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ABOUT COVER

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AIMS AND SCOPE

World Journal of Gastroenterology (*World J Gastroenterol*, *WJG*, print ISSN 1007-9327, online ISSN 2219-2840, DOI: 10.3748) is a peer-reviewed open access journal. The *WJG* Editorial Board consists of 642 experts in gastroenterology and hepatology from 59 countries.

The primary task of *WJG* is to rapidly publish high-quality original articles, reviews, and commentaries in the fields of gastroenterology, hepatology, gastrointestinal endoscopy, gastrointestinal surgery, hepatobiliary surgery, gastrointestinal oncology, gastrointestinal radiation oncology, *etc*. *WJG* is dedicated to become an influential and prestigious journal in gastroenterology and hepatology, to promote the development of above disciplines, and to improve the diagnostic and therapeutic skill and expertise of clinicians.

INDEXING/ABSTRACTING

The *WJG* is now indexed in Current Contents®/Clinical Medicine, Science Citation Index Expanded (also known as SciSearch®), Journal Citation Reports®, Index Medicus, MEDLINE, PubMed, PubMed Central, Scopus and Directory of Open Access Journals. The 2018 edition of Journal Citation Report® cites the 2017 impact factor for *WJG* as 3.300 (5-year impact factor: 3.387), ranking *WJG* as 35th among 80 journals in gastroenterology and hepatology (quartile in category Q2).

ORIGINAL ARTICLE

Observational Study

Lethal-7-related polymorphisms are associated with susceptibility to and prognosis of gastric cancer

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Abstract

BACKGROUND
The lethal-7 (*let-7*) family members and their targets are involved in the development and progression of tumors. *Let-7*-related polymorphisms have been reported to be associated with tumorigenesis and prognosis. In gastric cancer, however, the related studies are limited.

AIM
To investigate the role of *let-7*-related microRNA polymorphisms in the tumorigenesis and prognosis of gastric cancer in a Chinese population.

METHODS
A total of 898 gastric cancer patients and 992 tumor-free controls were recruited into this study from 2008 to 2013. Gastric cancer patients were followed periodically. Ten single nucleotide polymorphisms (SNPs) in the *let-7* gene region or their target mRNAs were genotyped using the MassARRAY system and their associations with the risk for or overall survival of gastric cancer were analyzed.

RESULTS
All the ten SNPs were in Hardy-Weinberg equilibrium. The C allele of the rs3811463 polymorphism in the 3′-untranslated region (UTR) of *LIN28A* was associated with a lower risk of gastric cancer [odds ratio (OR) = 0.74, 95% confidence interval (CI): 0.61-0.88, *P* = 0.001] after adjustment for age and *Helicobacter pylori* status. Seven hundred and thirty-five gastric cancer patients who had undergone radical tumorectomy were included in the survival analysis and their 5-year survival rate was 53.9% (95%CI: 50.1%-57.6%). The rs10889677 in

the 3'-UTR of *IL23R* was corresponded to the prognosis of gastric cancer in a dose-response manner, in which the death risk increased by 25% [hazard ratio (HR) = 1.25, 95%CI: 1.04-1.45, P = 0.011] with each increase in the number of C alleles after controlling for other potential clinicopathological parameters.

CONCLUSION
The *let-7*-related polymorphism rs3811463 in *LIN28A* is associated with the susceptibility to gastric cancer and the *let-7*-related polymorphism rs10889677 in *IL23R* is associated with the prognosis of gastric cancer.

Key words: Gastric cancer; Risk; Susceptibility; Prognosis; Polymorphism; Lethal-7; LIN28A; IL23R

Core tip: This study included a relatively large number of gastric cancer patients and tumor-free controls and explored the relationship of ten lethal-7 (*let-7*)-related single nucleotide polymorphisms with gastric carcinogenesis and prognosis. The results showed that the *LIN28A* rs3811463 polymorphism of the *let-7* target was associated with the development of gastric cancer and that the *IL23R* rs10889677 polymorphism was related to the overall survival of gastric cancer patients in a dose-dependent manner. This study adds evidence that polymorphisms represent a genetic factor that modifies the susceptibility to and prognosis of gastric cancer.

INTRODUCTION

Gastric cancer (GC) is the fifth most common malignancy and the third leading cause of cancer-related death worldwide[1]. It is estimated that there were 1 million new cases in 2018, and nearly half of the new cases were in China[1]. Despite advances in the diagnosis and treatment of GC in recent decades, the prognosis for GC patients is still poor, especially in China, with the five-year survival rate of only approximately 30%[2]. GC is a heterogeneous disease with distinct clinical, epidemiological, and molecular features and is thought to be a multifactorial disease influenced by environmental factors, microbial infection, and the host's genetic background[3]. Recently, increasing numbers of studies have identified that genetic variations, of which single nucleotide polymorphisms (SNPs) are the most common type, play an important role in the development and progression of tumors including GC[4,5].

