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Association of microRNA Polymorphisms with the Risk of Gastric Cancer in a Romanian Population

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ABSTRACT

Background & Aims: MicroRNAs (miRNAs) play an important role in the occurrence and progression of human cancers, including gastric cancer. Our hospital-based case-control study aimed to investigate whether four commonly studied single nucleotide polymorphisms (SNPs) have effects on susceptibility to gastric cancer in a Romanian population.

Method: We genotyped the miR-27a rs895819, miR-146a rs2910164, miR-196a2 rs11614913 and miR-499 rs3746444 SNPs by real-time PCR using predesignated TaqMan assays in 430 individuals (142 gastric cancer patients and 288 age and gender matched cancer-free controls). The associations between the investigated miRNA SNPs and gastric cancer risk were assessed by odds ratio (OR) with 95% confidence interval (CI) using logistic regression analysis.

Results: A higher frequency of the miR-27a rs895819 CC genotype (OR 1.98, 95% CI: 1.05-3.73, p=0.036) was found in the patients with gastric cancer compared with the controls. Similar results were observed in a recessive model, the CC genotype was correlated with gastric cancer susceptibility (OR 1.95, 95% CI: 1.07-3.55, p=0.032). In the stratified analysis, the association between miR-27a rs895819 SNP and gastric cancer risk was limited to noncardia (OR 2.08, 95% CI: 1.10-3.94, p=0.027) and intestinal (OR 2.27, 95% CI: 1.05-4.92, p=0.042) subgroups. However, after Bonferroni correction, all associations described above lost statistical significance. No correlation was observed for the remaining SNPs and risk of gastric cancer in any genetic model studied.

Conclusion: This study showed no association of the investigated miRNA SNPs with the risk of gastric cancer in a Romanian population.

Key words: gastric cancer – miRNA – single nucleotide polymorphism – genotype – susceptibility.

Abbreviations: GC: gastric cancer; miRNA: microRNA; SNP: single nucleotide polymorphism.

INTRODUCTION

Gastric cancer (GC) is one of the most common malignancies and a major cause of morbidity and mortality, being the fifth most frequent cancer and the third leading cause of cancer-related death in both sexes worldwide [1]. Gastric cancer incidence rates show significant variations across different countries and ethnic groups, between men and women or tumor subsites [2]. Gastric cancer is often diagnosed in advanced stages because the majority of patients are asymptomatic in early stages, with an overall five-year survival rate of less than 20–30% [3, 4]. The large majority (approximately 90%) of GCs are adenocarcinomas and according to Lauren, GC can be classified into two major types: intestinal type and diffuse type [5]. The mechanisms of GC pathogenesis are incompletely understood, yet, both genetic and environmental factors contribute to the occurrence and progression of this malignancy. The environmental factors, such as *Helicobacter pylori* (*H. pylori*) infection, dietary factors, tobacco smoking, alcohol intake, as well as host susceptibility genes are suggested to be key players in the GC carcinogenesis [6, 7].

MicroRNAs (miRNAs) are a class of small noncoding endogenous RNAs, 18–25 nucleotides in length, that negatively regulate gene expression post-transcriptionally through binding to target messenger RNAs (mRNAs). It was suggested that miRNAs may regulate 30% of all human genes and each

miRNA can control hundreds of genes, by inhibition of translation and/or degradation of the target mRNAs [8, 9]. miRNAs are involved in the pathogenesis of human cancers; more than 50% of miRNA genes are located at cancer-associated chromosomal regions [10]. miRNAs may function either as oncogenes whose expression is commonly upregulated or as tumor suppressor genes which are downregulated. The alterations in the expression of miRNAs were confirmed in tumorigenesis, progression, invasion, metastasis, diagnosis and prognosis of GC [11-13].

The single nucleotide polymorphisms (SNPs) in miRNA-coding regions can alter the expression levels or miRNA maturation and moreover these effects can be amplified through miRNA–mRNA interactions [14]. A large number of studies have been completed to assess the association of miRNAs with GC risk: the miR-27a rs895819, miR-149 rs2292832, miR-146a rs2910164, miR-196a2 rs11614913 and miR-499 rs3746444 SNPs are the most extensively investigated. These genetic variants have been previously studied mainly in Asian populations, and only few data is available in Caucasians.

Our aim was to investigate whether four commonly studied SNPs (miR-27a rs895819, miR-146a rs2910164, miR-196a2 rs11614913 and miR-499 rs3746444) have effects on the susceptibility to gastric cancer in a Romanian population.

METHODS

Subjects

In this hospital-based case-control study, we included 142 patients diagnosed with gastric adenocarcinoma and 288 unrelated cancer-free controls, recruited from the Clinical County Hospital of Craiova (Craiova, Romania). The controls were matched to GC cases by gender and age. The subjects with any gastrointestinal disease or different ethnic origins were excluded. The main GC diagnostic procedure was gastric endoscopy followed by gastric biopsy and histopathological examination to determine the histological subtype (intestinal, diffuse or mixed). Only *H. pylori*-positive patients were selected. *H. pylori* infection status was assessed by serology, histologic examination or the urea breath test. The study was approved by the Ethics Committee of the University of Medicine and Pharmacy of Craiova, Romania (ethical approval No.86-10/2014) and informed consent was obtained from each participant.

