Association between polymorphisms of thymidylate synthase gene 5′- and 3′-UTR and gastric cancer risk: meta-analysis

Ao Mo*, Yongliang Zhao*, Yan Shi*, Feng Qian*, Yingxue Hao*, Jun Chen*, Shiwei Yang*, Yuxing Jiang*, Ziyan Luo* and Peiwu Yu*

*Department of General Surgery and Center of Minimal Invasive Gastrointestinal Surgery, Southwest Hospital, Third Military Medical University, Chongqing 400038, China

Synopsis
Gastric cancer is the most common cancer and the most frequent cause of cancer death worldwide. Several studies have identified the role of thymidylate synthase (TS) 5′- and 3′-UTR and gastric cancer susceptibility; however, the results still remain inconclusive. The purpose of this meta-analysis was to reinvestigate this correlation. In the present study, online databases were searched to retrieve relevant articles published between January 2000 and 2016. The odds ratio (OR) and 95% confidence interval (CI) were employed to calculate the strength of association. Overall, a total of 13 articles were screened out, including 2382 gastric cancer patients and 3171 healthy controls. We found that polymorphisms of TS 5′-UTR 2R (double repeats)/3R (triple repeats) of a 28-bp sequence (11 articles) and 3′-UTR del6/ins6 (seven articles) were not significantly associated with increased risk of gastric cancer. Subgroup analysis by ethnicity showed that 2R allele and 2R/2R genotype in TS 5′-UTR were associated with gastric cancer susceptibility in Caucasian and African populations; del6 allele, del6/del6 and del6/ins6 genotypes were correlated with gastric cancer in Caucasian population. In conclusion, our result suggested that TS polymorphisms might be the risk factors for gastric cancer risk in Caucasian population, although this association needs further study, and future large-scale researches are still required.

Key words: gastric cancer, meta-analysis, polymorphism, thymidylate synthase gene.

Cite this article as: Bioscience Reports (2016) 36, e00429, doi:10.1042/BSR20160273

INTRODUCTION
Gastric cancer (or stomach cancer) is a disease in which malignant (cancer) cells develop from the lining of the stomach [1]. It remains a major health problem worldwide, and may spread from the stomach to other parts of the body, particularly the liver, lung, bone, lining of the abdomen and lymph nodes [2,3]. Gastric cancer is the fifth leading cause of cancer and the third leading cause of death worldwide making up 7% of cases and 9% of deaths [4]. According to the cancer statistics, there are approximately 26370 estimated new cases and 10730 estimated deaths in the United States in the year 2016 [5]. Infection with bacteria called Helicobacter pylori is a major cause of gastric cancer [6], and other risk factors include gender, cigarette smoking, atrophic gastritis and partial gastrectomy [7,8]. Up to now, gastric cancer remains a major diagnostic and therapeutic challenge [9,10]. The diagnosis is based on conventional white light endoscopy findings, and the prognosis depends on its stage [11]. Surgical resection is the only treatment modality that is potentially curative, but the majority of patients still relapse following resection [12]. In addition, treatment of advanced gastric cancer is controversial and there is no standard regimen for first- or second-line chemotherapy (CT) [13]. Detection of this disease in its early stage is helpful to improve the treatment outcome. Therefore, identifying some biomarkers associated with gastric cancer susceptibility can be used in predicting this disease and guiding the therapeutic strategies.

Abbreviations: CI, confidence interval; CT, chemotherapy; del6, 6-bp deletion; dNTP, deoxyribonucleoside triphosphates; 5-FU, 5-fluorouracil; HWE, Hardy–Weinberg equilibrium; ins6, 6-bp insertion; MeSH, Medical Subject Headings; OR, odds ratio; PCR-RFLP, PCR-restriction fragment length polymorphism; Ph, p-value of heterogeneity; 2R, double repeats; 3R, triple repeats; S-1, TS-1, Taiho Pharmaceutical; SNP, Single nucleotide polymorphism; TS, thymidylate synthase; TYMS, thymidylate synthase.

1To whom correspondence should be addressed (email peiwu_yu@163.com).
The development of gastric cancer is a complex and multistep process [14], and the mechanism under this disease is still relatively unknown. A small number of patients may have a genetic predisposition syndrome, and the total number of genome alterations was estimated at 4.18 for gastric cancer [15]. It is characterized by genomic instability that could be either microsatellite instability or chromosomal instability [16], involving multiple genetic and epigenetic alterations in oncogenes, tumour suppressor genes, DNA repair genes, cell-cycle regulators and signalling molecules [17,18]. Thymidylate synthase (TS) gene, located on chromosome 18p11.32, is the critical rate-limiting enzyme in the de novo synthesis of dTMP from deoxyuridine monophosphate (dUMP), which is required for DNA synthesis and repair [19]. It is a target for major chemotherapeutic drugs, and its expression is associated with tumour aggressiveness and poor prognosis [20]. In patients with advanced gastric cancer, TS has been identified as a prognostic marker, and high TS expression is associated with worse overall survival [21]. Several polymorphisms in the TS UTRs, which may influence TS mRNA transcription, message stability or protein expression, have been identified [22]. Two functionally important and ethnically diverse polymorphisms are the most extensively studied: TS enhancer region (TSER), a tandem-repeat polymorphism, which contains triple (3R) or double (2R) repeats of a 28-bp sequence in TS 5′ untranslated region, may be involved in modulation of TS mRNA expression [23]; and a 6-bp ins/del polymorphism on the 3′-UTR (position 5′-UTR) (position 51494, del6 or ins6), which may influence mRNA stability [24]. The presence of the 3R compared with 2R 28-bp repeat sequence has been shown to enhance mRNA transcription and protein expression in in vitro and in vivo studies [25].