MicroRNAs (miRNAs) are endogenous small noncoding RNAs that bind the mRNAs of the target genes to inhibit their translation and/or induce their decay[6]. It is estimated that more than 30% of human genes, involved in nearly all human physical and pathophysiological processes, are regulated by miRNAs[7]. The miRNA lethal-7 (*let-7*) is the first miRNA identified in humans, and more than ten members of the *let-7* family have been identified, including *let-7a-1*, *let-7a-2*, *let-7a-3*, *let-7b*, *let-7c*, *let-7d*, *let-7e*, *let-7f-1*, *let-7f-2*, *let-7g*, and *let-7i*[8]. *Let-7* often exhibits tumor-suppressor functions in tumorigenesis such as inhibiting proliferation, inducing apoptosis, and suppressing the invasion and metastasis of cancer cells[8]. The *let-7* family members are often downregulated in several cancers, thus derepressing the oncogenic targets such as K-ras, LIN28A, c-Myc, and HMGA2[9].

Previous studies have reported that miRNA-related SNPs could modulate the risk of tumors by altering the production of mature miRNAs or by affecting the binding affinity of miRNAs to their targets[10,11]. Xie *et al*[12] found that hepatocellular carcinoma patients carrying the C allele of rs10877887 in the promotor region of *let-7i* had a significantly increased death risk compared to patients with the TT genotype. The rs3811463 polymorphism is located in the 3'-untranslated region (UTR) of *LIN28A*, which is also near the target region of *let-7*. The C allele of rs3811463 could attenuate

the *let-7*-induced repression of *LIN28A* mRNA, resulting in the increased production of LIN28A protein, which could in turn downregulate the level of mature *let-7 via* an LIN28A/*let-7* double-negative feedback loop and alter breast cancer risk[13]. In GC, however, the related studies are limited. This study was aimed to determine the role of *let-7* related SNPs in the susceptibility to and prognosis of GC.

MATERIALS AND METHODS

Ethics statement
This study was approved by the Ethics Committee of the First Hospital of Jilin University (2013-005). All participants provided written informed consent prior to joining the study.

Subjects
GC patients who were hospitalized for potential tumor resection were invited to participate in the study from July 2008 to December 2013 at the First Hospital of Jilin University. A total of 898 patients who had not undergone chemotherapy or radiotherapy before surgery were recruited to the study, and all of them were histologically diagnosed with GC. Demographical and clinicopathological information was collected. The tumor histological type was evaluated by the World Health Organization criteria, and clinical stages were classified according to the 7th edition of the TNM staging system of the Union for International Cancer Control/American Joint Committee on Cancer (2010) based on postoperative pathologic examination.

During the same period, tumor-free controls were recruited from the Physical Examination Center of the same hospital. Controls were frequency-matched with cases by sex and age (± 5 years), and 992 controls were included in the study.

Follow-up
GC patients were followed periodically after tumorectomy. Follow-ups were performed at 3 mo, 6 mo, and 1 year after surgery and annually afterwards. Information on general status and postoperative chemotherapy was collected during each follow-up. If the patients had died, the date of death and potential cause were recorded. Survival time was defined as the duration from the date of surgery to the date of death. If the patient was alive, survival time was defined as the duration from the date of surgery to the date of the last follow-up. If the patient was lost to follow-up, survival time was defined as the duration from the date of surgery to the date of the last successful interview. Survival time was right-censored in the latter two cases. Patients were excluded from the survival analysis if they were lost to follow-up at the first interview or if they died of postoperative complications such as uncontrollable bleeding during the perioperative period.

Genotyping
All subjects donated blood samples. Genomic DNA was isolated using blood genomic DNA extraction kits (Axygen Biosciences, Union City, CA, United States). Serum immunoglobulin G antibodies to *Helicobacter pylori* (*H. pylori*) were assessed using an enzyme-linked immunosorbent assay (Biohit, Helsinki, Finland). Titers higher than 30 enzyme immunounits were defined as positive for *H. pylori* according to the manufacturer's instructions.

