

## RESEARCH COMMUNICATION

# 3R Variant of Thymidylate Synthase 5'-untranslated Enhanced Region Contributes to Colorectal Cancer Risk: A Meta-analysis

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### Abstract

**Background:** Studies investigating the association of 2R/3R polymorphism in the thymidylate synthase 5'-untranslated enhanced region (TSER) and colorectal cancer (CRC) risk have reported conflicting results. Thus, a meta-analysis was performed to summarize the data on the potential association. **Methods:** Pubmed, Embase and CBM databases were searched for all available studies. Links between the TSER 2R/3R polymorphism and CRC risk were estimated by odds ratios (ORs) with 95% confidence intervals (CIs). **Results:** Seven case-control studies with a total of 2723 cases and 4030 controls were included in this meta-analysis. The results showed that the 3R variant of TSER 2R/3R polymorphism contributes to CRC risk in two comparison models (OR 3R vs. 2R = 1.10, 95% CI 1.02-1.18, P = 0.015; OR Homozygote comparison model = 1.22 1.04-1.43, 95% CI 1.04-1.43, P = 0.012). Subgroup analyses by ethnicity further demonstrated a contribution in Caucasians with three comparison models (OR 3R vs. 2R = 1.10, 95% CI 1.02-1.19, P = 0.015; OR Homozygote comparison model = 1.21, 95% CI 1.03-1.41, P = 0.019; OR Recessive comparison model = 1.18, 95% CI 1.05-1.33, P = 0.008). However, the association in the Asian population was still uncertain due to the limited data (all P values were more than 0.05). **Conclusions:** Our meta-analysis suggests that the 3R variant of Thymidylate synthase 5'-untranslated enhanced region 2R/3R polymorphism contributes to gastric cancer risk in the Caucasian population, while any association in Asian populations needs further study.

**Keywords:** Colorectal cancer - thymidylate synthase - polymorphism - meta-analysis - ethnic groups

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### Introduction

Colorectal cancer (CRC) remains a major clinical and public health challenge, with 142,000 new cases and 51,000 deaths expected in the USA in 2010 (Siegel et al., 2012). CRC is the third most commonly diagnosed cancer in males and the second in females with over 1.2 million new cancer cases and 608,700 deaths estimated to have occurred in 2008 (Jemal et al., 2011). Thus, CRC still is a serious fatal disease worldwide and has caused serious damage to human health. As a complex and multi-factorial process, the colorectal carcinogenesis is still not fully understood. Epidemiological studies have revealed that smoking, diets and other environmental risk factors play important roles in the development of CRC (Chan and Giovannucci, 2010; Park et al., 2011). However, only a small proportion of individuals exposed to the known risk factors develop CRC, while many cases develop CRC among individuals without those risk factors, which suggest genetic factors also play an important role in the colorectal carcinogenesis (Markowitz and Bertagnolli, 2009; Feng et al., 2012).

Many key enzymatic regulators are involved in folate

metabolism, and thymidylate synthase (TYMS) catalyzes the conversion of deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP) in the DNA synthesis by using 5, 10-methylenetetrahydrofolate as a methyl donor (Costi et al., 2005). This process above is essential for the synthesis of thymidine which is a nucleotide needed for DNA synthesis and repair (Costi et al., 2005). Besides, TYMS is also the target for the widely used chemotherapeutic agent 5-fluorouracil (5-FU) (Gibson, 2006). Recent studies showed that functional polymorphisms in the TYMS gene may result in alterations in TYMS enzyme efficiency and/or expression level and may contribute to different cancers' risk via effects on nucleotide synthesis (Ho et al., 2011). A tandem-repeat polymorphism has been identified in the TYMS promoter enhancer region (TSER), which contains triple (3R) or double (2R) repeats of a 28-bp sequence and several rare alleles containing 4, 5, or 9 repeats (Marsh et al., 2001). Studies both in vitro and in vivo show the TYMS expression is TSER genotype-dependent and that the 3R allele is associated with an increase in TYMS expression (Horie et al., 1995; Marsh et al., 2001). Thus, considering the potential influence of

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altering TYMS activation on folate metabolism, many epidemiological studies have explored the association between the TSER 2R/3R polymorphism and CRC risk, but the results were conflicting (Chen et al., 2003; Matsuo et al., 2005; Ulrich et al., 2005; Carmona et al., 2008; Karpinski et al., 2010). Such inconsistency could be due to the small effect of the TSER 2R/3R polymorphism on CRC risk and the relatively small sample-size in each of the published studies. Meta-analysis is a statistical procedure for combining results from several published studies to acquire a precise estimation of the clinical interventions (Zintzaras and Lau, 2008). Thus, to establish a comprehensive picture of the relationship between the TSER 2R/3R polymorphism and CRC risk, we performed a meta-analysis of the published studies to summarize previous data and obtain a more precise estimation of this relationship.