SNPs genotyping

Peripheral blood samples were collected from all participants in EDTA (ethylenediaminetetraacetic acid) containing tubes. Genomic DNA of each individual was extracted from the peripheral blood leukocytes using Wizard® Genomic DNA Purification Kit (Promega, Madison, WI), according to the manufacturer protocols. DNA purity and concentration were determined by spectrophotometric absorbance measurements at 260 and 280 nm wave-lengths (Eppendorf Biophotometer, Eppendorf AG, Hamburg, Germany).

The miRNA SNPs were genotyped by real-time PCR using predesigned TaqMan assays (Applied Biosystems Foster City, CA) specific for each genetic variant: miR- 27a rs895819 T>C (assay C_3056952_20), miR-146a rs2910164 G>C (assay C_15946974_10), miR-196a2 rs11614913 C>T (assay C_31185852_10) and miR-499 rs3746444 A>G (assay C_2142612_30). Allelic discrimination was performed using a ViiA™ 7 RT-PCR System (Life Technologies, Carlsbad, USA) and genotype assignments were confirmed by visual inspection. To ensure quality control, the genotyping analysis was blindly performed with respect to case/control status. Positive controls and negative templates were added for each genotype. Moreover, approximately 10% samples were randomly chosen for repetitive analysis using another real-time PCR system (RotorGene 6200 HRM-Corbett) as reported previously [15], with 100% concordance rate for all the masked samples.

Statistical analysis

The χ^2 goodness-of-fit test was used to determine whether the individual SNPs were under Hardy-Weinberg equilibrium (HWE). Demographic data between groups were compared using χ^2 test for gender and ANOVA test for age. The strength of association between the investigated miRNA SNPs and GC risk was assessed by odds ratio (OR) with 95% confidence interval (CI) using logistic regression. The difference in the distribution of genotype frequencies between the cases and controls was compared using the χ^2 test. We used four inheritance genetic models (codominant – the most common genotype serves as reference, dominant, and recessive and allelic) to evaluate the associations between miRNA SNPs and risk of GC.

The SPSS statistical software package version 17 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. The Bonferroni-corrected alpha level was set at 0.013 (0.05/4 SNPs).

RESULTS

We successfully genotyped four miRNA SNPs in a total of 430 subjects, including 142 GC patients and 288 cancer-free controls. The selected characteristics of case and control groups are presented in Table I. No significant differences in gender and age were observed between the patients and controls (p=0.65 and p=0.13, respectively). All included cases were *H. pylori*-positive gastric adenocarcinoma. According to the tumor site, 111 were noncardia cancers and 31 were located in the cardia. Based on the Lauren classification, 79 of detected cancers were intestinal-type, 62 were diffuse-type and 1 was mixed type.

Table I. General characteristics of patients with gastric cancer and healthy controls

	Gastric adenocarcinoma (n=142)	Control (n=288)	P value
Male/Female	89/53	174/114	0.65
Age (years), mean±SD	66.26±8.65	64.49±7.94	0.13
Location			
- cardia	31		
- noncardia	111		
Histological type			
- intestinal	79		
- diffuse	62		
- mixed	1		

The genotype distributions complied well with Hardy-Weinberg equilibrium in control subjects, indicating that there was no genetic drift or any selective advantage (p>0.05). We assumed that the minor allele of each miRNA SNP was a risk factor compared to the wild-type allele. Table II presents the genotype frequencies and the association between miRNA SNPs and the risk of GC.

A higher frequency of the miR-27a rs895819 CC genotype (OR 1.98, 95% CI: 1.05 - 3.73, p=0.036) was found in patients with gastric adenocarcinoma compared with the controls. Also, in a recessive model, carriers of the CC genotype were associated with higher gastric adenocarcinoma risk (OR 1.95, 95% CI: 1.07 - 3.55, p=0.032). However, after Bonferroni correction for multiple testing, all associations described above lost statistical significance.

We found no correlation between GC cases and controls for miR-146a rs2910164 G>C, miR-196a2 rs11614913 C>T and miR-499 rs3746444 A>G SNPs in any genetic model studied (codominant, dominant, recessive or allelic).

We examined separately the associations of these SNPs with tumor site (noncardia and cardia) and histological type (intestinal and diffuse) (Tables III and IV). Without using Bonferroni correction, a positive association was found only for miR-27A rs895819 CC genotype, that was restricted to noncardia gastric adenocarcinoma (OR 2.06, 95% CI: 1.05 - 4.04; p=0.038) and intestinal type (OR 2.27, 95% CI: 1.05 - 4.92; p=0.042). In the allelic model, the frequency of the miR-27A rs895819 C allele was significantly higher in gastric cancer patients with an intestinal type compared to the healthy subjects (OR 1.47, 95% CI: 1.02 – 2.11; p=0.043). Following Bonferroni correction, no association was observed in the stratified analysis.