Let-7-related SNPs that were reportedly associated with cancer were selected and genotyped. Six SNPs are in the *let-7* gene region (rs13293512, rs562052, rs547008, rs1143770, rs629367, and rs10877887) and four SNPs are in the 3'-UTR of potential target genes of *let-7* (rs3811463 in *LIN28A*, rs10889677 in *IL23R*, rs7963551 in *RAD52*, and rs712 in *KRAS*). Genotypes were determined with the MassARRAY system (Sequenom Inc, CA, United States). The call rates were all greater than 95% (13 subjects for rs13293512, 10 for rs562052, 10 for rs547008, 26 for rs1143770, 2 for rs629367, 53 for rs10877887, 7 for rs10889677, 6 for rs7963551, and 31 for rs712, respectively, failed to genotype). Ten randomly selected samples were simultaneously genotyped twice, and the concordance rates were 100% for all loci.

Statistical analysis
Continuous variables are shown as the mean ± standard deviation (SD) and compared by Student's *t*-test. Categorical variables are presented as frequencies with percentages and were compared with the χ^2 test or Fisher's exact test when appropriate. Multivariate logistic regression analysis was employed to select the independent loci associated with the development of GC, and odds ratios (ORs) with their 95% confidence intervals (CIs) were calculated. Survival curves within each

stratification of variables were plotted by the Kaplan-Meier method and compared by log-rank test. The multivariate Cox proportional hazard model was used to evaluate the prognostic role of polymorphisms, and hazard ratios (HRs) with their 95% CIs were calculated. All analyses were performed using SAS 9.2 software (SAS Institute Inc, United States). A two-tailed *P*-value < 0.05 indicated statistical significance.

RESULTS

From 2008 to 2013, 898 GC cases and 992 tumor-free controls were enrolled in the study. Gender distribution was uniform between cases and controls (*P* = 0.886). The mean age of GC patients was 2 years older than that of the controls (61.1 years *vs* 59.2 years, respectively; *P* = 0.0002). Six hundred and three GC cases were positive for *H. pylori*, and the *H. pylori* positive rate (67.5%) was much higher in GC patients than in the control group (487/992 = 49.1%, *P* < 0.001) (Table 1).

LIN28A rs3811463 is associated with gastric cancer risk
Ten SNPs were genotyped in the study. All ten loci were in agreement with the Hardy-Weinberg equilibrium in the control group (*P* > 0.05). The distribution of the genotypes is presented in Table 2. The rs3811463 polymorphism in the 3'-UTR of *LIN28A* was distributed differently between GC cases and controls (*P* = 0.024). The proportions of T/C and C/C genotypes were lower in the GC group than in the control group (T/C: 22.6% *vs* 26.7%, respectively; C/C: 2.6% *vs* 3.8%, respectively). When compared to the population with the T/T genotype, individuals with the heterozygous T/C genotype had a decreased risk of GC (OR = 0.76, 95%CI: 0.61-0.94), and the risk was even lower for individuals with the C/C genotype (OR = 0.59, 95%CI: 0.34-1.01) after adjusting for age, gender, and *H. pylori* status. When the dominant model was used, the C allele (C/T + C/C) was associated with a reduced risk of GC (OR = 0.74, 95%CI: 0.60-0.91, *P* = 0.004). In stepwise logistic regression analysis, which included all loci, age, gender, and *H. pylori* status, rs3811463 was the only SNP site in the final model, and the risk of GC was lower by 26% with each increase of the number of C alleles (OR = 0.74, 95%CI: 0.61-0.88, *P* = 0.001), in addition to *H. pylori* status (OR = 2.20, 95%CI: 1.81-2.67, *P* < 0.001) and age (> 65 years *vs* ≤ 65 years, OR = 1.31, 95%CI: 1.07-1.61, *P* = 0.010) (Table 3). The other nine SNPs, however, were not associated with GC risk.

IL23R rs10889677 variant genotype predicts overall survival of gastric cancer
Given the prognostic value of surgical tumorectomy, survival analysis was performed in patients who had undergone radical tumorectomy (Figure 1). Among the 898 GC cases included in this study, 143 were excluded for one of the following reasons: (1) distant metastasis; (2) not receiving curative tumorectomy; or (3) positive surgical margins. Of the remaining 755 patients, 5 were lost to follow-up at the first interview, and 15 died of postoperative complications within one month of surgery. These 20 patients were also excluded from the survival analysis. Thus, 735 patients were included in the final survival analysis (Figure 1). The median follow-up time until September 2017 for these 735 patients was 59.8 mo. During the follow-up period, 334 patients (45.4%) died, 383 (52.1%) survived, and 18 (2.4%) were lost to follow-up. The 5-year survival rate was 53.9% (95% CI 50.1%-57.6%) and the median survival time was estimated to be 77.7 mo.

