	21	38	36	49	56	32
De Miranda[37]	2014	Caucasian	Brazil	central nervous system tumors	30/92	4	14	12	31	43	18
Karathanasis[54]	2014	Caucasian	Greece	ALL	35/48	9	16	10	15	18	15
Suthandiram[55]	2014	Asian	Malaysia	non-Hodgkin lymphoma	372/722	99	182	91	187	354	181
Montalvao[39]	2015	Caucasian	Brazil	nephroblastoma	77/222	13	38	26	76	103	43
Montalvao[39]	2015	Caucasian	Brazil	central nervous system tumors	64/222	11	33	20	76	103	43

ALL, acute lymphoblast leukemia; AML, acute myeloid leukemia

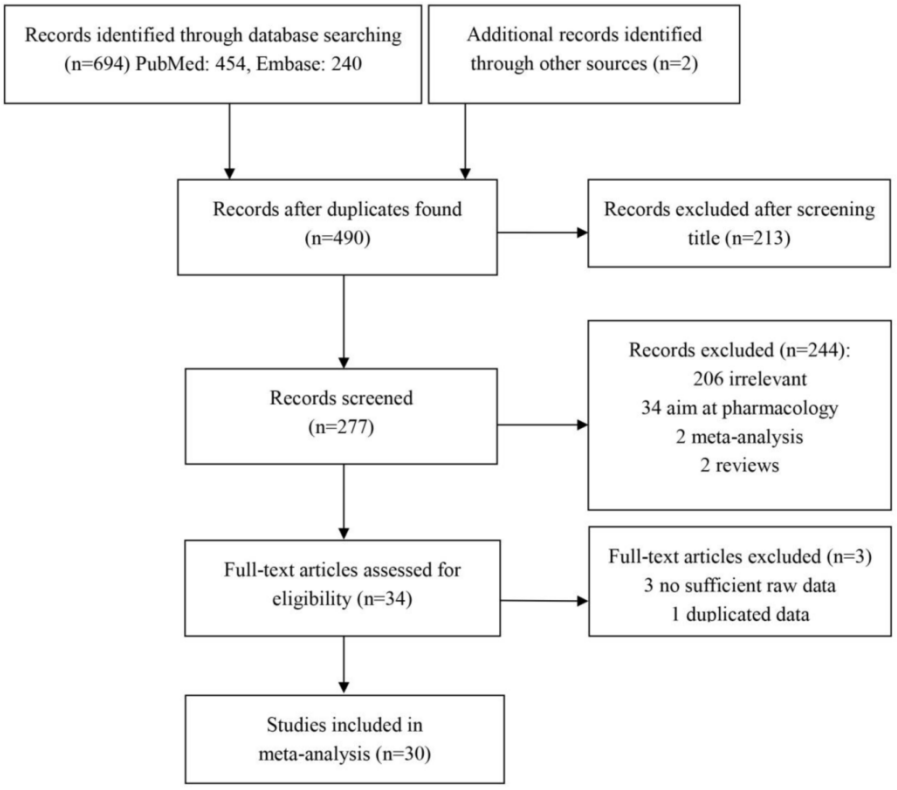

Figure 1. Flow chart of the study selection and inclusion process.