Although several molecular epidemiological studies were conducted to investigate the association between these two TS polymorphisms and gastric cancer risk. However, the results from different studies are divergent to some extent. Moreover, there is a marked geographical variation, with the highest rates reported in East Asia, South America and Eastern Europe and the lowest rates in the United States and Western Europe [26,27]. Therefore, we conducted this meta-analysis to clarify this issue and to obtain a more precise estimation of the relationship between TS polymorphisms and gastric cancer susceptibility.

**MATERIALS AND METHODS**

**Study identification**

We searched the electronic databases of Chinese language (CNKI and Wanfang) and English language (PubMed, Embase and Medline) to retrieve relevant articles published between January 2000 and 2016. The MeSH terms: “gastric cancer or gastric carcinoma or stomach cancer”, “thymidylate synthase or TS gene” and “polymorphism or variant” as well as their combinations were used as the searching words. The corresponding Chinese version was used in the Chinese databases. References of related articles were manually searched. When the same authors or laboratories reported this issue on the same population, only the recent full-text articles were included.

**Inclusion criteria**

The included studies must meet the following criteria: (1) case-control studies evaluating the effect of TS 5′-UTR and 3′-UTR polymorphisms in gastric cancer risk; (2) the diagnosis of gastric cancer should be made from gastroscopy or surgical biopsy reviewed by an experienced pathologist, and histology should be reported according to the World Health Organization criteria [28]; (3) controls should be unrelated, ethnically matched, healthy individuals; (4) the results were presented in odds ratio (OR) with its 95% corresponding confidence interval (CI); and (5) genotype information in patients and controls were available to extract.

**Data extraction**

Two authors independently assessed the information of each included study. Any disagreement was resolved by discussion with a third expert. Each item should be able to reach a final consensus. The following information was extracted from each article: first author, published year, country, mean age, sample size, genotyping methods, genotype distribution and Hardy–Weinberg equilibrium (HWE) in controls.

**Statistical analysis**

The association between TS polymorphisms and gastric cancer risk was measured by pooled OR with 95% CI. The significance of the pooled OR was determined by the Z-test, and a P value less than 0.05 was considered significant. The allelic model (M compared with m), homozygote model (MM compared with mm), heterozygote model (Mm compared with mm), dominant model (MM + Mm compared with mm) and recessive model (MM compared with Mm + mm) were calculated. The F² test and the Q-statistic test were used to determine the between-study heterogeneity. The fixed-effect model was used when the effects were assumed to be homogeneous (a P-value more than 0.10 for the Q-test and $F^2$ less than 50% for the $F^2$ test), otherwise, the random-effect model was employed. Funnel plot asymmetry was used to assess the publication bias. Analyses were performed using the software Review Manager 5.3 (Oxford). All P-values were two-sided.

**RESULTS**

**Characteristics of eligible studies**

Using the combined search, we first identified 185 relevant references. After applying the inclusion criteria, 13 studies were finally screened out into this meta-analysis, including 2382 gastric cancer patients and 3171 healthy controls. Figure 1 represents
A meta-analysis of association between polymorphisms of TS 5′- and 3′-UTR and gastric cancer risk

Figure 1 Flow chart of study selection process in this meta-analysis

the searching process. The 13 studies (one was written in Chinese language [29] and twelve in English language [30–41]) were performed in six countries (China, Korea, Tunisia, Turkey, U.S.A. and Italy). Nine case-control studies were from Asian population, three studies were from Caucasian population, whereas only one study was from African population. The sample size ranged from 86 to 810. Polymorphisms of TS gene were measured by the PCR-restriction fragment length polymorphism (PCR-RFLP). The genotype distribution in controls was in accordance with HWE except studies conducted by Gao et al. [31], Baroudi et al. [39] and Sumen et al. [41]. The detailed characteristics of included studies were summarized in Table 1. The information of alleles and genotypes of TS 5′-UTR and 3′-UTR polymorphisms was presented in Table 2.

Correlation of TS 5′-UTR 2R/3R polymorphism in gastric cancer susceptibility

The results of this meta-analysis were listed in Table 3. Eleven articles including 1859 patients and 2489 controls were eligible for pooling OR data. The between-study heterogeneity was calculated, the fixed-effect model was used in the heterozygote model and the dominant model, whereas the random-effect model was performed in other comparison models. Analyses of the 11 relevant studies showed that there was no obvious association between TS 5′-UTR 2R/3R polymorphism and gastric cancer risk under any genetic models as shown in Figure 2.