We tested the association between SNP genotypes and the overall survival of GC patients with radical resection. We observed that patients with different rs10889677 genotypes had different survival curves (log-rank *P* = 0.049, Figure 2). More precisely, patients had worse survival with each increase in the number of C alleles of rs10889677. By the end of the study, 41.7% of patients with no rs10889677 C allele (A/A genotype) had died, while 48.5% of patients with one C allele (A/C genotype) and 56.6% of patients with two C alleles (C/C genotype) had died (Table 4). The five-year survival rate for A/A genotype patients was 57.7%, which was higher than that of patients with the A/C genotype (50.3%) or C/C genotype (46.0%). We further transformed the rs10889677 genotypes to a quantifiable trait (the number of C alleles) and observed that the death HR increased by 23% with each additional C allele (HR = 1.23, 95%CI: 1.04-1.45, *P* = 0.015). The other nine SNP loci were not significantly associated with the overall survival of GC patients (Table 4).

Age (*P* < 0.001), tumor differentiation level (*P* = 0.010), depth of tumor invasion (T stage, *P* < 0.001), local lymph node metastasis (N stage, *P* < 0.001), TNM stage (*P* < 0.001), lymphovascular invasion (*P* < 0.001), and neural invasion (*P* < 0.001) were associated with the long-term survival of GC patients in univariate survival analysis (Table 5). Postoperative chemotherapy was marginally related to the outcome (*P* = 0.054). *H. pylori* status, which was previously reported to be related to GC

Table 1 General characteristics of subjects included in this study			
	Cases	**Controls**	***P* value**
n	898	992	
Gender			
Male	649 (72.3)	714 (72.0)	0.886
Female	249 (27.7)	278 (28.0)	
Age (yr)	61.1 ± 11.4	59.2 ± 10.0	< 0.001
≤ 65	582 (64.8)	703 (70.9)	0.005
> 65	316 (35.2)	289 (29.1)	
Helicobacter pylori			
Negative	290 (32.5)	505 (50.9)	< 0.001
Positive	603 (67.5)	487 (49.1)	

prognosis[14], was not associated with the long-term OS in our study (*P* = 0.364, Table 5).

Further multivariate analysis showed that the rs10889677 polymorphism was an independent prognostic predictor and that the death HR increased by 23% with each increase in the number of C alleles (HR = 1.25, 95%CI: 1.04-1.45, *P* = 0.012) (Table 6). In addition, old age (> 65 years, HR = 1.39, 95%CI: 1.10-1.75), high TNM stage [stage II (HR = 3.49, 95%CI: 1.88-6.48) or III (HR = 11.12, 95%CI: 6.0-20.61)], and positive lymphovascular invasion (HR = 1.89, 95%CI: 1.32-2.72) were also independently associated with decreased long-term survival. Additionally, chemotherapy after radical tumorectomy reduced the death hazard (HR = 0.75, 95%CI: 0.59-0.96, *P* = 0.021) (Table 6).

DISCUSSION

The *let-7* family members play an important role in several hallmarks of cancer, including repressing cellular proliferation, inducing cell apoptosis, suppressing aerobic glycolysis, inhibiting invasion and metastasis, and regulating tumor innate immune reactions by interacting with their targets[8]. *Let-7*-related SNPs, which are located in the *let-7* gene region or in the target gene region, were reported to be associated with the development or prognosis of tumors. In this study, we explored the association of *let-7*-related SNPs with the susceptibility to and long-term overall survival of GC in a Chinese population. We observed that the rs3811463 in the 3'-UTR of *LIN28A* was associated with the susceptibility of GC and that rs10889677 in the 3'-UTR of *IL23R* was associated with the OS of resectable GC.

The *let-7* family and LIN28A compose a double-negative feedback loop. LIN28A represses *let-7* by inhibiting the biogenesis and inducing the degradation of the *let-7* family; meanwhile, *let-7* inhibits the expression and function of LIN28A *via* binding to complementary sites at the 3'-UTR of *LIN28A* mRNA[8]. In contrast to the role of *let-7* in tumors, LIN28A promotes the proliferation of cancer cells and is related to the poor prognosis[15]. Rs3811463 is located in the 3'-UTR of *LIN28A*, near the binding region of *let-7*. Chen *et al*[13] reported that the C allele of rs3811463 corresponded to an increased risk of breast cancer by attenuating the *let-7*-induced LIN28A repression, which in turn downregulates the mature *let-7 via* the LIN28A/*let-7* double-negative feedback loop. A subsequent study on oral cancer did not report any association between oral cancer risk and rs3811463 polymorphism in 384 cases and 731 controls[16]. In our study, however, the C allele of rs3811463 was found to be associated with a reduced risk of GC. Moreover, this association demonstrated a dose-response manner in which the risk was lower with each increase in the number of C alleles compared to that of the patients without the C allele (*i.e.*, genotype T/T). The online bioinformatics tool SNPinfo (http://snpinfo.niehs.nih.gov/) predicts that the change from T to C might make *LIN28A* gain the target of miR-490, which would reduce the level of *LIN28A*, thus associating with a lower risk of GC. However, further studies are needed to verify this hypothesis.