## Materials and Methods

### Identification and eligibility of relevant studies

We searched PubMed, Embase and CBM database using the following search strategy: ('Colorectal carcinoma' or 'Colorectal cancer' or 'colon cancer' or 'rectal cancer') and ('thymidylate synthase' or 'TYMS' or 'TSER') and ('polymorphism' or 'polymorphisms' or 'mutation' or 'mutations') for papers published between 1983 and December 15, 2011. The language of the papers was not restricted. All references cited in these studies and previously published review articles were retrieved for additional eligible studies. The following criteria were used to select the eligible studies: (1) a case-control study on the association between the TSER 2R/3R polymorphism and CRC risk; (2) identification of CRC was confirmed histologically or pathologically; (3) an available genotype or allele frequency for estimating an odds ratio (OR) with a 95% confidence interval (CI); (4) a genotype distribution among the control populations consistent with Hardy-Weinberg Equilibrium (HWE). When the same authors reported two or more publications on possibly the same patient populations, only the most recent or complete study was included into this meta-analysis. The major reasons for exclusion of studies were: (1) family studies; (2) case only studies; (3) review papers; (4) containing overlapping data.

### Data extraction

Two reviewers independently evaluated the final articles included into this meta-analysis, and disagreements were resolved by reaching a consensus among all authors. Data retrieved from the articles included the following: first author's name, publication year, country of origin, source of controls, racial descent of the study population (categorized as Caucasian population and Asian population), genotyping method, eligible and genotyped cases and controls, the number for each TSER 2R/3R genotype, and the allele frequency of TSER 2R/3R.

### Statistical methods

For the control group of each study, the distributions of genotypes were tested for HWE using the Chi-square test. If controls of studies were found not to be in HWE, sensitivity analyses were performed with and without these studies to test the robustness of the findings. The strength of association between TSER 2R/3R polymorphism and CRC risk was estimated by Odds ratios (ORs) with 95% confidence intervals (CIs). Four different comparison models of ORs were calculated: the allele model (3R vs. 2R), the Homozygote comparison model (3R/3R versus 2R/2R), the Recessive genetic comparison model (3R/3R versus 2R/3R+2R/2R), and the Dominant genetic comparison model (3R/3R + 2R/3R versus 2R/2R). The  $\chi^2$ -based Q statistic was used to investigate the degree of heterogeneity between the studies, and a P value < 0.05 was interpreted as significant heterogeneity among the studies (Cochran, 1954). Besides, the  $I^2$  index expressing the percentage of the total variation across studies due to heterogeneity was also calculated further assess the between-study heterogeneity (Higgins et al., 2003).  $I^2$  values of 25, 50, and 75% were used as evidence of low, moderate, and high heterogeneity, respectively. If heterogeneity existed, the random effects model (the DerSimonian and Laird method), which yields wider confidence intervals, was adopted to calculate the overall OR value (DerSimonian and Laird, 1986). Otherwise, the fixed effects model (the Mantel-Haenszel method) was used (Mantel and Haenszel, 1959). In order to assess the stability of the results, sensitivity analyses were performed by reanalyzing the significance of ORs after omitting each study in turn. Begg's funnel plots and Egger's linear regression test were used to assess evidence for potential

