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Interaction between lncRNAs HOTAIR and MALAT1 tagSNPs in gastric cancer

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Gastric cancer is a multifactorial malignancy with several risk factors worldwide and over one million new cases in 2018 [1]. Long non-coding RNAs (lncRNAs) are non-coding transcripts of >200 nucleotides in length. The aberrant expression of lncRNAs may induce different cancers [2,3]. HOX transcript antisense RNA (HOTAIR) – a well-established oncogenic lncRNA – functions as a molecular scaffold whose overexpression causes invasiveness of cancer cell lines *in vitro* and triggers tumour growth and metastasis *in vivo* [4]. Metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) is involved in biological and cellular processes, such as retinal neurodegeneration, carcinogenesis, glycolysis, and vascular growth, thus it is a drug target in cancers. Various data show MALAT1 overexpression in gastric cancer, although the underlying mechanism is still unknown [5]. Single nucleotide polymorphisms (SNPs) are well recognized to regulate lncRNAs expression directly and change their corresponding function [6]. Although individual SNP effects have low prediction power, SNP–SNP interactions have a large effect on the mechanism of certain diseases [7]. We have shown that the HOTAIR rs17720428, rs7958904, and rs1899663 tagSNPs, but not rs4759314 are significantly linked to gastric cancer [8]. In this study, 200 new fully age- and sex-matched samples are genotyped for the HOTAIR SNPs and the data added to the previous set of 600 samples and then reanalysed. Moreover, in a case–control study (including 400 gastric cancer patients and 400 healthy matched controls), the novel MALAT1 rs619586 tagSNPs and their interactions with the HOTAIR rs17720428, rs7958904, rs1899663, and rs4759314 polymorphisms in gastric cancer susceptibility are assessed.

The patients were diagnosed with histologically confirmed gastric cancer and were recruited from October 2017 to March 2020. Individuals given routine physical examinations and having no self-reported history of cancer at any site were chosen as controls. Histologic subtypes were as previously described [8]. This study was approved by the Ethics Committee of the National Institute for Medical Research Development (NIMAD)/IR.NIMAD.REC.1396.097. The dbSNP database (http://www.ncbi.nlm.nih.gov/projects/SNP) was used to obtain the genetic polymorphism data of the entire sequence of lncRNAs. The sequences of the lncRNAs gene were downloaded employing the 1000 Genomes Browser (https://www.ncbi.nlm.nih.gov/variation/tools/1000genomes). The tagSNPs were chosen separately based on previously described criteria [8]. A tagSNP is a representative SNP in an area of the genome with a high LD (linkage disequilibrium), representing a group of SNPs called a haplotype. Thus, tagSNPs represent all of the SNPs within a haplotype and can be selected in each block in order to map the genetic variation responsible for complex diseases. The Infinium HTS platform was applied for genotyping samples as previously described [8]. Using χ2, genotyping outcomes of SNPs were examined for a significant departure from Hardy–Weinberg equilibrium. The selected exposure variables were assessed applying χ^2 to differences in basic characteristics' distribution among the patients and controls. The odds ratio and confidence intervals (CI) were employed to study the association between the HOTAIR and MALAT1 SNPs and gastric cancer. Allelic, dominant, codominant, over dominant, recessive, and log additive models for these tagSNPs were examined. In a linear regression model, the relationship between the dependent and the independent variable is linear on the logit scale. As a result, in the analysis of the association between a given SNP (codes 0, 1, 2) and a studied trait, the genetic effect where the trait is dichotomous will be log-additive. Haplotype frequencies were calculated using the SNPStats based on the expectation maximization algorithm (https://www.snpstats.net/start.htm). The lncRNA SNP-SNP pairwise interactions were then calculated by SPSS version 19.0 (IBM, Chicago, USA). Statistical significance

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Table 1. Genotype and allele frequencies of HOTAIR/MALAT1 SNPs in 800 cases and controls.