Interleukin-23 receptor (IL23R), a crucial subunit of the IL-23 receptor complex, is involved in innate and adaptive immune processes by interacting with IL-23, which plays a crucial role in many human diseases including autoimmune diseases and tumors[17,18]. Because IL23R presents tumor-promoting effects, knockdown of *IL23R* restricts tumor growth and decreases cancer metastasis[17,18]. The rs10889677

Table 2 The genotype distribution between gastric cancer cases and controls

Gene	SNP	Genotypes	Cases	Controls	*P* value[a]	OR (95% CI)[b]
let-7a-1	rs13293512	T/T	270 (30.3)	310 (31.5)	0.593	1.00
		T/C	442 (49.6)	494 (50.2)		1.03 (0.83-1.28)
		C/C	180 (20.2)	181 (18.4)		1.13 (0.86-1.48)
let-7a-2	rs562052	G/G	382 (43.0)	439 (44.3)	0.814	1.00
		G/A	413 (46.5)	446 (45.0)		1.06 (0.87-1.29)
		A/A	94 (10.6)	106 (10.7)		0.96 (0.70-1.32)
let-7a-2	rs547008	C/C	529 (59.2)	575 (58.3)	0.875	1.00
		C/T	321 (35.9)	358 (36.3)		0.96 (0.79-1.17)
		T/T	44 (4.9)	53 (5.4)		0.85 (0.56-1.30)
let-7a-2	rs1143770	T/T	255 (28.9)	282 (28.7)	0.262	1.00
		T/C	456 (51.8)	482 (49.0)		1.04 (0.83-1.29)
		C/C	170 (19.3)	219 (22.3)		0.83 (0.64-1.09)
let-7a-2	rs629367	A/A	532 (59.2)	597 (60.3)	0.583	1.00
		A/C	323 (36.0)	355 (35.9)		1.01 (0.83-1.22)
		C/C	43 (4.8)	38 (3.8)		1.21 (0.76-1.92)
let-7i	rs10877887	T/T	370 (42.3)	406 (42.2)	0.777	1.00
		T/C	399 (45.6)	449 (46.7)		0.96 (0.78-1.17)
		C/C	106 (12.1)	107 (11.1)		1.04 (0.76-1.42)
LIN28A	rs3811463	T/T	672 (74.8)	689 (69.5)	0.024	1.00
		T/C	203 (22.6)	265 (26.7)		0.76 (0.61-0.94)
		C/C	23 (2.6)	38 (3.8)		0.59 (0.34-1.01)
	Dominant	T/T	672 (74.8)	689 (69.5)	0.009	1.00
		T/C+C/C	226 (25.2)	303 (30.5)		0.74 (0.60-0.91)
IL23R	rs10889677	A/A	476 (53.2)	545 (55.2)	0.685	1.00
		A/C	356 (39.8)	375 (38.0)		1.10 (0.91-1.34)
		C/C	63 (7.0)	68 (6.9)		1.07 (0.74-1.55)
RAD52	rs7963551	T/T	597 (66.6)	672 (68.0)	0.666	1.00
		T/G	267 (29.8)	287 (29.0)		1.06 (0.87-1.31)
		G/G	32 (3.6)	29 (2.9)		1.18 (0.69-2.01)
KRAS	rs712	G/G	540 (61.0)	589 (60.5)	0.646	1.00
		G/T	296 (33.4)	339 (34.8)		0.94 (0.77-1.15)
		T/T	49 (5.5)	46 (4.7)		1.14 (0.74-1.75)

[a]*P* was computed using the $\chi2$ test.

[b]ORs with 95%CIs were computed using a logistic model after adjusting for age, gender, and *Helicobacter pylori* status.