DISCUSSION

In this study, we assessed whether four SNPs in functional regions of miRNA genes influence GC susceptibility in a Romanian population. Few studies have been published on the association of these SNPs with GC risk in Caucasian populations. We selected these SNPs based on previous association studies with GC risk, *in vitro* functional studies and suggested role of these molecules in GC pathogenesis. In our study, the allele distribution frequencies are in accordance with the NCBI databases (http://www.ncbi.nlm.nih.gov/projects/SNP).

We found a significant association between mir-27a rs895819 T>C and GC susceptibility. The carriers of CC genotype were at higher GC risk, mainly for the intestinal type, without Bonferroni correction. When Bonferroni correction for multiple testing was used, the associations lost statistical significance. miR-27a seems to act as an oncogene in GC by targeting BTG2 and prohibitin [16, 17]. The miR-27a rs895819 T>C (A>G in reverse orientation to genome) SNP seems to have a functional effect by affecting the stem-loop and further pre-microRNA maturation and expression of mature miR-27a [18-20]. Previous studies on the association between miR-27a rs895819 and the risk of GC have produced controversial results. The carriers of rs895819 C (G in reverse orientation) allele exhibited significantly increased risk of GC [18, 20]. Moreover, the carriers of CC genotype were associated with a higher risk of premalignant conditions as intestinal metaplasia and dysplasia [21] and a higher atrophy and metaplasia scores in men with chronic gastritis [22]. In contrast, the CC genotype showed a protective effect in a Chinese population [23]. However, other studies did not find an association between rs895819 and GC risk [24, 25]. Recently, a meta-analysis indicated that rs895819 was associated with an increased overall cancer risk, but not for GC in the subgroup analysis by cancer site [26].

We did not find any significant correlation between the remaining miRNA SNPs and GC susceptibility. The G/C variant of the rs895819 in the passenger strand seems to cause hairpins mispaired and alter miR-146a maturing process [27]. While some studies revealed a higher GC risk for individuals bearing G allele or GG genotype [28-30], others showed a protective effect for GG genotype [25] or C allele [31]. There are also studies with no detected relationship between miR-146a rs2910164 and GC in Asian [32, 33] and Caucasian populations

Table II. Association between miRNA SNPs and risk of gastric cancer under multiple models of inheritance

Polymorphism	Gastric cancer (n=142)	Control (n=288)	OR (95%CI)	p value
miR- 27a rs895819				
Codominant				
TT	63 (44.36%)	141 (48.96%)	Reference	-
TC	56 (39.44%)	121 (42.01%)	1.04 (0.67 - 1.60)	0.87
CC	23 (16.20%)	26 (9.03%)	1.98 (1.05 - 3.73)	0.036
Dominant				
TT	63 (44.36%)	141 (48.96%)	Reference	-
CC + TC	79 (55.64%)	147 (51.04%)	1.20 (0 .80 - 1.80)	0.37
Recessive				
TT + TC	119 (83.80%)	262 (90.97%)	Reference	-
CC	23 (16.20%)	26 (9.03%)	1.95 (1.07 - 3.55)	0.032
Allelic				
T	182 (64.08%)	403 (69.97%)	Reference	-
C	102 (35.92%)	173 (30.03%)	1.31 (0.97 - 1.76)	0.08

Table II (continued)