Gene/locus/model	Genotype	Controls	Patients	Odds ratio (95% CI)	*p*-value
HOTAIR					
rs17720428					
Allele	T	530 (66.3%)	475 (59.4%)	1.00	0.004
	G	270 (33.7%)	325 (40.6%)	1.34 (1.1–1.64)	
Codominant	TT	176 (44%)	137 (34.2%)	1.00	0.01
	TG	178 (44.5%)	201 (50.2%)	1.45 (1.07–1.96)	
	GG	46 (11.5%)	62 (15.5%)	1.73 (1.11–2. 69)	
Dominant	TT	176 (44%)	137 (34.2%)	1.00	0.005
	TG+GG	224 (56%)	263 (65.8%)	1. 51 (1.13–2.01)	
Recessive	TT+TG	345 (88.5%)	338 (84.5%)	1.00	0.01
	GG	46 (11.5%)	62 (15.5%)	1.41 (0.94–2.13)	
Over-dominant	TT+GG	222 (55.5%)	199 (56.3%)	1.00	0.1
	TG	178 (44.5%)	201 (50.2%)	1.26 (0.95–1.66)	
Log-Additive				1.35 (1.1–1.66)	0.004
rs7958904					
Allele	G	475 (59.5%)	419 (52.5%)	1.00	0.005
	C	323 (40.5%)	379 (47.5%)	1.33 (1.09–1.62)	
Codominant	GG	143 (35.8%)	107 (26.8%)	1.00	0.01
	GC	189 (47.4%)	205 (51.4%)	1.45 (1.05–1.99)	
	CC	67 (16.8%)	87 (21.8%)	1.74 (1.16–2.6)	
Dominant	GG	143 (35.8%)	107 (26.8%)	1.00	0.006
	GC+CC	256 (64.2%)	292 (73.2%)	1.52 (1.13–2.06)	
Recessive	GG+GC	332 (83.2%)	312 (78.2%)	1.00	0.07
	CC	67 (16.8%)	87 (21.8%)	1.38 (0.97–1.97)	
Over-dominant	GG+CC	210 (52.6%)	194 (48.6%)	1.00	0.26
	GC	189 (47.4%)	205 (51.4%)	1.17 (0.89–1.55)	
Log-Additive				1.33 (1.09–1.63)	0.005
rs1899663					
Allele	G	530 (66.3%)	474 (59.4%)	1.00	0.005
	T	270 (33.7%)	324 (40.6%)	1.34 (1.09–1.64)	
Codominant	GG	176 (44%)	137 (34.3%)	1.00	0.01
	GT	178 (44.5%)	200 (50.2%)	1.44 (1.07–1.95)	
	TT	46 (11.5%)	62 (15.5%)	1.73 (1.11–2. 69)	
Dominant	GG	176 (44%)	137 (34.2%)	1.00	0.005
	GT+TT	224 (56%)	262 (65.8%)	1. 5 (1.13–2.00)	
Recessive	GG+GT	345 (88.5%)	337 (84.5%)	1.00	0.09
	TT	46 (11.5%)	62 (15.5%)	1.42 (0.94–2.13)	
Over-dominant	GG+TT	222 (55.5%)	199 (49.9%)	1.00	0.11
	GT	178 (44.5%)	200 (50.1%)	1.26 (0.95–1.66)	
Log-Additive				1.35 (1.1–1.66)	0.04
MALATI					
rs619586					
Allele	A	755 (94.4%)	747 (93.6%)	1	0.52
	G	45 (5.6%)	51 (6.4%)	1.14 (0.76–1.73)	
Over-dominant	AA+GG	361 (90.2%)	352 (88.2%)	1	0.35
	AG	39 (9.8%)	47 (11.8%)	1.24 (0.79–1.94)	
Log-Additive				1.14 (0.76–1.71)	0.53

*Only analyses where the sum of subjects is 80 or more are shown.

at $p < 0.05$ was used. The potential impact of each SNP on the creation or destruction of the miRNA binding site was analysed by using the lncRNASNP2 database (http://bioinfo.life.hust.edu.cn/lncRNASNP2).

