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Review Article

Association of Methylenetetrahydrofolate reductase genetic polymorphisms and folate intake with susceptibility of esophageal squamous cell carcinoma: A meta-analysis

Yang Shujuan[1], Wen Yuanyuan[2], Zhang Jianxing[3], Chen Xin-yue[4]

ABSTRACT

Objectives: We conducted an update meta-analysis to investigate the association of MTHFR C677T and folate intake with esophageal cancer risk.

Methodology: PubMed, EMBASE and the CNKI were searched in our study. The pooled Odds ratio (ORs) and 95% confidence intervals (CIs) of MTHFR C677T and folate intake and ESCC risk from each study were pooled using meta-analysis.

Results: Twenty studies (4271 cases and 5559 controls) were included for meta-analysis. MTHFR 677 CT and TT genotypes were associated with an increased risk of ESCC, with OR(95%CI) of 1.48(1.25-1.77) and 1.65(1.24-2.20). By subgroup analysis, we found individuals carrying CT+TT genotype and low intake of folate had an increased risk of ESCC risk when compared with CC genotype [OR(95CI%)=1.55(1.02-2.57)]. There was a significant interaction between folate intake and ESCC. No publication bias was found among studies regarding MTHFR 677 CT and TT by Egger's test.

Conclusions: Our meta-analysis indicated *MTHFR 677CT* and/or *TT* genotypes are associated with the risk of ESCC, and folate could modify their association.

KEY WORDS: Methylenetetrahydrofolate reductase C677T, Folate intake, Esophageal squamous cell carcinoma, Meta-analysis.

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INTRODUCTION

Esophageal cancer has been ranked as one of the most common malignancies worldwide.[1] Comparing with Comparing with esophageal adenocarcinoma which is one of the most prevalent cancer in Western Countries, and the major phenotype in Asian countries, especially in China, is esophageal squamous cell carcinoma(ESCC). In China, and it is estimated there are new diagnosed 250,000 cases every year. Possible risk factors for ESCC include smoking and drinking habits, high intake of hot-temperature food, salted food and pickled vegetables, low intake of vegetable, chronic mucosal irritation.[2-5] It is also reported that deficiency of nutrients, such as vitamins and microelements, is association with an enhanced risk for ESCC.[5]

It is well known folate is a water-soluble vitamin element from green leafy vegetables, cereals, legumes and fruits.[6] Lack of folate may damage the function of DNA repair and chromosomal fragile site expression, and induce the breaks of chromosome and formation of micronucleus.[6] Folate is also function as a major methyl group donor and had a key role in DNA methylation.[7] Previous studies have shown the aberrant DNA methylation

1. Yang Shujuan,
2. Wen Yuanyuan
3. Zhang Jianxing,
4. Chen Xin-yue,

1-4: West China School of Public Heath, Sichuan University, Chengdu, China.

Correspondence:
Yang Shujuan,
West China School of Public Heath,
Sichuan University, Chengdu, China.
E-mail: rekinyyang@yahoo.com.cn

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is associated with cancer risk.[7] The common polymorphism for Methylenetetrahydrofolate reductase (MTHFR) gene is a C to T substitution at nucleotide 677 (MTHFR C677T), and this variation leads to a reduced activity of this enzyme. Thus the variation of folate metabolic enzyme activity altered by the genetic polymorphisms may have a role in the susceptibility to methylation-related carcinogenesis. However, the role of dietary folate in esophageal cancer and its relationship between MTHFR C677T were controversial.[8-10] The inconsistency of these results may be explained by differences in source of selected subjects, sample size, study design and random error. We therefore performed a systematic review to investigate potential genetic associations of MTHFR C677T and folate intake with ESCC risk by reducing random error and obtaining precise estimates.[11]

METHODOLOGY

Searching strategy: We searched PubMed (from Jan. 1966 to Oct. 2012), EMBASE (from January 1988 to Oct. 2012), and the Chinese Journal Net (CNKI; from January 1980 to Oct. 2012) by using the following key words for searching published papers: 'esophageal squamous cell carcinoma', 'esophagus' or 'oesophagus', 'carcinoma or cancer or neoplasm or tumour or tumor', 'Methylenetetrahydrofolate reductase', or 'MTHFR'. We only extract published paper in English and Chinese. All references cited in studies and published review articles were extracted for additional eligible studies. The criteria of including data were as follows: (1) a case-control study design; (2) reporting the association between *MTHFR C677T* polymorphisms and ESCC; (3) original data about the genotype distributions of *MTHFR C677T* genotypes. (4) If the data were published for more than twice, we only extracted the most complete data. Two reviewers independently evaluated and extracted the potential selected articles, and the disagreements were resolved by discussion. If the data were missing, we attempted to contact the authors of the articles by email or phone so as to request the relevant data. All the data were organized as first author's name, year of publication, study site, numbers of cases and controls, genotype frequencies of MTHFR C677T.

