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ORIGINAL ARTICLE

Genetic variant of TMBIM1 is associated with the susceptibility of colorectal cancer in the Chinese population

Jie Zhang[a,1], Yiwei Fu[a,1], Jiebin Chen[b], Qianjun Li[c], Huimin Guo[d], Bin Yang[a,*]

[a] *Department of Gastroenterology, Jiangsu Taizhou People's Hospital, Taizhou, PR China*
[b] *Department of Paediatrics, Jiangsu Taizhou People's Hospital, Taizhou, PR China*
[c] *Department of Gastroenterology, Huai'an First People's Hospital of Nanjing Medical University, Huai'an, PR China*
[d] *Department of Gastroenterology, The Drum Tower Hospital of Nanjing University Medical School, Nanjing, PR China*

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Colorectal cancer;
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Summary
Background and aims: Recent meta-analysis of genome-wide association studies (GWASs) identified a novel variant rs992157 at 2q35 that was associated with colorectal cancer (CRC) in the population of European ancestry. We aimed to replicate the association of rs992157 with CRC in the Chinese population and to further determine the real susceptible gene of CRC as indicated by this variant.
Methods: 824 CRC patients and 1063 healthy controls were included. The frequency of the genotype and the allele of rs992157 were compared between the patients and the controls and between different subgroups of patients classified by status of metastasis. Expression level of TMBIM1 was compared between the tumor tissue and the adjacent normal tissues collected from 43 patients during surgery. Besides, the relationship between genotypes of rs992157 and the tissue expression of TMBIM1 was analyzed.
Results: Patients were found to have significantly higher frequency of allele G than the controls (44.2% vs. 40.0%, $P = 0.009$; OR = 1.18). Moreover, allele G was associated with an increased risk of lymph node metastasis ($P = 0.02$) and distant metastasis of CRC ($P = 0.04$). The mean expression level of TMBIM1 was significantly higher in tumor tissue than in the adjacent normal tissues (0.0019 ± 0.00068 vs. 0.00041 ± 0.00024, $P < 0.001$). In addition, patients with genotype GG were found to have remarkably higher TMBIM1 expression in the tumors than those with genotype AA (0.0024 ± 0.00052 vs. 0.0015 ± 0.00078, $P = 0.005$).

* Corresponding author: Department of Gastroenterology, Jiangsu Taizhou People's Hospital, Hailing South road, No. 399, Taizhou, PR China.
E-mail address: yangbintaizhou@126.com (B. Yang).
[1] Dr. Zhang and Dr. Fu contribute equally to this work

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Conclusion: Variant rs992157 is significantly associated with the susceptibility and progression of CRC. It can increase the risk of CRC possibly via up-regulation of TMBIM1.

Introduction

Colorectal cancer (CRC) is a common malignancy leading to cancer-related death worldwide [1,2,3]. In previous studies, it has been well documented that family history is a strong risk factor for CRC, thus implicating the role of genetic factors in the development of the disease [4,5,6]. To date, many candidate genes have reported to be associated with CRC, including POLD1, APC, MUTYH and SMAD4 [7,8,9,10]. Limited by small sample sizes or by the selection bias, however, only a few of those reported variants can be successfully replicated in different populations.

Compared with high-penetrance mutations, common variants had weaker contribution to CRC risk. Theoretically, a combination of common variants could help differentiate individuals with higher risk of CRC among general populations. Recently, genome-wide association studies (GWASs) have been used to identify more CRC susceptible variants in different populations [11—13]. It was estimated that these newly identified loci can explain approximately 15% of the heritability for CRC [12]. It is noteworthy that most GWASs have been conducted in patients from European ancestry, the outcome of which may show weak association with CRC in other ancestry groups.

In a recent meta-analysis of GWASs, a common variant rs992157 at 2q35 was found to be associated with CRC in the population of European ancestry [14]. Subsequently, the association between rs992157 and CRC was replicated in five Northern European cohorts composed of 4,439 CRC cases and 15,847 controls [15]. Given the possibility of considerable differences in genetic architecture across populations such as allele frequencies or the extent of linkage disequilibrium (LD), replication of rs992157 in populations of non-European ancestry appears reasonable. Herein, for the first time, we carried out a case-control study to genotype rs992157 in 829 CRC cases and 1,345 controls from the Chinese Han population. Our purpose was to replicate the association of rs992157 with CRC in Chinese and to further determine the real susceptible gene of CRC as indicated by this variant.