Subgroup analysis was conducted based on ethnicity including Asian population and non-Asian population (Caucasian and African). Our result detected that 2R of TS 5′-untranslated enhanced region contributed to gastric cancer risk in non-Asian population under the allelic model (2R compared with 3R: OR = 0.66, 95% CI = 0.51–0.85, P = 0.001), homozygote model (2R/2R compared with 3R/3R: OR = 0.54, 95% CI = 0.34–0.88, P = 0.01) and recessive model (2R/2R compared with 3R/2R + 3R/3R: OR = 0.44, 95% CI = 0.29–0.67, P = 0.0001) in the fixed-effect model as shown in Figure 3. For the Asian population, there was no obvious association between TS 5′-UTR 2R/3R polymorphism and gastric cancer susceptibility under the five comparison models (Table 3).

Correlation of TS 3′-UTR del6/ins6 polymorphism in gastric cancer susceptibility

Seven articles contained 1587 gastric cancer patients and 1943 controls. Significant heterogeneity among included studies was detected, and the random-effect model was employed. Our result found that the TS 3′-UTR del6/ins6 variant was not associated with gastric cancer risk under any genetic models (Table 3). Subgroup analysis by ethnicity showed that TS 3′-UTR del6/ins6 variant was significantly related with gastric cancer risk under the allelic model (del6 compared with ins6: OR = 1.39, 95% CI = 1.17–1.66, P = 0.0002), homozygote model (del6/del6 compared with ins6/ins6: OR = 1.96, 95% CI = 1.35–2.82, P = 0.0003), dominant model (del6/del6 + del6/ins6 compared with ins6/ins6: OR = 1.44, 95% CI = 1.13–1.85, P = 0.003) and recessive model (del6/del6 compared with del6/ins6 + ins6/ins6: OR = 1.68, 95% CI = 1.20–2.36, P = 0.003) in Caucasian
Table 1 Main characteristics of included studies in this meta-analysis

| First author | Year | Country   | Mean age Cases | Mean age Controls | Sample size Cases | Sample size Controls | Genotyping Method |
|--------------|------|-----------|----------------|------------------|------------------|---------------------|-------------------|
| Gao, C.M.    | 2004 | China     | 36–81          | 36–81            | 155              | 223                 | PCR-RFLP          |
| Graziano, F. | 2004 | Italy     | 59 (30–83)     | 58 (33–77)       | 134              | 139                 | PCR-RFLP          |
| Zhang, J.H.  | 2004 | China     | 55.0 (105)     | 51.3 (10.7)      | 233              | 348                 | PCR-RFLP          |
| Tan, W.      | 2005 | China     | –              | –                | 231              | 492                 | PCR-RFLP          |
| Wang, L.D.   | 2005 | China     | 58 (40–80)     | 56 (46–76)       | 129              | 315                 | PCR-RFLP          |
| Zhang, Z.D.  | 2005 | China     | 58.7 (9.7)     | 58.1 (10.6)      | 322              | 337                 | PCR-RFLP          |
| Yang, L.     | 2008 | China     | –              | –                | 60               | 170                 | PCR               |
| Jung, H.     | 2010 | Korea     | 59.3 (12.4)    | 45.8 (16.0)      | 300              | 100                 | PCR               |
| Yim, D.J.    | 2010 | Korea     | 58.26 (12.75)  | 57.18 (12.65)    | 318              | 280                 | PCR-RFLP          |
| Baroudi, O.  | 2014 | Tunisia   | 56 (30–70)     | 47.02 (20–80)    | 52               | 88                  | PCR-RFLP          |
| Pan, X.      | 2014 | China     | 22–76          | 18–55            | 31               | 200                 | PCR-RFLP          |
| Sumen, I.C.  | 2014 | Turkey    | 59 (37–79)     | 53 (25–75)       | 38               | 48                  | PCR-RFLP          |
| Shen, R.     | 2015 | U.S.A.    | 59.7 (12.7)    | 59.1 (11.2)      | 379              | 431                 | PCR-RFLP          |

Table 2 Alleles and genotypes of TS 5’- and 3’-UTR polymorphisms in this meta-analysis