The quality of studies was evaluated by a previous defined scale reported by Jiang (Table-I).[12] The total quality score was ranged from 0 to 15. Score≤10 was regarded as low quality of study, and score >10 was regarded as high quality of study.

Table-I: Assessment scale for Quality of case-control studies

Criterion Score	*Score*
Source of cases (0-3)	
Selected from population or cancer registry	3
Selected from hospital	2
Selected from pathology archives, but without description	1
Not described	0
Source of control (0-3)	
Population-based	3
Blood donors or volunteers	2
Hospital-based (cancer-free patients)	1
Not described	0
Specimens used for determining genotypes (0-3)	
White blood cells or normal tissues	3
Tumor tissues or exfoliated cells of tissue	0
Hardy-Weinberg equilibrium in controls (0-3)	
Hardy–Weinberg equilibrium	3
Hardy–Weinberg disequilibrium	0
Total sample size (0-3)	
>1,000	3
>500 and <1,000	2
>200 and <500	1
<200	0
Total Score	Ranged from 0-15

Statistical analysis: All statistical analyses were performed using STATA statistical package (Version 9.1, STATA, College Station, TX). Variation of genotype frequency distribution in controls from the expected under Hardy-Weinberg equilibrium (HWE) were calculated using Chi-square test. The pooled Odds ratio (ORs) and 95% confidence intervals (CIs) were used to assess the association of polymorphisms in MTHFR C677T and folate intake with ESCC risk. The heterogeneity between studies was assessed by the Q-statistics, and P values<0.1 was defined as statistical significance. Subgroup analysis was conducted to explore the possible sources of heterogeneity. If there was heterogeneity between studies, a random effect model would be used to obtain the pooled OR and its 95% CI. Otherwise, a fixed-effect model would be applied. Funnel plot an Egger's test were used to assess the publication bias. Statistical significance was defined as a two-sided P value of less than 0.05.

RESULTS

60 studies were achieved by literature search from PubMed, EMBASE and CNKI database, using different combinations of key terms. However, 39 studies were excluded from analysis, mainly due

to reviews, overlapping data and being without meeting the criteria. Finally, a total of 20 studies were selected for meta-analysis, including 4271 cases and 5559 controls (Table-II). Among the 20 studies, frequencies of genotypes in controls of eight studies were in line with Hardy-Weinberg equilibrium, which suggested there might be population stratification and sample bias. Among 20 countries, we found only six studies had high quality, and the remained quality scores were ranged from 7 to 10. Of 20 case-control studies, 17 studies were conducted in China, one in India, one in Japan, one in German and one in Pakistan. MTHFR 677 CT and TT genotypes were associated with an increased risk of ESCC, with OR(95%CI) of 1.48(1.25-1.77) (Figure-1) and 1.65(1.24-2.20) (Figure-2). Significant heterogeneity was found among studies regarding *MTHFR 677 CT* and *TT* genotypes (P<0.05).

Subgroup analysis was taken in terms of folate intake and different quality of studies. We found individuals carrying CT+TT genotype and low intake of folate had a light increased risk of ESCC risk when compared with CC genotype [OR(95CI%)=1.55(1.02-2.57)] (Table-III), and a significant heterogeneity was found. However, a non-significant increased risk was found in individuals carrying CT+TT genotype. There was a significant interaction between folate intake and ESCC (*P*<0.05, data not shown). We also found significant heterogeneity after conducting stratification of quality of studies.

Egger's test was used to assess the publication bias, and it provided evidence that there was no publication bias among studies regarding MTHFR 677 CT and TT(*P*=0.46 and *P*=0.08, respectively).