Methods

Subjects

Under the approval of local institution review board, a total of 824 patients diagnosed as CRC between December 2010 and June 2018 at our clinic center were retrospectively reviewed. The diagnosis of CRC was based on a combination of physical condition, clinical laboratory tests, radiological examination and histological analysis. The inclusion criteria were as follows:

- receiving no chemotherapy or radiotherapy prior to surgery;
- with no history of other malignant tumor.

1063 healthy volunteers who underwent physical examination were recruited as normal controls. All the controls were excluded to have a familial history of CRC or of other malignant tumors. Baseline characteristics were collected for each participant.

Genotyping

All the patients signed the informed consent for the collection of peripheral blood. The genomic DNA was extracted from lymphocytes of 300 μL peripheral blood with commercial DNA extraction Kit (Qiagen, Tokyo, Japan) according to the manufacturer's instructions [16]. The variant rs992157 was genotyped for each participant using TaqMan Genotyping Assay as described in previous studies [17,18]. The genotyping outcome was interpreted by LightCycler® 480 Sequence Detection System (Roche Diagnostics GmbH, Mannheim, Germany). Twenty percent of the samples were randomly selected for replication. A duplication rate of 100% was observed, which indicated high reliability of the genotyping assay.

Tissue expression of PNKD and TMBIM1

Tumor tissue and the adjacent normal tissues were collected from 43 patients during the surgical intervention. Total RNA was then isolated from the frozen tissues using a commercial kit (CWBio. Co. Ltd, Beijing, China) [19]. Specifically, before the isolation of total RNA, parts of the tissues were paraffin-embedded to evaluate the percentage of cancer cells through Hematoxylin-Eosin staining. All samples were confirmed to have more than 90% cancer cells. Reverse transcription into the cDNA and the subsequent quantitative PCR was performed as previously described [19]. The quantitative gene expression level was measured using SYBR Master Mixture (TAKARA, Tokyo, Japan) on the LightCycler 480 (Roche Diagnostics GmbH, Mannheim, Germany). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as the internal control. The following specific primers are used: forward 5'—CTATCCCCGGAGCTGGAATAC -3', reverse 5'—GTGGCCTTTAGGGTAGCGATT -3' for PNKD, forward 5'—GAGAGAGCGGTGAGTGATAGC -3', reverse 5'—ACCTTTCGGATAAAAGTGTGTCG -3' for TMBIM1 and

Table 1 Baseline characteristics of the subjects.

	Patients ($n = 824$)	Controls ($n = 1063$)	P
Age (years)	56.4 ± 13.1	55.9 ± 11.3	0.37
Ratio of male to female	435/389	543/520	0.46
Lymph node metastasis		N/A	N/A
Negative	393 (47.7%)		
Positive	431 (52.3%)		
Distant metastasis		N/A	N/A
Negative	439 (53.2%)		
Positive	385 (46.8%)		

Table 2 Distribution of the genotype and allele frequency of rs992157 in the patients and controls.

	Genotype			P	Allele		P	Odds ratio (95% CI[a])
	GG	GA	AA		G	A		
Patients ($n = 824$)	154 (18.7%)	421 (51.1%)	249 (30.2%)	0.028	729 (44.2%)	919 (55.8%)	0.009	1.18 (1.04—1.35)
Controls ($n = 1063$)	168 (15.8%)	515 (48.4%)	380 (35.7%)		851 (40.0%)	1275 (60.0%)		

[a] Indicates confidential interval.

forward 5'—CCTCTGACTTCAACAGCGACAC-3', reverse 5'—TGGTCCAGGGGTCTTACTCC-3' for GAPDH. The amplification procedure consisted of a denaturation step of 95 °C for 10 min, 45 amplification cycles at 95 °C for 10 s, annealing at 60 °C for 30 s and elongation at 72 °C for 10 s. Relative mRNA expression was analyzed using the $2^{-\Delta\Delta Ct}$ method based on melting curve analyses. All amplifications were completed in triplicate.

Statistical analysis

Demographic data were compared between the cases and the controls by the Chi-square test or the student's t test. Hardy—Weinberg equilibrium (HWE) test was examined by a one-degree-of-freedom goodness-of-fit test. The allele and genotype frequencies were compared between the cases and the controls by the Chi-square test. In addition, the distribution of genotypes of rs992157 was compared between the subgroups of the patients stratified by metastasis with the Chi-square test. The odds ratio (OR) and 95% confidence intervals (95% CIs) was calculated with the minor allele as reference. The comparison of PNKD and TMBIM1 expression in the tumor and the adjacent normal tissues was carried out by the Student-t test. The One-way ANOVA test was used to analyze the relationship between genotypes of rs992157 and the tissue expression of PNKD and TMBIM1. SPSS version 19.0 (SPSS Inc., Chicago, IL, USA) was used for the data analysis. A P-value of < 0.05 was considered statistically significant.