| First author | Cases 3R/3R | 3R/2R | 2R/2R | 3R | 2R | Controls 3R/3R | 3R/2R | 2R/2R | 3R | 2R | HWE |
|--------------|-------------|-------|-------|----|----|---------------|-------|-------|----|----|-----|
| Graziano, F. | 38 | 76 | 18 | 152 | 112 | 31 | 74 | 31 | 136 | 136 | 0.303 |
| Zhang, J.H.  | 148 | 76 | 8 | 372 | 92 | 223 | 107 | 13 | 553 | 133 | 0.97 |
| Tan, W.      | 157 | 60 | 14 | 374 | 88 | 337 | 137 | 18 | 811 | 173 | 0.385 |
| Wang, L.D.   | 81 | 39 | 8 | 201 | 55 | 201 | 108 | 6 | 510 | 120 | 0.139 |
| Zhang, Z.D.  | 217 | 101 | 19 | 535 | 139 | 203 | 107 | 12 | 513 | 131 | 0.649 |
| Yang, L.     | 31 | 26 | 3 | 88 | 32 | 103 | 54 | 8 | 260 | 70 | 0.789 |
| Jung, H.     | 199 | 91 | 10 | 489 | 111 | 60 | 30 | 10 | 150 | 50 | 0.135 |
| Yim, D.J.    | 211 | 89 | 18 | 511 | 125 | 194 | 79 | 7 | 467 | 93 | 0.755 |
| Baroudi, O.  | 18 | 8 | 26 | 44 | 60 | 26 | 4 | 58 | 56 | 120 | 0.000 |
| Pan, X.      | 20 | 8 | 3 | 48 | 14 | 146 | 48 | 6 | 340 | 60 | 0.405 |
| Sumen, I.C.  | 7 | 18 | 13 | 32 | 44 | 9 | 6 | 33 | 24 | 72 | 0.000 |
| 3’-UTR       | ins6/ins6 | ins6/del6 | del6/del6 | ins6 | del6 | ins6/ins6 | del6 | del6/del6 | ins6 | del6 |
| Gao, C.M.    | 10 | 80 | 65 | 100 | 210 | 18 | 121 | 84 | 157 | 289 | 0.018 |
| Graziano, F. | 39 | 73 | 22 | 151 | 117 | 62 | 59 | 18 | 183 | 95 | 0.505 |
| Zhang, J.H.  | 24 | 105 | 104 | 153 | 313 | 34 | 155 | 159 | 223 | 473 | 0.671 |
| Zhang, Z.D.  | 53 | 143 | 141 | 249 | 425 | 30 | 139 | 153 | 199 | 445 | 0.846 |
| Yim, D.J.    | 29 | 130 | 159 | 188 | 448 | 19 | 121 | 140 | 159 | 401 | 0.294 |
| Pan, X.      | 3 | 10 | 18 | 16 | 46 | 22 | 90 | 88 | 134 | 266 | 0.888 |
| Shen, R.     | 144 | 163 | 72 | 451 | 307 | 192 | 190 | 49 | 574 | 288 | 0.847 |

Sensitivity analysis and publication bias

We conducted the sensitivity analysis to verify whether our results were affected by each included study. Our result indicated that single study could not influence the pooled OR qualitatively, suggesting that the result was stable. Funnel plot was used to assess publication bias, and the shape of the funnel plots was symmetrical, indicating no publication bias in this meta-analysis as shown in Figure 5.

DISCUSSION

In this meta-analysis, we totally screened out 13 relevant articles concerning TS 5’-UTR 2R/3R and 3’-UTR del6/ins6 polymorphisms. Our result found that both these two variants were not...
associated with gastric cancer risk. Subgroup analysis by ethnicity showed that 2R of TS 5'-UTR 2R/3R and del6 of TS 3'-UTR del6/ins6 variants were associated with increased risk of gastric cancer risk in Caucasian population. Our result was not consistent with three previous meta-analyses: one was conducted by Yang et al. [42] that contained six included articles and suggested that 2R of TYMS 5'-UTR 2R/3R contributed to gastric cancer risk in the Asian population, one was conducted by Lu et al. [43] that suggested the 3R variant of TS 5'-untranslated enhanced region del6/del6 compared with del6/ins6 increased risk of gastric cancer [48]. TS is one of the key enzymes that involved in the folate metabolism. Variants of TS gene differ not only biologically but also functionally in their ability to alter TYMS activation on folate metabolism, the intrinsic variability of DNA repair processes, the inflammatory response and the functioning of carcinogen detoxification and antioxidant protection [46]. Identifying the relevant genes can be used as a tool to search for genetic variations of the disease’s genes and susceptibility, thus to increase understanding of this disease’s mechanism [47].

The folate metabolic pathway is involved in the synthesis and methylation of DNA, and low folate levels were shown to be associated with an increased risk of gastric cancer [48]. TS is one of the key enzymes that involved in the folate metabolism. Variants of TS gene differ not only biologically but also functionally in their ability to alter TYMS activation on folate metabolism [49]. Furthermore, TS holds promise as a prognostic biomarker because of its role as the molecular target of 5-FU, a commonly used chemotherapeutic agent in gastric cancer [50]. High TS gene expression in tumours was associated with enhanced benefit from to the tumorigenesis of gastric cancer, such as the cell apoptotic pathway, cell proliferation ability, the intrinsic variability of DNA repair processes, the inflammatory response and the functioning of carcinogen detoxification and antioxidant protection [46]. Identifying the relevant genes can be used as a tool to search for genetic variations of the disease’s genes and susceptibility, thus to increase understanding of this disease’s mechanism [47].

The folate metabolic pathway is involved in the synthesis and methylation of DNA, and low folate levels were shown to be associated with an increased risk of gastric cancer [48]. TS is one of the key enzymes that involved in the folate metabolism. Variants of TS gene differ not only biologically but also functionally in their ability to alter TYMS activation on folate metabolism [49]. Furthermore, TS holds promise as a prognostic biomarker because of its role as the molecular target of 5-FU, a commonly used chemotherapeutic agent in gastric cancer [50]. High TS gene expression in tumours was associated with enhanced benefit from
Figure 2 Meta-analysis of the correlation between TS 5'-UTR polymorphism and gastric cancer susceptibility under the allelic model (A), homozygote model (B), heterozygote model (C), dominant model (D) and recessive model (E)
A meta-analysis of association between polymorphisms of TS 5′- and 3′-UTR and gastric cancer risk