Each gastric cancer and control group consisted of 400 subjects, of whom 75.5% were males. The average age (mean ± SD) was 67.1 ± 9.9 and 67.0 ± 9.34 years, respectively ($p = 0.39$). There were 302 men with cancer and 302 men in the control group ($p = 1.00$). The anatomical site of the tumour was 51.4% in the cardia gastric cancer, 45.1% in the non-cardia gastric cancer, and 3.5% in both. The prevalence of the intestinal-, the diffuse-, and the indeterminate-types was 53%, 21%, and 26%, respectively. All frequencies followed Hardy–Weinberg equilibrium.

Results showed that HOTAIR rs17720428 was associated with gastric cancer in allelic, codominant, dominant, recessive, and log-additive models. The G allele of rs17720428 significantly linked with gastric cancer relative to the T allele carriers. The TG+GG genotype was significantly linked with gastric cancer relative to the TT genotypes under the codominant models. In the dominant model, individuals carrying the TG+GG genotype of rs17720428 had a significantly higher link with gastric cancer in comparison with the TT genotype. Under the recessive model, the GG genotype of rs17720428 compared to TT+TG, significantly increased the risk of gastric cancer (Table 1). The HOTAIR rs7958904 tagSNPs were associated with gastric cancer in allelic, codominant, dominant, and log-additive models. The C allele of rs7958904 was significantly linked with gastric cancer

Table 2. SNP-SNP interaction models for lncRNA polymorphisms (N = 800).

SNP–SNP interaction	SNP genotype		Patients/controls		Odd's ratio (95% CI)	P value
rs17720428 and rs619586	**HOTAIR**	**MALAT1**				
	TT	AA	123	162	1.00	
	TG	AA	179	167	1.41 (1.03–1.93)	**0.03**
	GG	AA	59	43	1.81 (1.14–2.86)	**0.01**
	TG	AG	20	8	3.29 (1.4–7.25)	**0.004**
rs7958904 and rs619586	**HOTAIR**	**MALAT1**				
	GG	AA	94	132	1.00	
	GC	AA	184	175	1.48 (1.05–2.07)	**0.02**
	CC	AA	82	64	1.79 (1.82–2.74)	**0.006**
	GC	AG	19	11	2.42 (1.1–5.33)	**0.02**
rs1899663 and rs619586	**HOTAIR**	**MALAT1**				
	GG	AA	75	162	1.00	
	GT	AA	178	162	2.3 (1.63–3.25)	**< 0.001**
	TT	AA	108	43	5.42 (3.47–8.48)	**< 0.001**
	GT	AG	20	8	5.4 (2.27–12.82)	**< 0.001**
	TT	AG	12	3	8.64 (2.37–31.52)	**< 0.001**

Only analyses where the sum of subjects is 28 or more are shown.

compared with the G allele. In the codominant model, carrying the CC or GC genotype of rs7958904 had a significantly higher link with gastric cancer in comparison with the GG genotype. The genotype GC+CC of rs7958904 had a significantly higher link with gastric cancer relative to the GG genotype under the dominant model. The rs1899663 was associated with gastric cancer in allelic, codominant, dominant, and log-additive models. The T allele of rs1899663 was significantly associated with an increased link with gastric cancer compared with the G allele. In the codominant model, carrying the TT and GT genotypes were significantly linked with gastric cancer relative to GG. The GT+TT genotype of rs1899663 had a significantly higher link with gastric cancer relative to the GG genotype in the dominant model (Table 1). Haplotype analysis showed that the GCTA haplotype of HOTAIR rs17720428, rs7958904, rs1899663 and rs4759314 was significantly associated with gastric cancer (OR = 1.37; $p = 0.0036$). No significant association was found between the MALAT1 tagSNPs and gastric cancer under any model (Table 1).