SNP: Single nucleotide polymorphism; ORs: Odds ratios; CIs: Confidence intervals.

polymorphism, located in the 3'-UTR of the *IL23R* gene, has been reported to be associated with cancer risk, including the risk of breast cancer[19], esophageal cancer[20], bladder cancer[21], ovarian cancer[22], oral cancer[23], and GC[24,25]. The results of these studies, however, are controversial[26]. In GC, Chen *et al* showed that the presence of the C allele of rs10889677 predicted lower GC risk (A/C: OR = 0.81, 95%CI: 0.66–0.99; C/C: OR = 0.47, 95%CI: 0.31–0.71)[24], while another study by Dong *et al*[25] observed an opposite relationship; the presence of the C allele increased the GC risk by 24% (C_{allele}*vs* A_{allele}: OR = 1.24, 95%CI: 1.02–1.52). In our study, however, we did not find any significant association between rs10889677 and GC risk ($P > 0.05$). Nonetheless, we observed that the C allele was related to decreased survival of GC patients after curative tumorectomy in a dose-dependent manner, and the death HR increased by 25% (HR = 1.25, 95%CI: 1.05-1.49) with each increment in the number of the C alleles. This is the first study reporting that the rs10889677 of *IL23R* is related to the prognosis of GC, as most studies only focused on cancer susceptibility. Zwiers *et al*[27] showed that the C allele of rs10889677 was corresponded to reduced *IL23R* levels possibly through the target gain of *let-7*. However, this could not explain what we had observed, and another unknown mechanism might be involved.

Studies have reported a significant correlation between SNPs in the *let-7* gene

Table 3 Multivariate stepwise logistic regression analysis of gastric cancer risk

Factor	OR	95%CI	*P* value
rs3811463 (each increase in C allele)	0.74	0.61-0.88	0.001
Helicobacter pylori (positive *vs* negative)	2.20	1.81-2.67	< 0.001
Age (> 65 *vs* ≤ 65 yr)	1.31	1.07-1.61	0.010

OR: Odds ratio; CI: Confidence interval.

region and the susceptibility to or prognosis of malignant tumors, for example, rs629367 in *let-7a* and the risk of GC[10], rs10877887 in *let-7i* and the risk of papillary thyroid carcinoma[28] and survival of hepatocellular carcinoma[12], and rs1143770 in *let-7a* and the overall survival and disease-free survival of surgically resected non-small cell lung cancer[29]. Other studies, however, observed no such associations[30]. In our study of a relatively large number of GC patients, we did not find that the six polymorphisms in the *let-7* gene region (rs13293512, rs562052, rs547008, rs1143770, rs629367, and rs10877887) were associated with the risk of development and the overall survival of GC. Several factors might contribute to the discrepancies among studies, such as different origins of the tumors studied and study populations from different ethnic groups. Therefore, more studies with a large sample size, including populations from different ethnic groups and tumors from multiple origins, are warranted in the future.

Two limitations should be noted in our study. First, although we observed that two SNPs were associated with GC, we could not verify the underlying mechanism for these associations because of our study design. Second, GC patients and controls from only one hospital were included in the study. The generalization of these results to other populations should be cautious because there has been no external validation. Therefore, more studies are needed to verify the results.

In summary, this study provides evidence that polymorphisms represent a genetic factor that modifies the susceptibility to and prognosis of GC. Individuals carrying the C allele of rs3811463 in the 3'-UTR of *LIN28A* have a lower risk of GC, and GC patients with the C allele of rs10889677 in the 3'-UTR of *IL23R* have a shorter lifespan than patients without it.

Table 4 Genotypes of single nucleotide polymorphisms and overall survival of gastric cancer patients

SNP	Genotype	Total	Death (%)	5-yr survival rate	Median survival time	P value[a]
rs13293512	T/T	213	100 (46.9)	51.6%	85.2	0.508
	T/C	370	172 (46.5)	53.3%	75.8	
	C/C	148	60 (40.5)	59.0%	-[b]	
rs562052	G/G	313	150 (47.9)	51.5%	76.2	0.253
	G/A	337	141 (41.8)	56.9%	-[b]	
	A/A	77	40 (51.9)	50.7%	65.4	
rs547008	C/C	434	200 (46.1)	53.0%	85.2	0.148
	C/T	264	112 (42.4)	56.2%	-[b]	
	T/T	34	21 (61.8)	46.7%	33.8	
rs1143770	T/T	215	98 (45.6)	54.3%	-[b]	0.535
	T/C	370	164 (44.3)	54.4%	-[b]	
	C/C	138	68 (49.3)	51.3%	65.4	
rs629367	A/A	435	205 (47.1)	52.8%	75.8	0.834
	A/C	264	113 (42.8)	55.6%	-[b]	
	C/C	36	16 (44.4)	55.8%	85.2	
rs10877887	T/T	301	147 (48.8)	50.4%	64.7	0.235
	T/C	330	141 (42.7)	57.2%	-[b]	
	C/C	88	39 (44.3)	53.7%	-[b]	
rs3811463	T/T	555	251 (45.2)	53.7%	85.2	0.971
	T/C	162	75 (46.3)	54.9%	77.7	
	C/C	18	8 (44.4)	52.0%	-[b]	
rs10889677	A/A	379	158 (41.7)	57.7%	-[b]	0.049
	A/C	301	146 (48.5)	50.3%	65.4	
	C/C	53	30 (56.6)	46.0%	41.0	
rs7963551	T/T	508	236 (46.5)	52.56%	85.2	0.144
	T/G	199	82 (41.2)	59.35%	-[b]	
	G/G	27	16 (59.3)	38.22%	41.3	
rs712	G/G	452	215 (47.6)	51.51%	73.7	0.227
	G/T	235	95 (40.4)	58.64%	-[b]	
	T/T	36	17 (47.2)	55.01%	77.7	