Results

Demographic data of the subjects

As shown in Table 1, the cases and controls were matched in terms of age (56.4 ± 13.1 vs. 55.9 ± 11.3, $P = 0.37$) and gender (435/389 vs. 543/520, $P = 0.46$). The clinical pathologic data of the patients including lymph node metastasis and distant metastasis were also summarized in Table 1.

Association of rs992157 with the development and metastasis of CRC

HWE test showed no significant difference regarding the genotype frequency in the controls. As shown in Table 2, rs992157 were found to have significantly different frequencies of allele or genotype between the cases and the controls. Compared with the controls, CRC patients were found to have significantly higher frequency of genotype GG (18.7% vs.15.8%, $P = 0.028$). Besides, patients were found to have significantly higher frequency of allele G that the controls (44.2% vs. 40.0%, $P = 0.009$; OR = 1.18).

As shown in Table 3, patients with allele G were more likely to develop lymph node metastasis ($P = 0.02$), with an OR of 1.26 (95% CI = 1.04 — 1.54). Comparably, rs992157 was also significantly associated with an increased risk of distant metastasis of CRC ($P = 0.04$, OR = 1.22).

Relationship between genotypes of rs992157 and gene expression

As shown in Fig. 1, the mean expression level of TMBIM1 was significantly higher in tumor tissue than in the adjacent normal tissues (0.0019 ± 0.00068 vs. 0.00041 ± 0.00024, $P < 0.001$). There was no significant difference regarding the expression level of PNKD between the two groups (0.00043 ± 0.00031 vs. 0.00039 ± 0.00025, $P = 0.51$). Results of the comparison of the TMBIM1 expression among different genotypes of rs992157 were shown in Table 4. The mean value of TMBIM1 expression in tumors was 0.0024 ± 0.00052 for genotype GG, 0.0018 ± 0.00063 for genotype GA and 0.0015 ± 0.00078 for genotype AA. Patients with geno-

Table 3 Allele and genotype distribution of rs992157 in patients stratified by metastasis.

	Genotype			*P*	Allele		*P*	Odds ratio (95% CI[a])
	GG	GA	AA		G	A		
Lymphatic metastasis				0.06			0.02	1.26 (1.04—1.54)
Yes ($n=431$)	91 (21.1%)	223 (51.7%)	117 (27.2%)		405 (47.0%)	457 (53.0%)		
No ($n=393$)	63 (16.0%)	198 (50.4%)	132 (33.6%)		324 (41.2%)	462 (58.8%)		
Distant metastasis				0.02			0.04	1.22 (1.01—1.49)
Yes ($n=385$)	74 (19.2%)	213 (55.3%)	98 (25.5%)		361 (46.9%)	409 (53.1%)		
No ($n=439$)	80 (18.2%)	208 (47.4%)	151 (34.4%)		368 (42.0%)	510 (58.0%)		

[a] Indicates confidential interval.

Table 4 Tissue expression of TMBIM1 and its relationship with the genotype of rs992157.

Expression of TMBIM1	Genotypes of rs992157			*P*
	GG ($n=10$)	GA ($n=21$)	AA ($n=12$)	
Tumors	0.0024 ± 0.00052	0.0018 ± 0.00063	0.0015 ± 0.00078	0.01
Normal tissue	0.00045 ± 0.00025	0.00037 ± 0.00011	0.00035 ± 0.00015	0.32