DISCUSSION

Although previous epidemiologic studies investigated the role of *MTHFR C677T* for ESCC risk, the results were inconsistent. Most of these epidemiologic studies have limited sample size, and more importantly, are not power enough to give a precise estimated effect of gene variation on the ESCC risk. Therefore, meta-analysis could play a key role to obtain a more precise effect on the genetic polymorphisms on risk of disease. Three previous studies investigate the role of *MTHFR C677T* for ESCC risk.[29-31] The three meta-analyses showed individuals carrying variation of *MTHFR C677T* had an increased risk of ESCC, with an OR(95% CI) ranged from 1.58 to 1.78. However, the previous three meta-analyses only showed the association of *MTHFR C677T* on ESCC, and did not indicate the interaction between *MTHFR C677T*

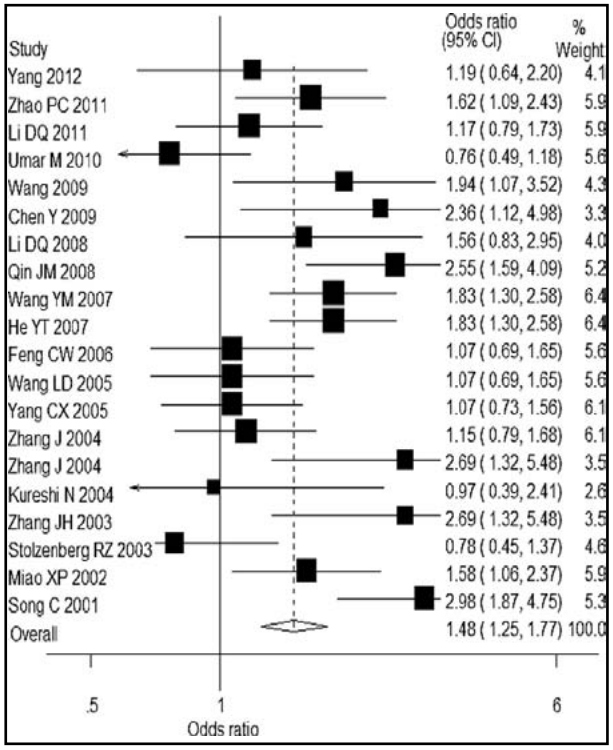

Fig.1: Pooled OR and 95% CI of studies on MTHFR 677CT vs CC

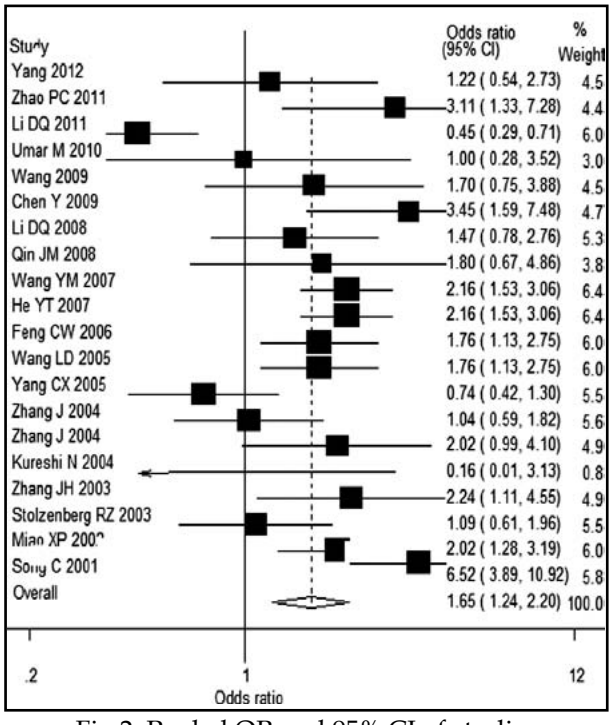

Fig.2: Pooled OR and 95% CI of studies on MTHFR 677TT vs CC

Table-II: Characteristics of studies of MTHFR C677T polymorphism and ESCC

Study ID	*County*	*Case*	*Control*	*Cases*			*Controls*			*PHWE*	*Quality*
				CC	*CT*	*TT*	*CC*	*CT*	*TT*		
Yang 201213	China	100	97	37	45	18	40	41	16	0.33	8
Zhao 201114	China	155	310	68	74	13	179	120	11	0.09	9
Li 201115	China	226	246	64	85	77	58	97	91	<0.1	9
Umar 201016	India	208	223	155	48	5	155	63	5	0.63	13
Wang 200917	China	102	108	39	47	16	58	36	14	0.77	9
Chen 200918	China	103	181	11	49	43	45	85	51	0.42	10
Qin 200819	China	120	204	60	53	7	170	59	11	0.06	11
Li 200820	China	126	169	22	52	52	41	62	66	<0.1	10
Wang 200721	China	584	540	73	263	248	119	234	187	<0.1	11
He 200722	China	584	540	73	263	248	119	234	187	<0.1	10
Feng 200623	China	275	315	51	105	119	74	143	98	0.12	8
Wang 200524	China	275	315	51	105	119	74	143	98	0.12	10
Yang 200510	Japan	165	493	63	82	20	186	227	80	0.45	9
Zhang 200425	German	241	256	94	116	31	107	115	34	0.72	10
Zhang 200425	China	189	141	16	93	80	25	54	62	<0.1	10
Kureshi 200426	Pakistan	34	54	22	12	0	32	18	4	0.52	8
Zhang 200327	China	198	141	16	93	89	25	54	62	<0.1	7
Stolzenberg 20039	China	129	398	23	58	48	65	209	124	0.14	8
Miao 200228	China	217	468	47	107	63	151	217	100	0.18	12
Song 20018	China	240	360	29	118	93	126	172	62	0.8	11
Total		4271	5559	1062	1896	1357	1886	2368	1357		

and folate intake.[29-31] Therefore, we conducted an updated meta-analysis by critically reviewing 20 individual case-control studies on MTHFR C677T and folate intake with esophageal cancer risk. Compared with previous three meta-analyses, this updated meta-analysis included another seven new case-control studies, and we have shown folate modify the association between polymorphism in MTHFR C677T and ESCC risk.