Figure 3 Forest plot of TS 5′-UTR variant and gastric cancer risk under the allelic model (A), homologous model (B), and recessive effect (C) in the fixed-effect model in non-Asian populations (Caucasian and African)

post-operative adjuvant S-1 treatment in gastric cancer [51], and might predict drug resistance and adverse prognosis in patients with advanced stages treated with FU-based CT [52]. TS expression was also associated with CT response, progression-free survival and overall survival in advanced gastric cancer patients treated with capecitabine alone CT [53]. Moreover, TS mRNA level in plasma can mirror tumour TS mRNA level, and both of them can be used to predict raltitrexed sensitivity in gastric cancer [54]. Several studies have identified the role of TS polymorphisms in gastric cancer risk; however, the results still remain inconclusive. Shen et al. [40] found that the del6/del6 genotype of TS 3′-UTR was associated with significantly increased gastric cancer risk, Sumen et al. [41] suggested that 3R allele, 2R/3R and 3R/3R genotypes were risk factors for gastric cancer, whereas Araújo et al. [55] did not obtain a significant relationship between TS 5′-UTR 2R/3R and 3′-UTR del6/ins6 polymorphisms and gastric cancer susceptibility. Studies have also shown that TS polymorphisms might be associated with other cancers’ risks such as colon cancer [56], lung cancer [57] and oral squamous cell carcinoma [58]. In addition, genetic variation in TS gene may affect carcinogenesis through the regulation of gene expression, the status of the dNTP pool and drug sensitivity. Gao et al. [59] first reported that TS 3′-UTR ins6/ins6 genotype could predict the poor survival of advanced gastric cancer patients treated with capecitabine plus paclitaxel. Huang et al. [60] found that the polymorphisms of TS 3′-UTR del6/ins6 might be potential prognostic factor in gastric cancer patients treated with 5-FU-based adjuvant CT. Kim et al. [61] demonstrated that TS genotyping could be of help in predicting toxicity in oral fluoropyrimidine-based CT in advanced gastric cancer patients.

Several limitations were presented in this meta-analysis. Firstly, the number of included studies for subgroup analysis was small, which might affect the accuracy of our result. Secondly, the stages of patients with gastric cancer could not be extracted from the included articles due to the lack of sufficient data. Thirdly, gene–gene interaction should be addressed in the future meta-analysis for the study of combined polymorphisms, instead of single low-penetrance variations in susceptibility that may lead to a high-risk classification for a specific population [62]. Lastly, other factors, such as gender, smoking and drinking should be considered as well in the future researches.

In conclusions, our meta-analysis suggested that 2R of TS 5′-UTR 2R/3R and del6 of TS 3′-UTR del6/ins6 might contribute to gastric cancer risk in the Caucasian population. However, future large-scale studies with more ethnicities are still needed to further investigate this association.
Figure 4 Forest plot of pooled OR with 95% CI for TS 3′-UTR polymorphism and gastric cancer risk under the allelic model (A), homozygote model (B), dominant model (C) and recessive model (D) in Caucasian population.

AUTHOR CONTRIBUTION

Peiwu Yu conceived and designed the entire study; Ao Mo and Yongliang Zhao analysed the data; Yan Shi, Feng Qian and Yingxue Hao performed the literature research and statistical analysis; Jun Chen and Shiwei Yang drafted the paper. Yuxing Jiang, Ziyan Luo and Peiwu Yu revised the whole paper. Peiwu Yu guided the whole study. All authors read and agreed with the final version of this manuscript.