miR-146a rs2910164				
Codominant				
GG	86 (60.56%)	160 (55.55%)	Reference	-
GC	48 (33.80%)	109 (37.85%)	0.82 (0.53 - 1.26)	0.36
CC	8 (5.64%)	19 (6.60%)	0.78 (0.33 - 1.86)	0.58
Dominant				
GG	86 (60.56%)	160 (55.55%)	Reference	-
CC + GC	56 (39.44%)	128 (44.45%)	0.81 (0.54 - 1.23)	0.32
Recessive				
GG + CG	134 (94.36%)	269 (93.40%)	Reference	-
CC	8 (5.64%)	19 (6.60%)	0.85 (0.36 - 1.99)	0.69
Allelic				
G	220 (77.46%)	429 (74.48%)	Reference	-
C	64 (22.54%)	147 (25.52%)	0.85 (0.61 - 1.19)	0.34
miR-196a2 rs11614913				
Codominant				
CC	61 (42.96%)	121 (42.01%)	Reference	-
CT	63 (44.37%)	128 (44.45%)	0.97 (0.63 - 1.50)	0.91
TT	18 (12.67%)	39 (13.54%)	0.92 (0.48 - 1.73)	0.79
Dominant				
CC	61 (42.96%)	121 (42.01%)	Reference	-
TT + CT	81 (57.04%)	167 (57.99%)	0.96 (0.64 - 1.44)	0.85
Recessive				
CC + CT	124 (87.33%)	249 (86.46%)	Reference	-
TT	18 (12.67%)	39 (13.54%)	0.92 (0.51 - 1.69)	0.80
Allelic				
C	185 (65.14%)	492 (64.24%)	Reference	-
T	99 (34.86%)	206 (35.76%)	0.96 (0.71 - 1.29)	0.79
miR-499a rs3746444				
Codominant				
AA	80 (56.34%)	173 (60.07%)	Reference	-
AG	58 (40.84%)	107 (37.15%)	1.17 (0.77 - 1.78)	0.45
GG	4 (2.82%)	8 (2.78%)	1.08 (0.32 - 3.70)	0.90
Dominant				
AA	80 (56.34%)	173 (60.07%)	Reference	-
GG + AG	62 (43.66%)	115 (39.93%)	1.17 (0 .78 – 1.75)	0.46
Recessive				
AA + AG	138 (97.18%)	280 (97.22%)	Reference	-
GG	4 (2.82%)	8 (2.78%)	1.01 (0.30 – 3.43)	0.98
Allelic				
A	218 (76.76%)	453 (78.65%)	Reference	-
G	66 (23.24%)	123 (21.35%)	1.15 (0.79 - 1.57)	0.53

[24, 34]. Several meta-analyses have pointed out that miR-146a rs2910164 was significantly associated with an increased risk in GC with somehow conflicting findings between Caucasians and Asians [35-39].

The functional mature miR-196a, generated by both miR-196a-1 and miR-196a-2, was found upregulated in GC tissues [40]. The miR-196A2 rs11614913 seems to affect the binding of mature mir-196a2-3p to its target mRNA, leading to an impaired mir-196a2 function, in cancer cells [41]. More positive reports on the association of the miR-196a rs11614913 and GC risk have been published in a Greek cohort [34] and Asian populations [42-44]. These findings are in contrast with others studies in Chinese populations [25, 31] and updated meta-analyses [37, 45, 46] where no correlations were found.

Table III. Association between miRNA SNPs and risk of cardia and non-cardia adenocarcinoma

Polymorphism	Non-cardia (n=111) (%) OR (95%CI); p value		Cardia (n=31) n (%) OR (95%CI); p value	
miR- 27a rs895819				
TT	50 (45.04%)	Reference	13 (41.94%)	Reference
TC	42 (37.84%)	0.98 (0.61-1.58); 0.93	14 (45.16%)	1.26 (0.57-2.77); 0.57
CC	19 (17.12%)	2.06 (1.05-4.04); 0.038	4 (12.90%)	1.67 (0.50-5.52); 0.42
CC + TC vs TT	61 (54.96%)	1.17 (0.75-1.82); 0.48	18 (58.06%)	1.33 (0.63-2.81); 0.46
CC vs TT+ TC	92 (82.88%)	2.08 (1.10-3.94); 0.027	27 (87.10%)	1.49 (0.49-4.60); 0.50
T	142 (63.96%)	Reference	40 (64.52%)	Reference
C	80 (36.04%)	1.31 (0.95-1.82); 0.11	22 (35.48%)	1.28 (0.74-2.22); 0.38
miR-146a rs2910164				
GG	67 (60.36%)	Reference	19 (61.29%)	Reference
GC	38 (34.23%)	0.83 (0.52-1.33); 0.44	10 (32.26%)	0.77 (0.35-1.73); 0.53
CC	6 (5.41%)	0.75 (0.29-1.97); 0.56	2 (6.45%)	0.89 (0.19-4.10); 0.88
CC + GC vs GG	44 (39.64%)	0.82 (0.53-1.28); 0.38	12 (38.71%)	0.79 (0.37-1.69); 0.54
CC vs GG + CG	105 (94.59%)	0.81 (0.31-2.08); 0.66	29 (93.55%)	0.98 (0.22-4.41); 0.98
G	172 (77.48%)	Reference	48 (77.42%)	Reference
C	50 (22.52%)	0.85 (0.59-1.22); 0.38	14 (22.58%)	0.85 (0.46-1.59); 0.61
miR-196a2 rs11614913				
CC	46 (41.44%)	Reference	15 (48.39%)	Reference
CT	49 (44.15%)	1.01 (0.63-1.62); 0.98	14 (45.16%)	0.88 (0.41-1.91); 0.75
TT	16 (14.41%)	1.08 (0.55-2.12); 0.83	2 (6.45%)	0.41 (0.09-1.89); 0.21
TT + CT vs CC	65 (58.56%)	1.02 (0.66-1.60); 0.92	16 (51.61%)	0.77 (0.37-1.62); 0.50
TT vs CC + CT	95 (85.59%)	1.08 (0.57-2.02); 0.82	29 (93.55%)	0.44 (0.10-1.92); 0.23
C	141 (63.51%)	Reference	44 (70.97%)	Reference
T	81 (36.49%)	1.03 (0.75-1.42); 0.85	18 (29.03%)	0.74 (0.41-1.31); 0.29
miR-499 rs3746444				
AA	65 (58.56%)	Reference	15 (48.39%)	Reference
AG	44 (39.64%)	1.09 (0.70-1.72); 0.70	14 (45.16%)	1.51 (0.70-3.25); 0.30
GG	2 (1.80%)	0.67 (0.14-3.22); 0.60	2 (6.45%)	2.88 (0.56-14.82); 0.25
GG + AG vs AA	46 (41.44%)	1.07 (0.68-1.66); 0.78	16 (51.61%)	1.61 (0.76-3.37); 0.21
GG vs AA + AG	109 (98.20%)	0.64 (0.13-3.07); 0.56	29 (93.55%)	2.14 (0.49-11.91); 0.32
A	174 (78.38%)	Reference	44 (70.97%)	Reference
G	48 (21.62%)	1.02 (0.70-1.48); 0.93	18 (29.03%)	1.51 (0.84-2.70); 0.18