It is reported that folate mediates the DNA methylation reactions and function of DNA synthesis, replication, and repair,[32] which may enhance the susceptibility of cancer for individuals with low intake of folate. Previous studies have reported a high intake of food full of folate can prevent the development of various cancers.[33] Ours study has shown individuals carrying MTHFR 677TT genotype who have high intake of folate are associated with a non-significant increased risk of ESCC, and the results suggest folate intake modify the function of polymorphism in MTHFR C677T with ESCC risk.

In our study, we found significant heterogeneity between studies. However, the heterogeneity decreased or disappeared after stratifying by the intake of folate, suggesting that the folate intake was an important factor of influencing the association between variation of MTHFR C677T and ESCC risk. We found a significant heterogeneity after stratifying by quality of study, and thus this factor was not a source of heterogeneity. Some other potential factors may alter the association of this gene polymorphism and ESCC risk, such as smoking, drinking, hot food and salted food, and

Table-III: Subgroup analysis of MTHFR C677T polymorphism and ESCC

Items	*Cases*		*Control*		*OR(95% CI)*	*P for heterogeneity*
	CC	*CT+TT*	*CC*	*CT+TT*		
Folate intake						
Low folate intake	74	130	109	129	1.55(1.02-2.57)	0.41
Moderate folate intake	28	33	63	64	-	-
High folate intake	88	273	160	333	1.62 (0.80-3.23)	0.13
Quality of study						
High quality	407	1170	667	1278	1.15(1.11-1.20)	<0.05
Low quality	659	2185	1107	2565	1.37(1.12-1.67)	<0.05

they would induce between-study heterogeneity in the meta-analysis. Therefore, further studies should consider these potential risk factors.

Some possible limitations should be considered in our meta-analysis. Firstly, the eligibility criteria for inclusion of ESCC patients differed for each study, which might influence the obvious consistency of effects across the included studies and cause obvious between-study heterogeneity in this meta-analysis. Secondly, there might be misclassification during our study. Some controls in our study were selected from non-cancer inpatients, and some were selected from residents. Finally, owing to lack of other genetic and environmental data of ESCC risk, the gene-environment and gene-gene interaction for esophageal cancer could not be well assessed and the outcomes from this study might be affected by risk of selection biases. Further studies are warranted to interpret their interaction.

In conclusion, our meta-analysis indicated *MTHFR 677CT* and/or *TT* genotypes are associated with the risk of ESCC, and folate could modify their association. Further large sample size and well designed study are urgently needed to further identify the association of folate intake and polymorphism of MTHFR C677T with ESCC risk.