REFERENCES

1 Ajani, J.A., Bentrem, D.J., Besh, S., D’Amico, T.A., Das, R., Denlinger, C., Fakih, M.G., Fuchs, C.S., Gerdes, H., Glasgow, R.E., et al. (2013) Gastric cancer, version 2.2013. J. Natl. Compr. Canc. Netw. 11, 531–546
2 Maruyama, K., Gunven, P., Okabayashi, K., Sasako, M. and Kinoshita, T. (1989) Lymph node metastases of gastric cancer. General pattern in 1931 patients. Ann. Surg. 210, 596–602 CrossRef
3 Korenaga, D., Okamura, T., Baba, H., Saito, A. and Sugimachi, K. (1988) Results of resection of gastric cancer extending to adjacent organs. Br. J. Surg. 75, 12–15 CrossRef
4 Stewart, B.W. and Wild, C.P. (2015), World Cancer Report 2014. IARC. http://tinyurl.com/p8x23zk
5 Siegel, R.L., Miller, K.D. and Jemal, A. (2016) Cancer statistics, 2016. CA Cancer J. Clin. 66, 7–30 CrossRef
6 Conteduca, V., Sansonno, D., Lauletta, G., Russi, S., Ingravallo, G. and Dammacco, F. (2013) HS infection and gastric cancer: state of the art (review). Int. J. Oncol. 42, 5–18
7 Guggenheim, D.E. and Shah, M.A. (2013) Gastric cancer epidemiology and risk factors. J. Surg. Oncol. 107, 230–236 CrossRef
8 de Martel, C., Forman, D. and Plummer, M. (2013) Gastric cancer: epidemiology and risk factors. Gastroenterol. Clin. North Am. 42, 219–240 CrossRef
9 Fock, K.M. (2014) Review article: the epidemiology and prevention of gastric cancer. Aliment. Pharmacol. Ther. 40, 250–260 CrossRef
10 Takahashi, T., Saikawa, Y. and Kitagawa, Y. (2013) Gastric cancer: current status of diagnosis and treatment. Cancers (Basel) 5, 48–63 CrossRef
11 Yada, T., Yokoi, C. and Uemura, N. (2013) The current state of diagnosis and treatment for early gastric cancer. Diagn. Ther. Endosc. 2013, 241320 CrossRef
12 Waddell, T., Verheij, M., Allum, W., Cunningham, D., Cervantes, A. and Arnold, D. (2014) Gastric cancer: ESMO-ESSO-ESTRO clinical practice guidelines for diagnosis, treatment and follow-up. Radiother. Oncol. 110, 189–194 CrossRef
13 Cervantes, A., Roda, D., Tarazona, N., Roselló, S. and Pérez-Fidalgo, J.A. (2013) Current questions for the treatment of advanced gastric cancer. Cancer Treat. Rev. 39, 60–67 CrossRef
14 Piazzuelo, M.B. and Correa, P. (2013) Gastric cancer: overview. Colomb. Med. (Cali.) 44, 192–201
15 Nishimura, T. (2008) Total number of genome alterations in sporadic gastrointestinal cancer inferred from pooled analyses in the literature. Tumor Biol. 29, 343–350 CrossRef
16 Hudler, P. (2012) Genetic aspects of gastric cancer instability. Scientific World J. 2012, 761900 CrossRef
17 Mocellin, S., Verdi, D., Pooley, K.A. and Nitti, D. (2015) Genetic variation and gastric cancer risk: a field synopsis and meta-analysis. Gut 64, 1209–1219 CrossRef
18 Nagni, S. (2012) Carcinoma of the stomach: a review of epidemiology, pathogenesis, molecular genetics and chemoprevention. World J. Gastrointest. Oncol. 4, 156–169 CrossRef
19 Kamb, A., Finer-Moore, J., Calvert, A.H. and Stroud, R.M. (1992) Structural basis for recognition of polyglutamylation folates by thymidylate synthase. Biochemistry 31, 9883–9890 CrossRef
20 Li, N., Wang, X., Zhai, J., Shi, Y., Wang, Y., Zhu, J., Sha, L., Zhang, I., Jiang, L. and Zhang, Y. (2014) Prognostic role of thymidylate synthase expression in gastric cancer: a meta-analysis. Biomed. Res. 25, 97–103
21 Hu, H.-B., Wang, Y., Zeng, X.-M., Li, B., Liu, E.-Y. and Zhong, M.-Z. (2012) Predictive value of thymidylate synthase expression in gastric cancer: a systematic review with meta-analysis. Asian Pac. J. Cancer Prev. 13, 261–267 CrossRef
22 Zhou, J.Y., Shi, R., Yu, H.L., Zeng, Y., Zheng, W.L. and Ma, W.L. (2012) The association between two polymorphisms in the TS gene and risk of cancer: a systematic review and pooled analysis. Int. J. Cancer 131, 2103–2116 CrossRef
23 Chu, E., Koeller, D.M., Casey, J.L., Drake, J.C., Chabner, B.A., Elwood, R.C., Zinn, S. and Allegra, C.J. (1991) Autoregulation of human thymidylate synthase messenger RNA translation by thymidylate synthase. Proc. Natl. Acad. Sci. U.S.A. 88, 8977–8981 CrossRef
24 Ulrich, C.M., Bigler, J., Velicer, C.M., Greene, E.A., Farin, F.M. and Potter, J.D. (2000) Searching expressed sequence tag databases: discovery and confirmation of a common polymorphism in the thymidylate synthase gene. Cancer Epidemiol. Biomarkers Prev. 9, 1381–1385
25 Horie, N., Alba, H., Oguro, K., Hojo, H. and Takeishi, K. (1995) Functional analysis and DNA polymorphism of the tandemly repeated sequences in the 5′-terminal regulatory region of the human gene for thymidylate synthase. Cell Struct. Funct. 20, 191–197 CrossRef
26 Forman, D. and Burley, V.J. (2006) Gastric cancer: global pattern of the disease and an overview of environmental risk factors. Best Pract. Res. Clin. Gastroenterol. 20, 633–649 CrossRef
27 Gorrell, R.J., Zwickel, N., Reynolds, J., Bulach, D. and Kwok, T. (2016) Helicobacter pylori Cagl hypervariable motif: a global analysis of geographical diversity and association with gastric cancer. J. Infect. Dis. 213, 1927–1931 CrossRef
28 Dodge, O.G. (1979) Histological typing of gastric and esophageal tumours (International Classification of Tumours No. 18). Brit. J. Cancer. 39, 204–210
29 Pan, X. (2014), TS and MTHFR Gene Polymorphisms and their Association with mRNA and Protein Expression in Yunnan Gastric Cancer Patients. Master’s Thesis, Kunming Medical University, Yunnan Sheng, China
30 Graziano, F., Kawakami, K., Watanabe, G., Ruzzo, A., Humar, B., Santini, D., Catalano, V., Ficarelli, R., Merriman, T., Panunzi, S. et al. (2004) Association of thymidylate synthase polymorphisms with gastric cancer susceptibility. Int. J. Cancer 112, 1010–1014 CrossRef
31 Gao, C., Takezaki, T., Wu, J.Z., Liu, Y.T., Ding, J.H., Li, S.P., Su, P., Hu, X., Kai, H.T., Li, Z.Y. et al. (2004) Polymorphisms in thymidylate synthase and methylenetetrahydrofolate reductase genes and the susceptibility to esophageal and stomach cancer with smoking. Asian Pac. J. Cancer Prev. 5, 133–138
32 Zhang, J., Cui, Y., Kuang, G., Li, Y., Wang, N., Wang, R., Guo, W., Wen, D., Wei, L., Yu, F. and Wang, S. (2004) Association of the thymidylate synthase polymorphisms with esophageal squamous cell carcinoma and gastric cardiac adenocarcinoma. Carcinogenesis 25, 2479–2485 CrossRef
33 Tan, W., Xiao, X., Wang, L., Yu, C., Xiong, P., Liang, G., Sun, T., Zhou, Y., Zhang, X., Li, H. and Lin, D. (2005) Significant increase in risk of gastricesophageal cancer is associated with interaction between promoter polymorphisms in thymidylate synthase and serum folate status. Carcinogenesis 26, 1430–1435 CrossRef
34 Wang, L.D., Guo, R.F., Fan, Z.M., He, X., Gao, S.S., Guo, H.Q., Matsuo, K., Yin, L.M. and Li, J.L. (2005) 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 18, 177–184 CrossRef
35 Zhang, Z., Xu, Y., Zhou, J., Wang, X., Wang, L., Hu, X., Guo, J., Wei, Q. and Shen, H. (2005) Polymorphisms of thymidylate synthase in the 5′-and 3′-untranslated regions associated with risk of gastric cancer in South China: a case-control analysis. Carcinogenesis 26, 1764–1769 CrossRef
36 Yang, L., Xiao, M., Ni, R., Tan, Q., Wei, J., Wang, J. and Ge, B. (2008) Correlation between thymidylate synthase genotype and susceptibility to gastric carcinoma. Chinese J. Clin. Oncol. 5, 448–452 CrossRef
37 Yin, D.J., Kim, O.J., An, H.J., Kang, H., Ahn, D.H., Hwang, S.G., Oh, D. and Kim, N.K. (2010) Polymorphisms of thymidylate synthase gene 5′ and 3′-untranslated region and risk of gastric cancer in Koreans. Anticancer Res. 30, 2325–2330
38 Jung, H., Lee, J.I., Lee, H.H., Kim, S.H., Hur, H. and Jeon, H.M. (2010) Gastric cancer susceptibility according to methylenetetrahydrofolate reductase and thymidylate synthase gene polymorphism. J. Korean Surg. Soc. 79, 27–34 CrossRef