The miR-499 gene was mapped to 20q11.22 and a T to C change in the miR-499 (A to G in the opposite strand) results in a mismatch in the stem region [14, 41]. A positive association for C allele and TC genotype with GC tumor size was found in a population of Asian origin [47]. Our findings are in line with other previous results, where no significant correlation was found between this SNP and GC risk [48-50], including two meta-analyses [46, 51].

A potential explanation for our results and controversial published findings on populations of different geographic origins could be the haplotype context. miRNA SNPs can function in a specific haplotype block and functional haplotypes differ across ethnicities. On the other hand, our data may reflect genetic heterogeneity in the GC pathogenesis.

This study has some limitations. First, the small sample size limited the subgroup analysis of rare genotypes. Second, the biases associated with subjects' selection and lack of information for other environmental factors to analyze gene-environment interactions. Thirdly, contribution of a genetic risk variant to disease development can be influenced by the genetic effect of other SNPs or genes that were not investigated in this research. To attenuate the small size of subgroups we used three genetic models in data analysis. Both medical records and interview questionnaire were used to collect more reliable clinical data.

CONCLUSION

Our findings suggest that the investigated miRNA SNPs have no effect on susceptibility to gastric cancer in our Romanian population. Larger well-designed studies on different ethnic groups and functional studies are warranted to clarify the role of these polymorphisms in gastric carcinogenesis.

Conflicts of interest: No conflict to declare.

Table IV. Association between miRNA SNPs and risk of intestinal and diffuse gastric adenocarcinoma

Polymorphism	Intestinal (n=79)n (%) OR (95%CI); p value		Diffuse (n=62) n (%) OR (95%CI); p value	
miR- 27a rs895819				
TT	31 (39.24%)	Reference	31 (50.00%)	Reference
TC	35 (44.30%)	1.32 (0.77-2.26); 0.32	21 (33.87%)	0.79 (0.43-1.44); 0.44
CC	13 (16.46%)	2.27 (1.05-4.92); 0.042	10 (16.13%)	1.75 (0.77-4.00); 0.20
CC + TC vs TT	48 (60.76%)	1.49 (0.89-2.47); 0.12	31 (50.00%)	0.96 (0.55-1.66); 0.88
CC vs TT+ TC	66 (83.54%)	1.99 (0.97-4.07); 0.07	52 (83.87%)	1.94 (0.88-4.26); 0.11
T	97 (61.39%)	Reference	83 (66.94%)	Reference
C	61 (38.61%)	1.47 (1.02-2.11); 0.043	41 (33.06%)	1.15 (0.76-1.74); 0.51
miR-146a rs2910164				
GG	47 (59.49%)	Reference	39 (62.90%)	Reference
GC	28 (35.45%)	0.87 (0.52-1.48); 0.62	19 (30.65%)	0.72 (0.39-1.30); 0.27
CC	4 (5.06%)	0.72 (0.23-2.21); 0.55	4 (6.45%)	0.86 (0.28-2.68); 0.80
CC + GC vs GG	32 (40.51%)	0.85 (0.51-1.41); 0.53	23 (37.10%)	0.74 (0.42-1.30); 0.29
CC vs GG + CG	75 (94.94%)	0.76 (0.25-2.29); 0.61	58 (93.55%)	0.98 (0.32-2.98); 0.97
G	122 (77.22%)	Reference	97 (78.23%)	Reference
C	36 (22.78%)	0.86 (0.57-1.31); 0.48	27 (21.77%)	0.81 (0.51-1.29); 0.38
miR-196a2 rs11614913				
CC	35 (44.30%)	Reference	25 (40.32%)	Reference
CT	34 (43.04%)	0.92 (0.54-1.57); 0.75	29 (46.78%)	1.01 (0.61-1.98); 0.76
TT	10 (12.66%)	0.89 (0.40-1.95); 0.76	8 (12.90%)	0.99 (0.41-2.38); 0.99
TT + CT vs CC	44 (55.70%)	0.91 (0.55-1.50); 0.72	37 (59.68%)	1.07 (0.61-1.88); 0.81
TT vs CC + CT	69 (87.34%)	0.93 (0.44-1.95); 0.84	54 (87.10%)	0.95 (0.42-2.14); 0.89
C	104 (65.82%)	Reference	79 (63.71%)	Reference
T	54 (34.18%)	0.93 (0.64-1.35); 0.71	45 (35.29%)	1.02 (0.68-1.53); 0.91
miR-499 rs3746444				
AA	49 (62.02%)	Reference	31 (50.00%)	Reference
AG	29 (36.71%)	0.96 (0.57-1.61); 0.87	29 (46.77%)	1.51 (0.86-2.65); 0.15
GG	1 (1.27%)	0.44 (0.05-3.62); 0.40	2 (3.23%)	1.40 (0.28-6.88); 0.69
GG + AG vs AA	30 (37.98%)	0.92 (0.55-1.54); 0.75	31 (50.00%)	1.50 (0.87-2.61); 0.15
GG vs AA + AG	78 (98.73%)	0.45 (0.06-3.64); 0.41	60 (96.77%)	1.17 (0.24-5.63); 0.85
A	127 (80.38%)	Reference	91 (73.39%)	Reference
G	31 (19.62%)	0.90 (0.58-1.40); 0.63	33 (26.61%)	1.34 (0.86-2.09); 0.21