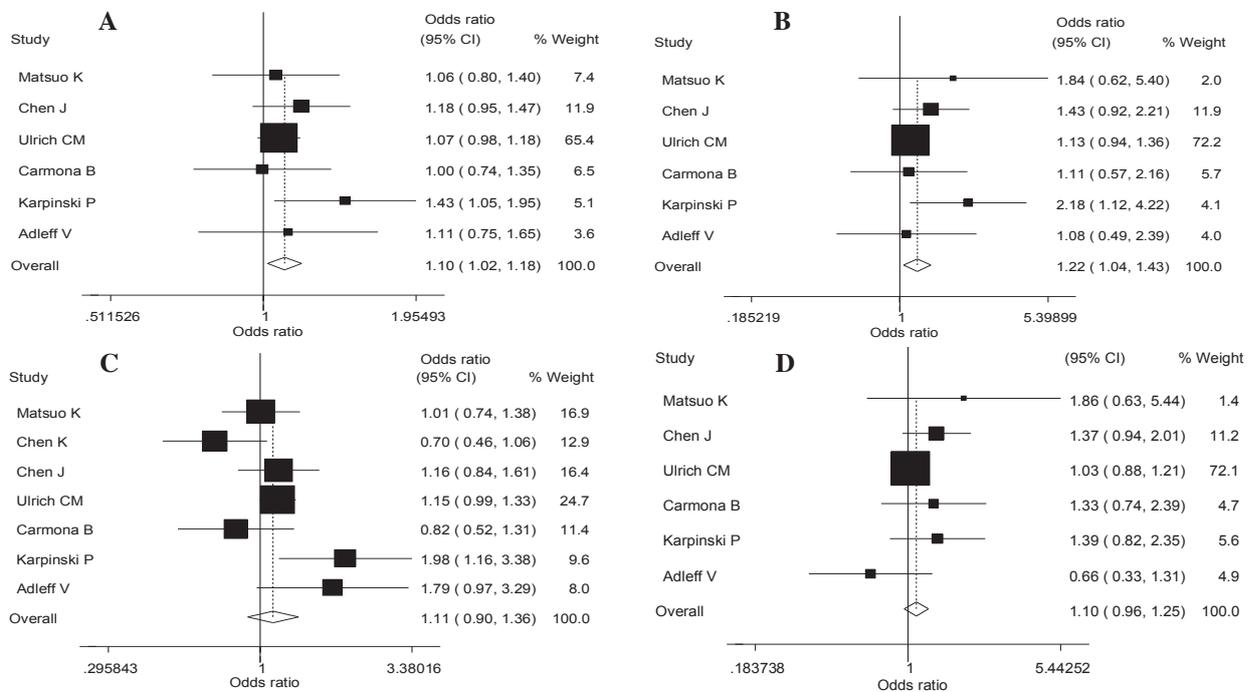
**Table 1. Characteristics of Seven Case-control Studies Included Into the Meta-analysis**

Study	Year	Ethnicity (Country)	Case group	Control group	HWE
Chen J	2003	Caucasian(USA)	270 patients with histologically confirmed CRC	454 non-cancer controls recruited from hospital inpatients	0.443
Matsuo K	2005	Asian(Japan]	257 patients with histologically confirmed CRC	771 healthy controls recruited from normal population	0.339
Ulrich CM	2005	Caucasian(USA)	1600 patients with histologically confirmed CRC	1962 healthy individuals	0.828
Carmona B	2008	Caucasian (Portugal)	173 patients with histologically confirmed CRC	170 healthy controls recruited from normal population	0.578
Karpinski P	2010	Caucasian (Poland)	186 patients with histologically confirmed CRC	140 healthy controls recruited from normal population	0.078
Adleff V	2004	Caucasian (Hungary)	98 patients with histologically confirmed CRC	102 healthy controls recruited from normal population	0.101
Chen K	2006	Asian (China)	139 patients with histologically confirmed CRC	431 healthy controls recruited from normal population	Unclear

**Table 2. Summary of Pooled Odds Ratios (OR) with Confidence Interval (CI) in the Meta-analysis**

Comparison Model	Studies (No. of cases / controls)	Odds Ratio		M*	Heterogeneity		P <sub>Egger's test</sub>
		OR[95%CI]	POR		I <sup>2</sup> (%)	P <sub>H</sub> †	
<b>All studies</b>							
3R vs. 2R	6(2584/3599)	1.10(1.02-1.18)	0.015	F	0	0.548	0.438
Homozygote comparison model	6(2584/3599)	1.22(1.04-1.43)	0.012	F	0	0.44	0.211
Recessive genetic comparison model	7(2723/4030)	1.11(0.90-1.36)	0.321	R	56.8	0.031	0.942
Dominant genetic comparison model	6(2584/3599)	1.10(0.96-1.25)	0.172	F	18.4	0.294	0.254
<b>Caucasian</b>							
3R vs. 2R	5(2327/2828)	1.10(1.02-1.19)	0.015	F	0	0.415	0.397
Homozygote comparison model	5(2327/2828)	1.21(1.03-1.41)	0.019	F	5.4	0.376	0.359
Recessive genetic comparison model	5(2327/2828)	1.18(1.05-1.33)	0.008	F	48.9	0.098	0.377
Dominant genetic comparison model	5(2327/2828)	1.09(0.95-1.24)	0.227	F	22.9	0.269	0.507
<b>Asians</b>							
3R vs. 2R	1 (257/771)	1.06(0.80-1.40)	0.697	F	NA	NA	NA
Homozygote comparison model	1 (257/771)	1.84(0.62-5.40)	0.269	F	NA	NA	NA
Recessive genetic comparison model	2(396/1202)	0.88(0.69-1.14)	0.336	F	47.7	0.167	NA
Dominant genetic comparison model	1 (257/771)	1.86(0.63-5.44)	0.259	F	NA	NA	NA