Meta-analysis results

Since the pathogenesis of solid cancer and hematological malignance vary greatly, we explored the association of *RFC1* G80A polymorphism with solid cancer and hematological malignance separately. The pooled ORs and 95% CIs indicated that there was no significant association between *RFC1* G80A polymorphism and overall solid cancer risk (**Figure 2**). When stratified by cancer type, subgroup analysis indicated that *RFC1* G80A polymorphism was associated with the decreased risk of digestive system cancer under the heterozygous model (OR=0.89, 95%CI=0.81-0.99, *P*=0.03), while no significant association was detected for other cancer types (**Table 2**). However, *RFC1* G80A polymorphism was shown to significantly increase the risk of developing hematological malignance (comparisons of allele frequencies: A vs. G: OR=1.11, 95%CI=1.003-1.23, *P*=0.045; heterozygous: GA vs. GG: OR=1.18, 95%CI=1.06-1.31, *P*=0.002; dominant: AA+GA vs. GG: OR=1.18, 95%CI=1.07-1.29, *P*=0.001), as are shown in **Figure 3**. Moreover, stratification analysis was performed by cancer type and ethnicity, as shown in **Table 3**. The association became stronger among Caucasians, but not valid among Asians (GA vs. GG: OR=1.28, 95%CI=1.12-1.45, $P<0.001$; AA+GA vs. GG: OR=1.21, 95%CI=1.08-1.36, *P*=0.001). With respect to cancer type, the studied polymorphism imparted increased genetic susceptibility to ALL (GA vs. GG: OR=1.13, 95%CI=1.001-1.28, *P*=0.048; AA+GA vs. GG: OR=1.28, 95%CI=1.13-1.46, $P<0.001$) and AML (GA vs. GG: OR=2.57, 95%CI=1.37-4.85, *P*=0.003).

Sensitive analysis and publication bias

The funnel plot and Egger's test were conducted to test publication biases. The funnel plots of the association between *RFC1* G80A polymorphism and solid cancer and hematological malignance were symmetrical in the allele model (**Figure 4** and **Figure 5**). The Egger's test value indicated that there was no significant publication bias in our meta-analysis (*P*=0.304; 0.287; 0.768; 0.476 and 0.273 for allele, homozygous, heterozygous, dominant ,and recessive models of *RFC1* G80A polymorphism and solid cancer risk respectively; *P*=0.219; 0.374; 0.183; 0.201; 0.686 for allele, homozygous, heterozygous, dominant, and recessive models of *RFC1* G80A polymorphism and hematological malignance respectively). The sensitive analyses indicated that no single study could qualitatively change the results, suggesting the robustness of the meta-analysis.

Table 2. Meta-analysis of *RFC1* G80A polymorphism and solid cancer

Variables	A vs. G			AA vs. GG			GA vs. GG			AA+GA vs. GG			AA vs. AG+GG		
	OR	95%CI	P	OR	95%CI	P	OR	95%CI	P	OR	95%CI	P	OR	95%CI	P
Overall	1.04	0.94-1.14	0.439	1.08	0.90-1.29	0.432	0.94	0.88-1.01	0.102	0.98	0.87-1.10	0.727	1.12	0.96-1.30	0.155
Ethnicity															
Mixed	0.94	0.87-1.02	0.144	0.89	0.76-1.05	0.164	0.93	0.82-1.05	0.249	0.92	0.82-1.03	0.161	0.94	0.82-1.07	0.343
Caucasian	1.06	0.91-1.23	0.469	1.10	0.83-1.47	0.500	0.99	0.90-1.09	0.838	1.04	0.83-1.29	0.760	1.06	0.89-1.28	0.510
Asian	1.07	0.89-1.27	0.485	1.15	0.82-1.62	0.429	0.87	0.74-1.01	0.063	0.99	0.84-1.16	0.899	1.24	0.88-1.75	0.213
Cancer type															
Digestive system cancer	1.04	0.93-1.17	0.456	1.07	0.88-1.31	0.497	**0.89**	**0.81-0.99**	**0.030**	0.94	0.86-1.03	0.200	1.18	0.92-1.51	0.194
Urinary system cancer	1.41	0.85-2.63	0.187	1.97	0.72-5.51	0.190	1.15	0.95-1.39	0.165	1.60	0.73-3.53	0.245	1.49	0.86-2.59	0.159
Breast cancer	0.94	0.77-1.15	0.539	0.91	0.59-1.40	0.677	0.91	0.79-1.04	0.163	0.86	0.64-1.14	0.285	1.02	0.74-1.40	0.903
Central nervous system tumors	1.48	0.82-2.68	0.194	2.16	0.66-7.09	0.206	1.50	0.96-2.35	0.078	1.78	0.74-4.27	0.197	1.53	0.74-3.16	0.248
Others	0.81	0.66-0.98	0.204	0.72	0.43-1.22	0.224	0.93	0.75-1.15	0.513	0.82	0.56-1.19	0.289	0.81	0.57-1.14	0.226