Authors' contributions: I.R., F.B. and M.I. designed and supervised the study; C.C.V., M.C.G. and R.A.C. collected the data; M.G.C. and M.I. performed the laboratory work; I.R., F.B., C.C.V. analyzed the data; I.R. F.B. and R.A.C. wrote the manuscript All authors approved the final version of the manuscript.

REFERENCES

1. Ferlay J, Soerjomataram I, Dikshit R, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 2015; 136:E359-86. doi: 10.1002/ijc.29210
2. Colquhoun A, Arnold M, Ferlay J, Goodman KJ, Forman D, Soerjomataram I. Global patterns of cardia and non-cardia gastric cancer incidence in 2012. Gut 2015;64:1881-1888. doi:10.1136/gutjnl-2014-308915
3. GASTRIC (Global Advanced/Adjuvant Stomach Tumor Research International Collaboration) Group, Oba K, Paoletti X, et al. Role of chemotherapy for advanced/recurrent gastric cancer: an individual-patient-data meta-analysis. Eur J Cancer 2013;49:1565-1577. doi:10.1016/j.ejca.2012.12.016
4. Park JY, von Karsa L, Herrero R. Prevention strategies for gastric cancer: a global perspective. Clin Endosc 2014;47:478-489. doi:10.5946/ce.2014.47.6.478
5. Lauren P. The two histological main types of gastric carcinoma: diffuse and so-called intestinal-type carcinoma. An attempt at a histo-clinical classification. Acta Pathol Microbiol Scand 1965;64:31-49.
6. Karimi P, Islami F, Anandasabapathy S, Freedman ND, Kamangar F. Gastric cancer: descriptive epidemiology, risk factors, screening, and prevention. Cancer Epidemiol Biomarkers Prev 2014;23:700-713. doi:10.1158/1055-9965.epi-13-1057
7. Cheng XJ, Lin JC, Tu SP. Etiology and Prevention of Gastric Cancer. Gastrointest Tumors 2016;3:25-36. doi:10.1159/000443995