\*M, model of meta-analysis; R, random-effects model; F, Fixed-effects model; †PH, the P value of heterogeneity test; NA, not applicable



**Figure 1. Forest Plot of Pooled OR with 95% CI for TSER 2R/3R Polymorphism and CRC Risk with CRC Risk (A, 3R vs. 2R, Fixed effects model; B, Homozygote comparison model, Fixed effects model; C, Recessive genetic comparison model, Random effects model; D, Dominant genetic model, Fixed effects model) (The squares and horizontal lines corresponded to the study-specific OR and 95% CI. The area of the squares reflected the study-specific weight (inverse of the variance). The diamond represented the pooled OR and 95% CI)**

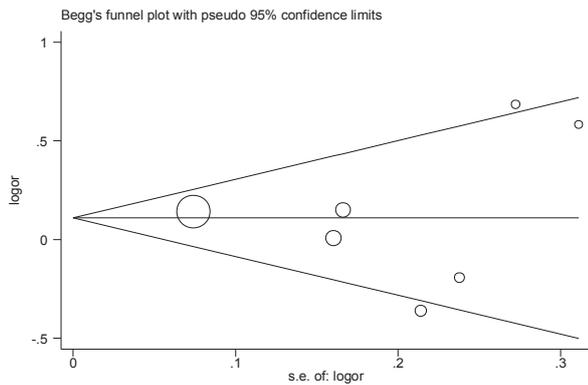
publication bias (Egger et al., 1997). The analysis was conducted using version 9.2 of STATA (Biostat, NJ, USA). All P values were two-sided and a P value of less than 0.05 was deemed statistically significant.

## Results

### Characteristics of included studies

619 unique references were initially identified by the search. After discarding overlapping references and those which clearly did not meet the criteria, 15 studies were further assessed for eligibility. After reviewing each original paper and extracting data, eight studies were

excluded including two studies for overlapping data (Curtin et al., 2007; Curtin et al., 2007) and six studies for studies on colorectal adenoma (Ulrich et al., 2002; Chen et al., 2004; Goode et al., 2004; Hubner et al., 2006; Hubner et al., 2007; van den Donk et al., 2007). Finally, seven case-control studies with a total of 2723 cases and 4030 controls were included into this meta-analysis (Chen et al., 2003; Adleff et al., 2004; Matsuo et al., 2005; Ulrich et al., 2005; Chen et al., 2006; Carmona et al., 2008; Karpinski et al., 2010). The detailed characteristics of these studies are summarized in Table 1. There were five case-control studies from Caucasian population (a total of 2327 cases and 2828 controls), and two study was from



**Figure 2. Funnel Plot for Publication Bias Test in the Meta-analysis Investigating the Association Between TSER 2R/3R Polymorphism and CRC Risk**

Asian population (a total of 396 cases and 1202 controls) (Table1).

#### Meta-analysis results

The results of this meta-analysis were shown in Table 2. When all seven studies were pooled into meta-analysis, there was no significant heterogeneity in three genetic comparison models, including 3R vs. 2R, the dominant genetic comparison model and the homozygote comparison model; thus, the fixed effects model was used to pool the results in these three comparison models except the recessive genetic comparison model. The combined results based on all studies showed that the 3R variant of TSER 2R/3R polymorphism contributes to CRC risk under two comparison models (OR 3R vs. 2R = 1.10, 95%CI=1.02-1.18, P=0.015; OR Homozygote comparison model = 1.22 1.04-1.43, 95%CI=1.04-1.43, P=0.012) (Figure 1).

Subgroup analyses by ethnicity showed that the 3R variant contributes to CRC risk in Caucasian population under three comparison models (OR 3R vs. 2R = 1.10, 95%CI=1.02-1.19, P=0.015; OR Homozygote comparison model = 1.21, 95%CI=1.03-1.41, P=0.019; OR Recessive comparison model = 1.18, 95%CI=1.05-1.33, P=0.008). However, the association in the Asian population was still uncertain due to the limited data (All P values were more than 0.05). The sensitivity analysis by sequential omission of individual studies found that the significance of ORs didn't change when omitting individual studies, which suggested that the outcomes in this meta-analysis was credible.