Table 3. Meta-analysis of *RFC1* G80A polymorphism and hematological malignance

Variables	A vs. G			AA vs. GG			GA vs. GG			AA+GA vs. GG			AA vs. AG+GG		
	OR	95%CI	P	OR	95%CI	P	OR	95%CI	P	OR	95%CI	P	OR	95%CI	P
Overall	**1.11**	**1.00-1.23**	**0.045**	1.21	0.99-1.47	0.063	**1.18**	**1.06-1.31**	**0.002**	**1.18**	**1.07-1.29**	**0.001**	1.07	0.90-1.27	0.439
Ethnicity															
Caucasian	1.12	0.99-1.26	0.070	1.24	0.97-1.58	0.092	**1.28**	**1.12-1.45**	**<0.001**	**1.21**	**1.08-1.36**	**0.001**	1.09	0.86-1.38	0.493
Asian	1.10	0.90-1.34	0.372	1.17	0.80-1.71	0.424	1.01	0.84-1.22	0.898	1.11	0.94-1.31	0.212	1.05	0.82-1.34	0.696
Cancer type															
ALL	1.15	0.99-1.34	0.069	1.29	0.96-1.79	0.095	**1.13**	**1.00-1.28**	**0.048**	**1.28**	**1.13-1.46**	**<0.001**	1.09	0.84-1.40	0.537
NHL	1.06	0.96-1.17	0.245	1.13	0.93-1.38	0.228	1.16	0.88-1.52	0.285	1.06	0.92-1.23	0.425	1.10	0.93-1.31	0.266
PCNSL	0.89	0.67-1.18	0.398	0.75	0.42-1.31	0.308	1.34	0.94-1.91	0.102	0.96	0.64-1.45	0.851	0.72	0.43-1.20	0.204
AML	1.71	0.89-3.30	0.109	2.68	0.73-9.90	0.139	**2.57**	**1.37-4.85**	**0.003**	2.37	0.75-7.43	0.140	1.64	0.61-4.42	0.327

ALL, acute lymphoblast leukemia; NHL, Non- Hodgkin leukemia; PCNSL, primary central nervous system lymphoma; AML, acute myeloid leukemia