8. Bartel B. MicroRNAs directing siRNA biogenesis. Nat Struct Mol Biol 2005;12:569-571. doi:10.1038/nsmb0705-569
9. Garzon R, Calin GA, Croce CM. MicroRNAs in Cancer. Annu Rev Med 2009;60:167-179. doi:10.1146/annurev.med.59.053006.104707
10. Calin GA, Sevignani C, Dumitru CD, et al. Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. Proc Natl Acad Sci U S A 2004;101:2999-3004. doi:10.1073/pnas.0307323101
11. Zhang Z, Li Z, Li Y, Zang A. MicroRNA and signaling pathways in gastric cancer. Cancer Gene Ther 2014;21:305-316. doi:10.1038/cgt.2014.37
12. Tong F, Cao P, Yin Y, Xia S, Lai R, Liu S. MicroRNAs in gastric cancer: from benchtop to bedside. Dig Dis Sci 2014;59:24-30. doi:10.1007/s10620-013-2887-3
13. Link A, Kupcinskas J, Wex T, Malfertheiner P. Macro-role of microRNA in gastric cancer. Dig Dis 2012;30:255-267. doi:10.1159/000336919
14. Ryan BM, Robles AI, Harris CC. Genetic variation in microRNA networks: the implications for cancer research. Nat Rev Cancer 2010;10:389-402. doi:10.1038/nrc2867
15. Burada F, Dumitrescu T, Nicoli R, et al. IL-1RN +2018T>C polymorphism is correlated with colorectal cancer. Mol Biol Rep 2013;40:2851-2857. doi:10.1007/s11033-012-2300-x
16. Liu T, Tang H, Lang Y, Liu M, Li X. MicroRNA-27a functions as an oncogene in gastric adenocarcinoma by targeting prohibitin. Cancer Lett 2009;273:233-242. doi:10.1016/j.canlet.2008.08.003
17. Zhou L, Liang X, Zhang L, et al. MiR-27a-3p functions as an oncogene in gastric cancer by targeting BTG2. Oncotarget 2016;7:51943-1954. doi:10.18632/oncotarget.10460
18. Sun Q, Gu H, Zeng Y, et al. Hsa-mir-27a genetic variant contributes to gastric cancer susceptibility through affecting miR-27a and target gene expression. Cancer Sci 2010;101:2241-2247. doi:10.1111/j.1349-7006.2010.01667.x
19. Zhang N, Huo Q, Wang X, et al. A genetic variant in pre-miR-27a is associated with a reduced breast cancer risk in younger Chinese population. Gene 2013;529:125-130. doi:10.1016/j.gene.2013.07.041
20. Song B, Yan G, Hao H, Yang B. rs11671784 G/A and rs895819 A/G polymorphisms inversely affect gastric cancer susceptibility and miR-27a expression in a Chinese population. Med Sci Monit 2014;20:2318-2326. doi:10.12659/msm.892499
21. Song MY, Su HJ, Zhang L, et al. Genetic polymorphisms of miR-146a and miR-27a, H. pylori infection, and risk of gastric lesions in a Chinese population. PLoS One 2013;8:e61250. doi:10.1371/journal.pone.0061250
22. Arisawa T, Tahara T, Shibata T, et al. A polymorphism of microRNA 27a genome region is associated with the development of gastric mucosal atrophy in Japanese male subjects. Dig Dis Sci 2007;52:1691-1697. doi:10.1007/s10620-006-9648-5
23. Zhou Y, Du WD, Chen G, et al. Association analysis of genetic variants in microRNA networks and gastric cancer risk in a Chinese Han population. J Cancer Res Clin Oncol 2012;138:939-945. doi:10.1007/s00432-012-1164-8
24. Kupcinskas J, Wex T, Link A, et al. Gene polymorphisms of micrornas in Helicobacter pylori-induced high risk atrophic gastritis and gastric cancer. PLoS One 2014;9:e87467. doi:10.1371/journal.pone.0087467
25. Jiang J, Jia ZF, Cao DH, Wu YH, Sun ZW, Cao XY. Association of the miR-146a rs2910164 polymorphism with gastric cancer susceptibility and prognosis. Future Oncol 2016;12:2215-2226. doi:10.2217/fon-2016-0224
26. Chen M, Fang W, Wu X, et al. Distinct effects of rs895819 on risk of different cancers: an update meta-analysis. Oncotarget 2017. doi:10.18632/oncotarget.17454
27. Jazdzewski K, Murray EL, Franssila K, Jarzab B, Schoenberg DR, de la Chapelle A. Common SNP in pre-miR-146a decreases mature miR expression and predisposes to papillary thyroid carcinoma. Proc Natl Acad Sci U S A 2008;105:7269-7274. doi:10.1073/pnas.0802682105
28. Zeng Y, Sun QM, Liu NN, et al. Correlation between pre-miR-146a C/G polymorphism and gastric cancer risk in Chinese population. World J Gastroenterol 2010;16:3578-3583. doi:10.3748/wjg.v16.i28.3578
29. Ahn DH, Rah H, Choi YK, et al. Association of the miR-146aC>G, miR-149T>C, miR-196a2T>C, and miR-499A>G polymorphisms with gastric cancer risk and survival in the Korean population. Mol Carcinog 2013;52 Suppl 1:E39-E51. doi:10.1002/mc.21962
30. Zhou F, Zhu H, Luo D, et al. A functional polymorphism in Pre-miR-146a is associated with susceptibility to gastric cancer in a Chinese population. DNA Cell Biol 2012;31:1290-1295. doi:10.1089/dna.2011.1596
31. Ni Q, Ji A, Yin J, Wang X, Liu X. Effects of Two Common Polymorphisms rs2910164 in miR-146a and rs11614913 in miR-196a2 on Gastric Cancer Susceptibility. Gastroenterol Res Pract 2015;2015:764163. doi:10.1155/2015/764163
32. Soleimani A, Ghanadi K, Noormohammadi Z, Irani S. The correlation between miR-146a C/G polymorphism and UHRF1 gene expression level in gastric tumor. J Dig Dis 2016;17:169-174. doi:10.1111/1751-2980.12329
33. Xia ZG, Yin HF, Long Y, et al. Genetic variant of miR-146a rs2910164 C>G and gastric cancer susceptibility. Oncotarget 2016;7:34316-34321. doi:10.18632/oncotarget.8814
34. Dikeakos P, Theodoropoulos G, Rizos S, Tzanakis N, Zografos G, Gazouli M. Association of the miR-146aC>G, miR-149T>C, and miR-196a2T>C polymorphisms with gastric cancer risk and survival in the Greek population. Mol Biol Rep 2014;41:1075-1080. doi:10.1007/s11033-013-2953-0
35. Fu B, Song P, Lu M, Wang B, Zhao Q. The association between miR-146a gene rs2910164 polymorphism and gastric cancer risk: a meta-analysis. Biomed Pharmacother 2014;68:923-928. doi:10.1016/j.biopha.2014.10.002
36. Xie WQ, Tan SY, Wang XF. MiR-146a rs2910164 polymorphism increases risk of gastric cancer: a meta-analysis. World J Gastroenterol 2014;20:15440-15447. doi:10.3748/wjg.v20.i41.15440
37. Wei Y, Li L, Gao J. The association between two common polymorphisms (miR-146a rs2910164 and miR-196a2 rs11614913) and susceptibility to gastric cancer: A meta-analysis. Cancer Biomark 2015;15:235-248. doi:10.3233/cbm-150470
38. Chen ZF, Ma LL, Xue HB. Common polymorphisms of the microRNA genes (miR-146a and miR-196a-2) and gastric cancer risk: an updated meta-analysis. Genet Mol Res 2015;14:8589-8601. doi:10.4238/2015.July.31.7
39. Xie WQ,Wang XF. MiR-146a rs2910164 polymorphism increases the risk of digestive system cancer: A meta-analysis. Clin Res Hepatol Gastroenterol 2017;41:93-102. doi:10.1016/j.clinre.2016.06.007
40. Yao Y, Suo AL, Li ZF, et al. MicroRNA profiling of human gastric cancer. Mol Med Rep 2009;2:963-970. doi:10.3892/mmr_00000199
41. Hu Z, Chen J, Tian T, et al. Genetic variants of miRNA sequences and non-small cell lung cancer survival. J Clin Invest 2008;118:2600-2608. doi:10.1172/jci34934
42. Wang F, Sun GP, Zou YF, Fan LL, Song B. Quantitative assessment of the association between miR-196a2 rs11614913 polymorphism and gastrointestinal cancer risk. Mol Biol Rep 2013;40:109-116. doi:10.1007/s11033-012-2039-4