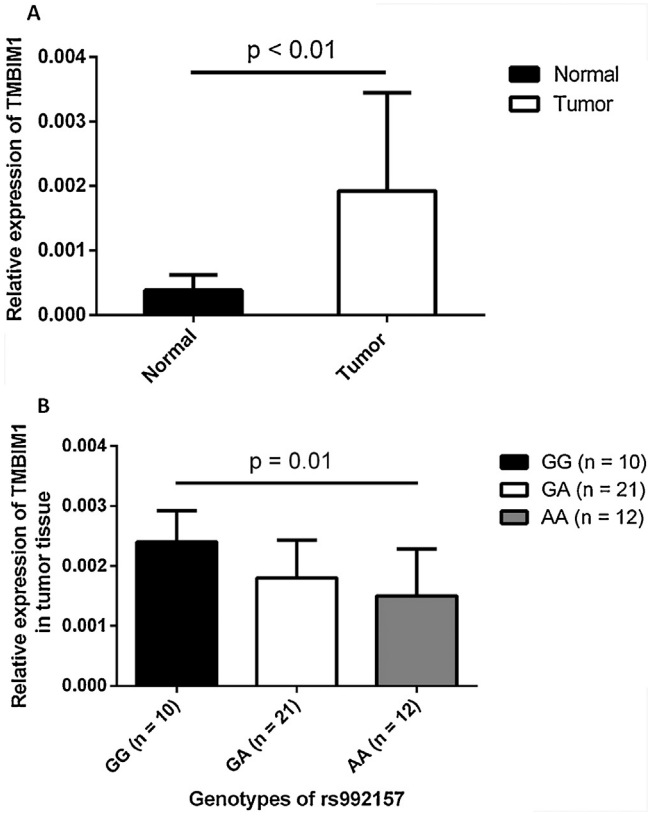

Figure 1 Tissue expression of TMBIM1. A. TMBIM1 was significantly over-expressed in tumor tissues as compared with normal tissues (fold change = 4.63, $P < 0.001$). B. For tumor tissues, genotype GG was indicative of remarkably higher expression of TMBIM1 than genotype AA ($P < 0.001$).

type GG were found to have remarkably higher TMBIM1 expression in the tumors than those with genotype AA ($P = 0.005$).

Discussion

Many new genetic variants associated with the development of CRC have been reported through GWASs, most of which were performed in European descents [20,21]. Replication study is important for the validation of these reported variants, which could help expand the understanding of the genetic architecture of CRC. Recently, through a meta-analysis of seven GWASs in European, Orlando et al [14] identified that rs992157 was a new risk variant of CRC which was located in the intron of PNKD and TMBIM1. Consistently, Tanskanen et al [15] validated that rs992157 was significantly associated with an increased risk of CRC in the Finnish population. In this study, we evaluated the association of rs992157 with the susceptibility of CRC in the Chinese population. For the first time, we confirmed that rs992157 was significantly associated with CRC in our cohort. The allele G was found to confer a 1.18-fold increased risk to the development of CRC, which was in line with the OR value of 1.1 and 1.14 reported in previous studies [14,15].

As rs992157 is intronic to both PNKD and TMBIM1, it remains undetermined which of the two genes could be involved in the development of CRC. In the current study, we analyzed the expression level of these two genes in both CRC tissues and the adjacent normal tissue. It was observed that TMBIM1 was remarkably over-expressed in the tumor tissues, while there was no significant difference regarding the expression of PNKD. In the study of Orlando et al [14], rs992157 was predicted to have allele specific cis-regulatory relationship with the expression of PNKD and TMBIM1. Therefore, we further investigated whether rs992157 was the expression quantitative trait loci in the CRC tissues. Patients with genotype GG were found to have significantly higher expression of TMBIM1 in the tumor tissues. Data from online database HaploReg showed that rs992157 was located within a regulatory region marked by a number of histone marks and motifs [22]. Further investigations are needed to clarify

the functional role of rs992157 in the regulation of TMBIM1 expression in CRC tissues.

TMBIM1, also named as RECS1, encodes a membrane protein localized in endosomes and lysosomes [23]. To date, there was a lack of study investigating the relationship between TMBIM1 and the development of cancer. TMBIM1 was reported to play a role in the suppression of matrix metalloproteinase production and regulation of Fas-mediated apoptosis and inflammation, which was also known as a physiological process prior to the onset of CRC [24]. Interestingly, in our study, variant rs992157 was found associated with the metastasis of CRC. Patients with genotype GG of rs992157 had a higher incidence of both lymph metastasis and distant metastasis. It is therefore possible that TMBIM1 may function as a trigger of CRC and promote cell migration. The precise mechanisms underlying potential regulatory effect of TMBIM1 on cell proliferation and migration of CRC is worthy of further investigation.

The findings of our study shed new light on the genetic predisposition of the Chinese population to CRC. The sample size of recruited subjects was sufficient for the reliability of the results. We validated that rs992157 of TMBIM1 gene was associated with the risk of CRC in Chinese population. Besides, we found that rs992157 could be a functional variant regulating the expression of TMBIM1 in CRC tissues. Further investigation into the role of TMBIM1 in the biological features of CRC is warranted to uncover the etiology of CRC.

Conclusion

Variant rs992157 is significantly associated with the susceptibility and progression of CRC. It can increase the risk of CRC possibly via up-regulation of TMBIM1. The precise mechanisms underlying the regulatory effect of TMBIM1 on CRC cells are worthy of further investigations.

Disclosure of interest

The authors declare that they have no competing interest.

Acknowledgment

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