39 Baroudi, O., Baroudi, T., Omrane, I., Moussa, A., Mezini, A., Ayari, H., Guermazi, S., Bahloul, A., Bouzaïenne, H., Urrhammer, N. et al. (2014) Thymidylate synthase polymorphism in sporadic colorectal and gastric cancer in Tunisian population: a predictive role in 5-fluorouracil based chemotherapy treatment. Med. Oncol. 31, 825 CrossRef

40 Shen, R., Liu, H., Wen, J., Liu, Z., Wang, L.-E., Wang, Q., Tan, D., Ajaní, J.A. and Wei, Q. (2015) Genetic polymorphisms in the microRNA binding-sites of the thymidylate synthase gene predict risk and survival in gastric cancer. Mol. Carcinog. 54, 880–888 CrossRef

41 Sumen, I.C., Arikoglu, H., Arslan, E., Ata, O., Kıyıcı, A. and Kayis, A. (2014) The association of methylenetetrahydrofolate reductase (MTHFR) and thymidylate synthase (TS) gene polymorphisms with gastric cancer. Int. J. Mevlana Med. Sci. 2, 15–19

42 Yang, Z., Liu, H.-X. and Zhang, X.-F. (2012) 2R of thymidylate synthase 5′-untranslated enhanced region contributes to gastric cancer risk: a meta-analysis. Asian Pac. J. Cancer Prev. 13, 1923–1927 CrossRef

43 Lu, M., Sun, L., Yang, J. and Li, Y.Y. (2012) 3R variant of thymidylate synthase 5′-untranslated enhanced region contributes to colorectal cancer risk: a meta-analysis. Asian Pac. J. Cancer Prev. 13, 2605–2610 CrossRef

44 Zhuang, W., Wu, X.-T., Zhou, Y., Liu, G.-J., Wu, T.-X., Yao, X., Du, L. and Wei, M.-L. (2009) Polymorphisms of thymidylate synthase in the 5′-and 3′-untranslated regions and gastric cancer. Dig. Dis. Sci. 54, 1379–1385 CrossRef

45 Bevan, S. and Houlston, R.S. (1999) Genetic predisposition to gastric cancer. QJM 92, 5–10 CrossRef

46 González, C.A., Sala, N. and Capellá, G. (2002) Genetic susceptibility and gastric cancer risk. Int. J. Cancer 100, 249–260 CrossRef