REFERENCE

1. Jemal A, Siegel R, Ward E, et al. Cancer statistics 2007. CA Cancer J Clin, 2007, 57, 43–66.
2. Morita M, Kumashiro R, Kubo N, Nakashima Y, Yoshida R, Yoshinaga K, et al. Alcohol drinking, cigarette smoking, and the development of squamous cell carcinoma of the esophagus: epidemiology, clinical findings, and prevention. Int J Clin Oncol. 2010;15(2):126-134.
3. Falk GW. Risk factors for esophageal cancer development. Surg Oncol Clin N Am. 2009;18(3):469-485.
4. De Stefani E, Aune D, Boffetta P, Deneo-Pellegrini H, Ronco AL, Acosta G, et al. Salted meat consumption and the risk of cancer: a multisite case-control study in Uruguay. Asian Pac J Cancer Prev. 2009;10(5):853-857.
5. Yu X, Zhang T, Zhang H, Hu A, Hu Y, Guo W, et al. Comparison of lifestyle and living environment among high risk immigrant and low risk host residents: implications for esophageal cancer etiology. Asian Pac J Cancer Prev. 2010;11(6):1827-1831.
6. Aune D, Deneo-Pellegrini H, Ronco AL, Boffetta P, Acosta G, Mendilaharsu M, De, et al. Dietary folate intake and the risk of 11 types of cancer: a case-control study in Uruguay. Ann Oncol. 2011; 22(2):444-451.
7. Larsson SC, Giovannucci E, Wolk A. Folate intake, MTHFR polymorphisms, and risk of esophageal, gastric, and pancreatic cancer: A meta-analysis. Gastroenterology. 2006;131:1271-1283.
8. SongC,XingD,TanW,WeiQ,LinD.Methylenetetrahydrofolate reductase polymorphisms increase risk of esophageal squamous cell carcinoma in a Chinese population. Cancer Res. 2001; 61(8):3272-3275.
9. Stolzenberg-Solomon RZ, Qiao YL, Abnet CC, Ratnasinghe DL, Dawsey SM, Dong ZW, et al. Esophageal and gastric cardia cancer risk and folate- and vitamin B(12)-related polymorphisms in Linxian, China. Cancer Epidemiol Biomarkers Prev. 2003;12(11 Pt 1):1222-1226.
10. Yang CX, Matsuo K, Ito H, Shinoda M, Hatooka S, Hirose K, et al. Gene-environment interactions between alcohol drinking and the MTHFR C677T polymorphism impact on esophageal cancer risk: results of a case-control study in Japan. Carcinogenesis. 2005; 26(7):1285-1290.
11. Egger M, Smith GD, Phillips AN. Meta-analysis, principles and procedures. BMJ.1997; 315(7121): 1533-1537.
12. Jiang DK, Ren WH, Yao L, Wang WZ, Peng B, Yu L. Meta-analysis of association between TP53 Arg72Pro polymorphism and bladder cancer risk. Urology. 2010;76(3):765.e1-7.
13. Yang JB, Shen YW, Zeng M, Zhang H, Mu B, Song GJ, et al. Relationship between MTHFR C677T polymorphisms and susceptibility of esophageal cancer in north Sichuan population. Sichuan Medical Journal. 2012;33(1): 26-27.
14. Zhao P, Lin F, Li Z, Lin B, Lin J, Luo R. Folate intake, methylenetetrahydrofolate reductase polymorphisms, and risk of esophageal cancer.Asian Pac J Cancer Prev. 2011;12(8):2019-2023.
15. Li QD, Li H, Wang MS, Diao TY, Zhou ZY, Fang QX, et al. Multi-susceptibility genes associated with the risk of the development stages of esophageal squamous cell cancer in Feicheng County. BMC Gastroenterol. 2011;11:74.
16. Umar M, Upadhyay R, Khurana R, Kumar S, Ghoshal UC, Mittal B. Evaluation of MTHFR677C>T polymorphism in prediction and prognosis of esophageal squamous cell carcinoma: A case-control study in a northern Indian population. Nutr Cancer. 2010;62(6):743-749.
17. Wang Q, Li D, Ye WJ, Chen MH, Jing XA. Effect of MTHFR C677T mutation, interaction between MTHFR C677T mutation and environmental factors on esophageal cancer. Chin J Public Health, 2009, 25(11): 1308-1310.
18. Chen Y, Yin D, Deng YC, A HL, Wang HJ, Ma YQ, et al. Relationship between MTHFR gene polymorphisms and susceptibility of esophageal cancer of Han nationality in Xinjiang. J Toxicol. 2009; 23(6):429-432.
19. Qin JM, Yang L, Chen B, Wang XM, Li F, Liao PH, et al. Interaction of methylenetetrahydrofolate reductase C677T, cytochrome P4502E1 polymorphism and environment factors in esophageal cancer in Kazakh population. World J Gastroenterol. 2008; 14(45):6986-6992.
20. Li DQ, Diao YT, Li H, Fang XQ, Li HQ. Association of the Polymorphisms of MTHFR C677T, VDR C352T, and MPO G463A with Risk for Esophageal Squamous Cell Dysplasia and Carcinoma. Archives of Medical Research. 2008;39(6):594-600.
21. Wang Y, Guo W, He Y, Chen Z, Wen D, Zhang X, et al. Association of MTHFR C677T and SHMT(1) C1420T with susceptibility to ESCC and GCA in a high incident region of Northern China.Cancer Causes Control. 2007;18(2):143-152.
22. He YT, Wang YM, Zhang JH, Li Y, Guo W, Wang N. Correlation between a Polymorphism in the Methylene Tetrahydrofolate Reductase Gene and Susceptibility to Carcinoma of the Esophagus and Gastric Cardia. Chinese Journal of Clinical Oncology. 2007;34(4):194-197.
23. Feng CW, Fan ZM, Gao SS, He X, Guo Huaqin, Yin LM, et al. Analysis of methylenetetrahydrofolate reductase and thymidylate synthase gene polymorphisms of esophageal and cardia cancerpatients. Journal of Zhengzhou University(Medical Sciences). 2006; 41(1):10-14.