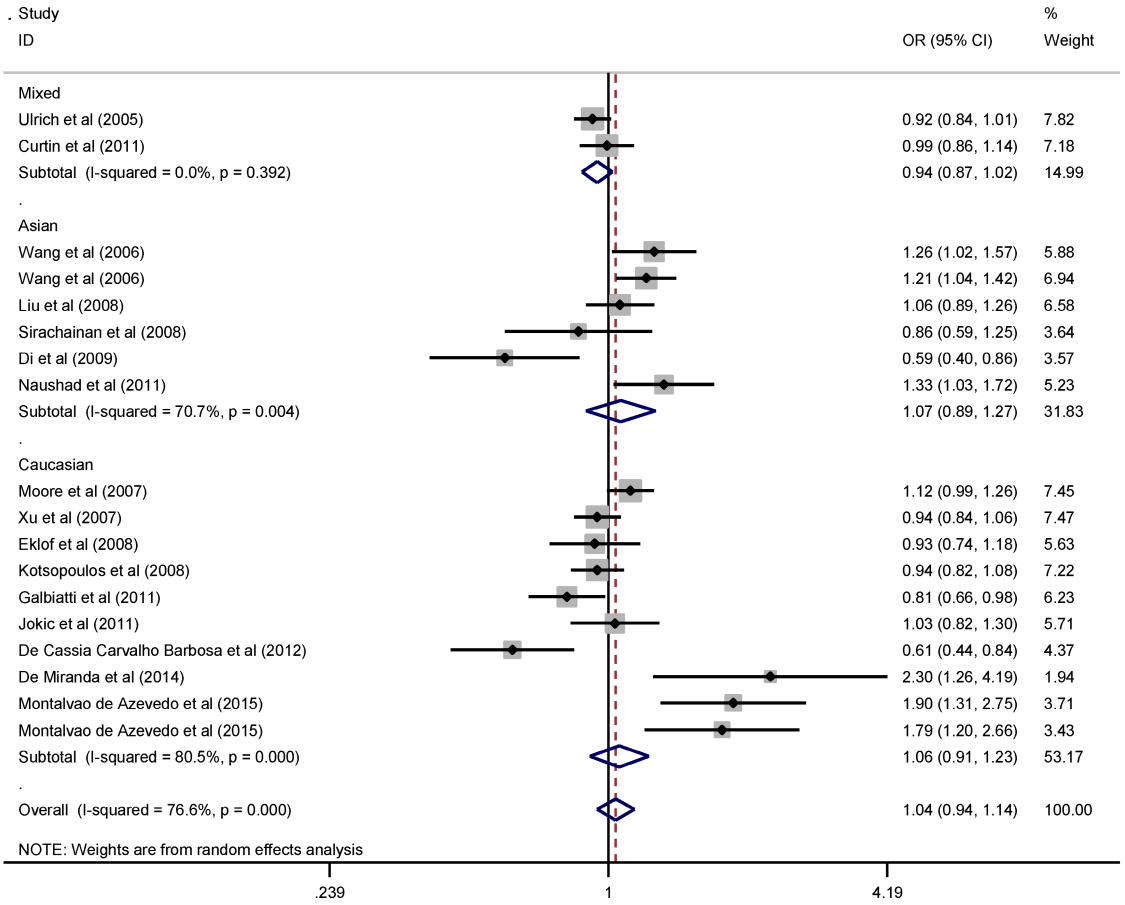

Figure 2. Forest plot of solid cancer risk related with *RFC1* G80A polymorphism stratified by ethnicity in allele model (A vs. G)