#### Publication bias

Begg's funnel plot and Egger's test were used to assess publication bias. The shape of the funnel plots was symmetrical (Figure 2), and the Egger test further provided evidence that there was no publication bias among the studies included (P = 0.942 > 0.05). Thus, the publication bias was not obvious in this meta-analysis.

## Discussion

Recent studies showed that functional polymorphisms in the TYMS gene may result in alterations in TYMS enzyme efficiency and/or expression level and may

contribute to different cancers' risk via effects on nucleotide synthesis (Marsh et al., 2001). Considering the potential influence of altering TYMS activation on folate metabolism, many epidemiological studies have explored the association between the TSER 2R/3R polymorphism and CRC risk, but the results were conflicting. Such inconsistency could be due to the small effect of the TSER 2R/3R polymorphism on CRC risk and the relatively small sample-size in each of the published studies. Meta-analysis is a statistical procedure for combining results from several published studies to acquire a precise estimation of the clinical interventions (Petitti, 2000; Attia et al., 2003). Therefore, we performed a meta-analysis of seven published case-control studies covering 2723 cases and 4030 controls to obtain a more precise estimation of the relationship between the TSER 2R/3R polymorphism and CRC risk. The results of meta-analyses showed that the 3R variant of TSER 2R/3R polymorphism contributes to CRC risk in two comparison models (OR 3R vs. 2R = 1.10, 95%CI=1.02-1.18, P=0.015; OR Homozygote comparison model = 1.22 1.04-1.43, 95%CI=1.04-1.43, P=0.012). Subgroup analyses by ethnicity showed that the 3R variant contributes to CRC risk in Caucasian population under three comparison models (OR 3R vs. 2R = 1.10, 95%CI=1.02-1.19, P=0.015; OR Homozygote comparison model = 1.21, 95%CI=1.03-1.41, P=0.019; OR Recessive comparison model = 1.18, 95%CI=1.05-1.33, P=0.008). However, the association in the Asian population was still uncertain due to the limited data (All P values were more than 0.05). This association was further identified by sensitivity analysis. Thus, the outcome of this meta-analysis suggests that the 3R variant of TSER 2R/3R polymorphism contributes to gastric cancer risk in the Caucasian population.

The 2R or 3R genetic variants are the most common genetic mutations of TSER gene and known to be involved in modulation of TYMS mRNA expression (Marsh et al., 2001). The two alleles of TSER 2R/3R differ not only biologically but also functionally in their ability to alter TYMS activation on folate metabolism. Thus, there is obvious biological evidence for the different effects on cancer development between the two different variants (Marsh et al., 2001). In 2008, Ioannidis JP et al suggested an interim guideline to develop guidance criteria for assessing cumulative epidemiologic evidence in genetic associations, such as the amount of biological evidence, epidemiological credibility and clinical public-health impact (Ioannidis et al., 2008). As is argued above, there is obvious biological evidence that the variants of TSER might be involved in modulation of TYMS mRNA expression and have different effects on cancer development. In addition, our pooled analysis adds strong epidemiological evidence for the association between the TSER 2R/3R polymorphism and CRC risk. Finally, there is also convincing evidence of clinical relevance between the TSER 2R/3R polymorphism and CRC (Park et al., 2010; Goto et al., 2012). The TSER 2R/3R polymorphism were associated with the prognosis of patients with CRC, which further indicated the TSER 2R/3R polymorphism might play an important role in the colorectal carcinogenesis (Afzal et al., 2011; Jennings et

al., 2012). Thus, biological evidence, epidemiological evidence, and clinical evidence all confirm the association between the TSER 2R/3R polymorphism and CRC risk.

However, some possible limitations in our meta-analysis should be acknowledged. Firstly, the eligibility criteria for inclusion of controls were different from each other. The controls in some studies were selected from non-cancer patients who underwent gastroscopy, while the controls in other several studies were just selected from asymptomatic individuals. Additionally, misclassification bias was possible. For example, most studies could not exclude latent CRC cases in the controls. Finally, gene-gene and gene-environmental interactions were not fully addressed in this meta-analysis for the lack of sufficient data. As we know, aside from genetic factor, smoking is a major risk factor for CRC (Arafa et al., 2011); however we didn't perform subgroup analyses in smokers or nonsmokers owing to the limited reported information on such associations in the included studies.

Despite of those limitations, this meta-analysis suggests the 3R variant of Thymidylate synthase 5'-untranslated enhanced region 2R/3R polymorphism contributes to gastric cancer risk in the Caucasians. Besides, large and carefully designed case-control studies among other racial groups are needed to provide the best evidence for such a possible association in other ethnicity.

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The author(s) declare that they have no competing interests.

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