43. Li M, Li RJ, Bai H, et al. Association between the pre-miR-196a2 rs11614913 polymorphism and gastric cancer susceptibility in a Chinese population. Genet Mol Res 2016;15:gmr7516. doi:10.4238/gmr.15027516
44. Parlayan C, Ikeda S, Sato N, Sawabe M, Muramatsu M, Arai T. Association analysis of single nucleotide polymorphisms in miR-146a and miR-196a2 on the prevalence of cancer in elderly Japanese: a case-control study. Asian Pac J Cancer Prev 2014;15:2101-2107. doi:10.7314/APJCP.2014.15.5.2101
45. Zhang L, Gao J, Zhou D, Bao F. Lack of association of two common polymorphisms rs2910164 and rs11614913 with susceptibility to gastric cancer: A meta-analysis. Turk J Gastroenterol 2015;26:378-385. doi:10.5152/tjg.2015.6603
46. Xu Q, Liu JW,Yuan Y. Comprehensive assessment of the association between miRNA polymorphisms and gastric cancer risk. Mutat Res Rev Mutat Res 2015;763:148-160. doi:10.1016/j.mrrev.2014.09.004
47. Cai M, Zhang Y, Ma Y, et al. Association between microRNA-499 polymorphism and gastric cancer risk in Chinese population. Bull Cancer 2015;102:973-978. doi:10.1016/j.bulcan.2015.09.012
48. Okubo M, Tahara T, Shibata T, et al. Association between common genetic variants in pre-microRNAs and gastric cancer risk in Japanese population. Helicobacter 2010;15:524-531. doi:10.1111/j.1523-5378.2010.00806.x
49. Pu JY, Dong W, Zhang L, Liang WB, Yang Y, Lv ML. No association between single nucleotide polymorphisms in pre-mirnas and the risk of gastric cancer in Chinese population. Iran J Basic Med Sci 2014;17:128-133.
50. Wu XJ, Mi YY, Yang H, et al. Association of the hsa-mir-499 (rs3746444) polymorphisms with gastric cancer risk in the Chinese population. Onkologie 2013;36:573-576. doi:10.1159/000355518
51. Li L, Sheng Y, Lv L, Gao J. The association between two microRNA variants (miR-499, miR-149) and gastrointestinal cancer risk: a meta-analysis. PLoS One 2013;8:e81967. doi:10.1371/journal.pone.0081967