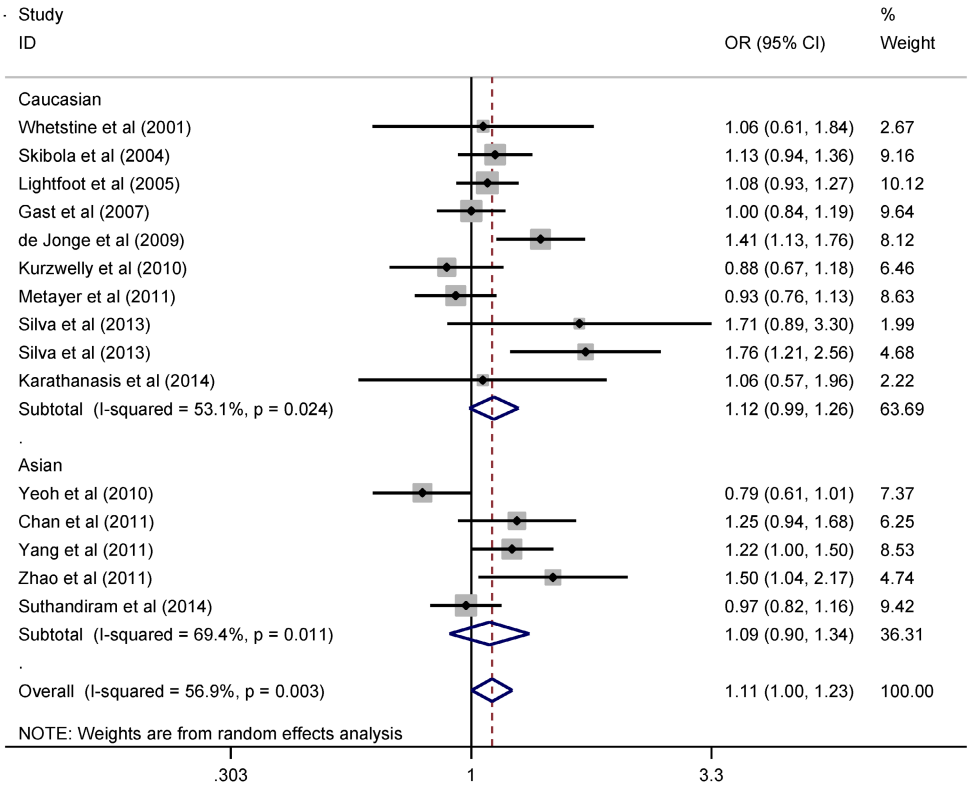

Figure 3. Forest plot of hematological malignance risk related with *RFC1* G80A polymorphism stratified by ethnicity in allele model (A vs. G)

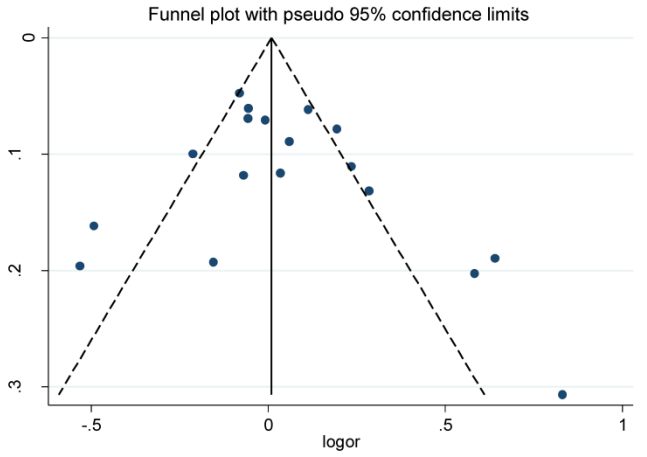

Figure 4. Funnel plot for *RFC1* G80A polymorphism and solid cancer risk

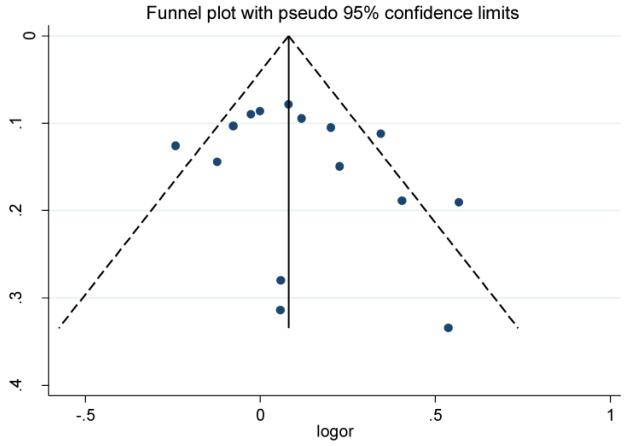

Figure 5. Funnel plot for *RFC1* G80A polymorphism and hematological malignance risk

Discussion

RFC is a typical facilitative transmembrane protein delivering 5-methyltetrahydrofolate from plasma into cells. The dysfunction of RFC has been shown to be related to several disease such as neural tube defects [33], congenital heart defect [34], Alzheimer's Disease [35] and homocysteinemia [36]. Moreover, RFC is also responsible for transporting Methotrexate from extracellular fluid into intracellular fluid, which is an effective treatment for rheumatoid arthritis and acute lymphoblastic leukemia. Although *RFC1* G80A polymorphism, leading to a histidine-to-arginine substitution at position 27 of the RFC protein, was first identified in as early as 2000 [36], the effect of this alteration is not fully clarified. Chango et al. [36] indicated that individuals carrying AA genotype had higher plasma folate levels than those carrying GG genotype. Recently, increasing evidences have highlighted the importance of *RFC1* G80A polymorphism in the pathogenesis of malignance [37-39].