47 Loder, N. (1999) Genetic variations can point the way to disease genes. Nature 401, 734 CrossRef

48 Götzte, T., Röcken, C., Röhl, F.W., Wex, T., Hoffmann, J., Westphal, S., Malfertheiner, R., Ebert, M.P. and Dierkes, J. (2007) Gene polymorphisms of folate metabolizing enzymes and the risk of gastric cancer. Cancer Lett. 251, 228–236 CrossRef

49 Ho, V., Massey, T.E. and King, W.D. (2011) Thymidylate synthase gene polymorphisms and markers of DNA methylolation capacity. Mol. Genet. Metab. 102, 481–487 CrossRef

50 Pietrantonio, F., De Braud, F., Da Prat, V., Perrone, F., Pierotti, M.A., Gariboldi, M., Fanetti, G., Biondi, P., Pellegrinelli, A., Bossi, L. and Di Bartolomeo, M. (2013) A review on biomarkers for prediction of treatment outcome in gastric cancer. Anticancer Res. 33, 1257–1266

51 Sasako, M., Terashima, M., Ichikawa, W., Ochiai, A., Kitada, K., Kurahashi, I., Sakuramoto, S., Katai, H., Sano, T. and Imamura, H. (2015) Impact of the expression of thymidylate synthase and dihydropyrimidine dehydrogenase genes on survival in stage II/III gastric cancer. Gastric Cancer 18, 538–548 CrossRef

52 Gao, Y., Cui, J., Xi, H., Cai, A., Shen, W., Li, J., Zhang, K., Wei, B. and Chen, L. (2016) Association of thymidylate synthase expression and clinical outcomes of gastric cancer patients treated with fluoropyrimidine-based chemotherapy: a meta-analysis. Onco Targets Ther 9, 1339–1350 CrossRef

53 Liu, X.F., Zhang, H., Sun, J.Q., Yin, C., Liu, T.F., Yang, H. and Chen, L.H. (2014) Correlation between expression of thymidylate synthase and clinical outcome of advanced gastric cancer treated with capecitabine alone chemotherapy. Tumor Biol. 35, 12409–12414 CrossRef

54 Shen, J., Wang, H., Wei, J., Yu, L., Xie, L., Qian, X., Zou, Z., Liu, B. and Guan, W. (2012) Thymidylate synthase mRNA levels in plasma and tumor as potential predictive biomarkers for raltitrexed sensitivity in gastric cancer. Int. J. Cancer 131, E938–E945 CrossRef

55 Araújo, M., Borges, B.N., Rodrigues-Antunes, S., Burbano, R. and Harada, M.L. (2015) Thymidylate synthase and methylenetetrahydrofolate reductase gene polymorphisms and gastric cancer susceptibility in a population of Northern Brazil. Genet. Mol. Res. 14, 10001–10006 CrossRef

56 Gao, C.-M., Ding, J.-H., Li, S.-P., Liu, Y.-T., Cao, H.-X., Wu, J.-Z. and Tajima, K. (2012) Polymorphisms in the thymidylate synthase gene and risk of colorectal cancer. Asian Pac. J. Cancer Prev. 13, 4087–4091 CrossRef

57 Qasem, W.A., Youssef, A.-M., Youssef, M. and Manasreh, I. (2015) Thymidylate synthase polymorphisms and risk of lung cancer among the Jordanian population: a case control study. Asian Pac. J. Cancer Prev. 16, 8287–8292 CrossRef

58 Bezerra, A.M., Sant’Aña, T.A., Gomes, A.V., de Lacerda Vidal, A.K. and Muniz, M.T. (2014) Tmys double (2R) and triple repeat (3R) confers risk for human oral squamous cell carcinoma. Mol. Biol. Rep. 41, 7737–7742 CrossRef

59 Gao, J., He, Q., Hua, D., Mao, Y., Li, Y. and Shen, L. (2013) Polymorphism of TS 3′-UTR predicts survival of Chinese advanced gastric cancer patients receiving first-line capecitabine plus paclitaxel. Clin. Transl. Oncol. 15, 619–625 CrossRef

60 Huang, Z.-H., Hua, D. and Li, L.-H. (2009) The polymorphisms of TS and MTHFR predict survival of gastric cancer patients treated with fluorouracil-based adjuvant chemotherapy in Chinese population. Cancer Chemother. Pharmacol. 63, 911–918 CrossRef

61 Kim, G.M., Jeung, H.-C., Kwon, W.S., Rha, S.Y., Kim, H.S., Jung, T., Nam, B.-H., Lee, K. H. and Chung, H. C. (2012) Association of thymidylate synthase gene polymorphisms with efficacy and toxicity of oral fluoropyrimidine based chemotherapy in advanced gastric cancer. Cancer Res 72, 3689 CrossRef

62 Yuan, J., Li, Y., Tian, T., Li, N., Zhu, Y., Zou, J., Gao, J. and Shen, L. (2016) Risk prediction for early-onset gastric carcinoma: a case-control study of polygenic gastric cancer in Han Chinese with hereditary background. Oncotarget 7, 33608–33615

Accepted Manuscript online 14 November 2016, doi 10.1042/BSR20160273

Received 24 July 2016/31 October 2016; accepted 14 November 2016