[a]*P* was computed by log-rank test.
[b]Median survival time could not be estimated as fewer than 50% of patients died.

Table 5 Associations between clinical factors and overall survival of gastric cancer patients

Variable	Classification	n[a]	Death (%)	5-yr survival (%)	P value[b]
Age (yr)	≤ 65	494	197 (39.9)	59.7 (55.0-64.0)	< 0.001
	> 65	241	137 (56.8)	42.3 (35.6-48.8)	
Gender	Female	195	82 (42.1)	56.8 (49.2-63.7)	0.261
	Male	540	252 (46.7)	52.9 (48.4-57.2)	
Differentiation	Poor	508	241 (47.4)	50.9 (46.2-55.3)	0.010
	Moderate and high	209	85 (40.7)	61.6 (54.4-68.0)	
WHO type	Tubular adenocarcinoma	614	275 (44.8)	54.8 (50.6-58.8)	0.205
	Signet ring cell	72	32 (44.4)	52.8 (39.8-64.2)	
	Other	45	25 (55.6)	44.5 (29.2-58.6)	
T stage	T1	100	13 (13.0)	85.8 (76.5-91.6)	< 0.001
	T2	102	16 (15.7)	84.4 (74.8-90.6)	
	T3	423	220 (52.0)	48.1 (43.1-52.9)	
	T4	103	78 (75.7)	17.4 (9.2-27.8)	
N stage	N0	219	38 (17.4)	84.2 (78.4-88.5)	< 0.001
	N1	184	63 (34.2)	66.0 (58.2-72.6)	

	N2	150	87 (58.0)	38.7 (30.3-47.1)	
	N3	175	139 (79.4)	17.3 (11.7-23.9)	
TNM	I	137	12 (8.8)	90.6 (83.9-94.6)	< 0.001
	II	278	92 (33.1)	68.3 (62.1-73.6)	
	III	313	223 (71.2)	25.8 (20.7-31.2)	
Lymphovascular invasion	Negative	213	38 (17.8)	83.0 (77.0-87.6)	< 0.001
	Positive	511	287 (56.2)	42.6 (38.1-47.0)	
Neural invasion	Negative	328	100 (30.5)	70.2 (64.6-75.0)	< 0.001
	Positive	396	225 (56.8)	41.1 (36.0-46.2)	
Chemotherapy	No	488	218 (44.7)	54.8 (50.0-59.3)	0.054
	FOLFOX-4	136	63 (46.3)	52.1 (43.2-60.3)	
	XELOX	73	28 (38.4)	61.7 (49.1-72.0)	
	Other	38	25 (65.8)	34.0 (19.1-49.4)	
Helicobacter pylori	Negative	228	109 (47.8)	49.8 (42.7-56.4)	0.364
	Positive	503	222 (44.1)	56.1 (51.4-60.4)	

[a]Some of the variables have missing values (The missing number was 4 for the variable of WHO type, 18 for differentiation, 7 for T stage, 7 for N stage, 7 for TNM stage, 11 for lymphovascular invasion, 11 for neural invasion, and 4 for *Helicobacter pylori*, respectively).
[b]*P* was computed by log-rank test.