To the best of our knowledge, this is the first meta-analysis focusing on the association of *RFC1* G80A polymorphism and the susceptibility of solid cancer. Overall, there was no significant association between the *RFC1* G80A polymorphism and solid cancers which was consistent with most of previously published results. On the contrary, some other studies considered *RFC1* G80A polymorphism as a risk factor for tumorigenesis. The controversial results of these studies might be partially due to the variations among the different populations. In recent years, increasing researches have investigated the relationship of *RFC1* G80A polymorphism and cancer risk; however, the association remains inconclusive. Thus, it is urgent to perform a pooled analysis to clarify the association of interest and give a general envisage of the impact of *RFC1* G80A polymorphism on tumorigenesis. In part, this conclusion maybe results from the small sample size in each study. Moreover, a significantly decreased risk of digestive system cancer related to *RFC1* G80A polymorphism was observed. Surprisingly, in the studies conducted by Xu et al. [28], Di et al. [40] and Galbiatti et al. [41] AA genotype was considered as wild type, while in the majority of studies GG genotype was regarded as wild type. This discrepancy may lead to conflicting results which may affect the relationship between *RFC1* G80A polymorphism and susceptibility of solid cancer in the pooled analysis.

Previous studies have shown that *RFC1* G80A polymorphism is related to children acute lymphoblastic leukemia, and this polymorphism may affect the prognosis of acute lymphoblastic leukemia treated with MTX [18, 20]. Similarly, our meta-analysis demonstrated that the studied polymorphism increased the risk of hematological malignance. Koppen et al. [42] and Vijayakrishnan et al. [43] also performed comprehensive analysis on the influence of folate-related gene polymorphisms including the effect of *RFC1* G80A polymorphism on susceptibility to leukemia. Koppen et al. [42] failed to find clear association between *RFC1* G80A polymorphism and acute lymphoblastic leukemia partly because only one relevant study was included in their meta-analysis. Vijayakrishnan et al. [43] indicated that *RFC1* G80A polymorphism was related with acute lymphoblastic leukemia. Compared to the two previous meta-analyses, our study included more eligible studies and provided a more comprehensive and powerful evaluation on the relationship between *RFC1* G80A polymorphism and hematological malignance. In the stratification analysis, *RFC1* G80A polymorphism was found to be associated with increased leukemia risk in Caucasians under the heterozygous and dominant model, implying the interaction of the ethnic background and genetic variation in leukemia tumorigenesis. As for cancer type, *RFC1* G80A polymorphism was associated with acute lymphoblastic leukemia under the heterozygous and dominant model, but not non-Hodgkin lymphoma. These results suggested that the *RFC1* G80A polymorphism may play a different role in the pathogenesis of different type of hematological malignance. Besides, due to relatively small sample size of homozygous genotype AA carriers, the data presented in our meta-analysis cannot demonstrate more severe phenotype in homozygous non-synonymous mutation carriers than heterozygous carriers.

Despite the interesting results in our meta-analysis, several limitations of the current study should be acknowledged. First, this meta-analysis only included published studies so that potential publication bias might not be avoided. Second, the influence of a single genetic variant on tumorigenesis is complicated by the gene-gene or gene-environment, which may lead to an unmeasurable deviation while evaluating the relationship between *RFC1* G80A polymorphism and cancer risk in this meta-analysis. Third, we performed this meta-analysis with crude ORs since studies included in this meta-analysis lacked sufficient data for adjustment for confounding factors, which might affect the stability of our results. Last, random effect model were used in some genetic models, which might present unstable results. Therefore, validation for our findings from large, well-designed studies is needed in the future.

In conclusion, the comprehensive meta-analysis confirmed the notion that *RFC1* G80A polymorphism may play a significant role in hematopoietic system malignance. In addition, the relationship between *RFC1* G80A polymorphism and cancer grade and patient prognosis can be a promising research to clarify the role of *RFC1* G80A polymorphism in pathogenesis of cancer. Although the mechanism of how *RFC1* G80A polymorphism contributes to cancer risk is not entirely clear, it is may be used as a potential biomarker for cancer diagnosis in the future.

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Competing Interests

The authors have declared that no competing interest exists.

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