24. Wang LD, Guo RF, Fan ZM, He X, Gao SS, Guo HQ, et al. Association of methylenetetrahydrofolate reductase and thymidylate synthase promoter polymorphisms with genetic susceptibility to esophageal and cardia cancer in a Chinese high-risk population. Dis Esophagus. 2005;18(3):177-184.
25. Zhang J, Zotz RB, Li Y, Wang R, Kiel S, Schulz WA, et al. Methylenetetrahydrofolate reductase C677T polymorphism and predisposition towards esophageal squamous cell carcinoma in a German Caucasian and a northern Chinese population. J Cancer Res Clin Oncol. 2004;130(10):574-580.
26. Kureshi N, Ghaffar S, Siddiqui S, Salahuddin I, Frossard PM. Head and neck cancer susceptibility: a genetic marker in the methylenetetrahydrofolate reductase gene. ORL J Otorhinolaryngol Relat Spec. 2004;66(5):241-245.
27. Zhang JH, Li Y, Guo W, Wang R, Sarabia K, Wen DG, et al. The Association of Methylenetetrahydrofolate Reductase C677T Polymorphism and Esophageal Squamous Cell Carcinoma Analyzed by LightCycler. Progress In Biochemistry and Biophysics. 2003; 30(4): 555-559.
28. Miao X, Xing D, Tan W, Qi J, Lu W, Lin D. Susceptibility to Gastric Cardia Adenocarcinoma and Genetic Polymorphisms in Methylenetetrahydrofolate Reductase in an At-Risk Chinese Population. Cancer Epidemiol Biomarkers Prev. 2002; 11(11):1454-1458.
29. Liu YX, Wang B, Wan MH, Tang WF, Huang FK, Li C. Meta-analysis of the relationship between the Metholenetetrahydrofolate reductase C677T genetic polymorphism, folate intake and esophageal cancer. Asian Pac J Cancer Prev. 2011;12(1):247-252.
30. Fang Y, Xiao F, An Z, Hao L. Systematic review on the relationship between genetic polymorphisms of methylenetetrahydrofolate reductase and esophageal squamous cell carcinoma. Asian Pac J Cancer Prev. 2011;12(7):1861-1866.
31. Langevin SM, Lin D, Matsuo K, Gao CM, Takezaki T, Stolzenberg-Solomon RZ, et al. Review and pooled analysis of studies on MTHFR C677T polymorphism and esophageal cancer. Toxicol Lett. 2009;184(2):73-80.
32. Wang J, Sasco AJ, Fu C, Xue H, Guo G, Hua Z, et al. Aberrant DNA methylation of P16, MGMT, and hMLH1 genes in combination with MTHFR C677T genetic polymorphism in esophageal squamous cell carcinoma. Cancer Epidemiol Biomarkers Prev. 2008;17(1):118-125.
33. Mason JB. Folate, cancer risk, and the Greek god, Proteus: a tale of two chameleons. Nutr Rev. 2009; 67(4): 206-212.