Table 6 Multivariate Cox regression analysis of gastric cancer survival

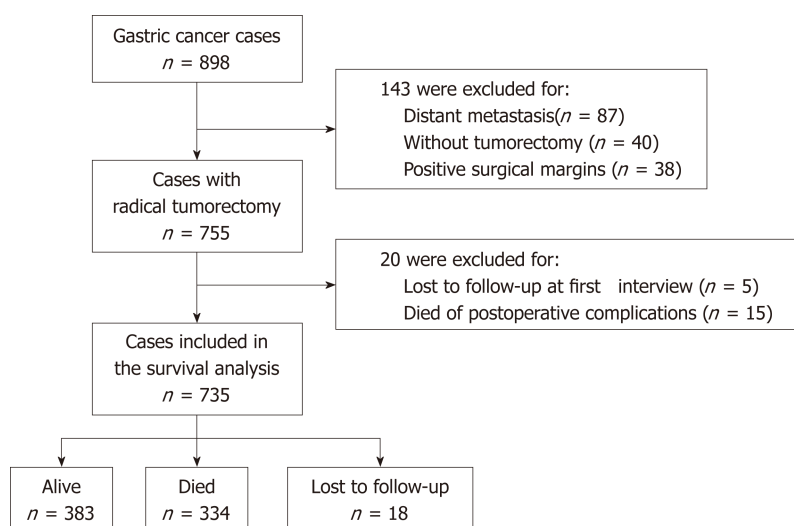| Variable	Comparison	HR	95%CI	*P* value
rs10889677	each increase in C allele	1.25	1.05-1.49	0.012
Age (yr)	> 65 *vs* ≤ 65	1.39	1.10-1.75	0.005
TNM stage	II *vs* I	3.49	1.88-6.48	< 0.001
	III *vs* I	11.12	6.00-20.61	< 0.001
Lymphovascular invasion	Positive *vs* Negative	1.89	1.32-2.72	0.001
Chemotherapy	Yes *vs* No	0.74	0.59-0.95	0.016

HR: Hazard ratio; CI: Confidence interval.

Figure 1 The flow chart of patients included in the survival analysis.
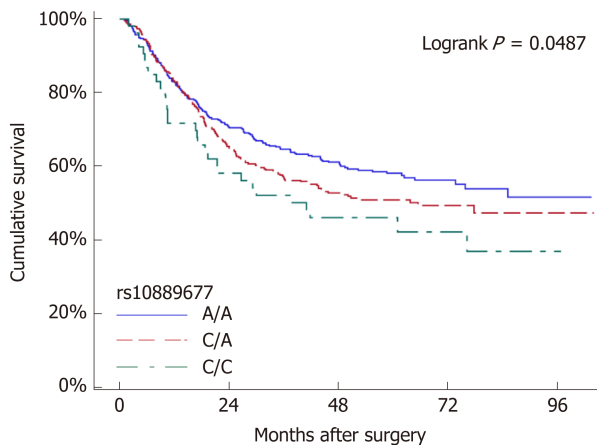
Figure 2 Survival plot of gastric cancer patients stratified by rs10889677 genotypes. Compared with the survival of patients with no C allele of rs10889677 (genotype A/A, blue line), patients with one C allele (genotype C/A, red line) exhibited a decreased survival rate and patients with two C alleles (genotype C/C, cyan line) exhibited the lowest survival rate.

ARTICLE HIGHLIGHTS

Research background

Gastric cancer (GC) is one of the most common malignancies worldwide. Despite the advances in diagnosis and treatment of GC in recent decades, prognosis of GC patients is still poor. It is of great importance to identify biomarkers that could be helpful in the improvement of screening of high-risk individuals, early diagnosis, and predicting outcome for the individualized therapy.

Research motivation

The microRNA lethal-7 (*let-7*) often exhibits tumor-suppressor functions in tumorigenesis. Single nucleotide polymorphisms (SNPs) in the *let-7* gene region or *let-7* target genes have been reported to modulate the risk of several cancers including breast cancer and lung cancer. In GC, the related studies are limited

Research objectives

By including a relatively large number of GC patients, this study aimed to determine the role of *let-7*-related polymorphisms in GC in a Chinese population.

Research methods

From 2008 to 2013, 898 consecutive GC patients and 992 tumor-free controls were recruited into the study. GC patients were followed periodically to determine their prognosis. Ten SNPs in the *let-7* gene region or their target mRNAs were genotyped using MassArray system and the associations with the risk or overall survival of GC were analyzed.

Research results

Two SNPs in *let-7* target genes were associated with GC in a dose-dependent manner. Rs3811463 in the 3'-UTR of *LIN28A* was associated with lower risk of GC and the risk was reduced by 26% with each increase of the C allele of rs3811463. The rs10889677 in the 3'-UTR of *IL23R* was corresponded to the prognosis of GC, and the death risk increased by 25% with each increment of the C allele of rs10889677, after controlling for clinicopathological parameters.

Research conclusions

Let-7-related SNPs were related to GC. The rs3811463 in *LIN28A* is associated with the susceptibility to and rs10889677 in *IL23R* is associated with the prognosis of GC.

Research perspectives

Our research adds evidence that polymorphisms represent a genetic factor to modify the susceptibility to and prognosis of GC. The underlying mechanisms of the associations should be elucidated in future studies.

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