Table 2 shows interaction between tagSNPs on gastric cancer. The SNP-SNP interactions of HOTAIR rs17720428 and MALAT1 rs619586 were associated with gastric cancer (rs17720428 TG-rs619586 AA; rs17720428 GG-rs619586 AA; and rs17720428 TG-rs619586 AG). The interactions of HOTAIR rs7958904 and MALAT1 rs619586 significantly increased the link with gastric cancer (rs7958904 GC-rs619586 AA; rs7958904 CC-rs619586 AA; and rs7958904 GC-rs619586 AG). Moreover, the interaction of HOTAIR rs1899663 and MALAT1 rs619586 tagSNPs strongly increased the link with gastric cancer (rs1899663 GT-rs619586 AA; rs1899663 TT-rs619586 AA; rs1899663 GT-rs619586 AG, and rs1899663 TT-rs619586 AG; Table 2). Bioinformatic analysis showed that the MALAT1 rs619586 tagSNPs might cause miR-3619-5p, miR-761, and miR-214-3p target gain and miR-4645-3p and miR-331-5p target loss.

As compared with our previous study [8], the HOTAIR tagSNPs-associated risk estimates of gastric cancer did not change inherently, which shows the high credibility of the results. Increasing the sample size, however, reduced the *p*-value substantially. The SNP–SNP interactions of HOTAIR and MALAT1 significantly increased the link with gastric cancer. Interactions of HOTAIR rs17720428 and rs7958904 with MALAT1 rs619586 tagSNPs were significantly linked with gastric cancer. The interactions of HOTAIR rs1899663 with MALAT1 rs619586 tagSNPs also strongly increased the link with gastric cancer. MALAT1 rs619586 SNP was indicated as a notable protective factor in papillary thyroid cancer (PTC). Functional experiments showed that the rs619586 G allele significantly reduces MALAT1 expression, decreases proliferation, and increases the apoptosis of PTC [9]. MALAT1 rs664589 G allele shows altered binding to miR-194-5p in the nucleus, increasing the MALAT1 expression and enhancing colorectal cancer development [10]. Wei-Jing et al. found no association between the rs618586 SNP and lung cancer. In contrast, it was associated with platinum-based chemotherapy response of lung cancers [11]. MALAT1 rs619586 G allele functions as a competing endogenous RNA in pulmonary arterial hypertension, exhibiting the higher affinity towards miR-214, hence inhibiting the vascular endothelial cell proliferation and migration *in vitro* [12].

Using bioinformatic analysis, we showed that MiR-331-5p and miR-4645-3p have lost their potential binding sites for MALAT1 due to the rs619586 SNP. Little information was found about the functional role of miR-4645-3p and miR-

331-5p. MiR-3619-5p, miR-761, and MiR-214-3p have acquired a possible binding site for MALAT1 due to the rs619586 SNP. MiR-3619-5p and miR-761 play the role of gastric cancer tumour suppressors [13,14] and their binding to MALAT1 may cause the destruction of these molecules; however, the functional studies are warranted to establish it. In contrast, miR-214-3p has been regarded as an oncogene to enhance tumorigenesis and gastric cancer progression [15]. As previously described, no miRNA target gain or loss was recognized for the HOTAIR rs1899663 tagSNPs [8]. The SNP-SNP interaction of HOTAIR rs1899663 with MALAT1 rs619586 was strongly associated with gastric cancer. Similar results were also found for the SNP-SNP interaction of HOTAIR rs1899663 with HOTTIP rs1859168 in our previous study [8]. This may emphasize the significant role of the HOTAIR rs1899663 tagSNPs in increasing the risk of gastric cancer. However, their functional role should be clarified in the future by controlling the presence of the MALAT1 rs619586 and HOTTIP rs1859168 SNPs.

In sum, we introduce novel SNP-SNP interactions between the oncogenic lncRNAs HOTAIR and MALAT1 tagSNPs associated with an increased susceptibility to gastric cancer. The combined impact of these variants on the risk of gastric cancer was often larger than each SNP alone. In-depth functional experiments are recommended to validate all the assumptions with respect to the mechanism involved. These interactions may be used as potential biomarkers to predict an individual's risk of gastric cancer and to trace the inheritance of disease genes within families. However, the large studies from different ethnic populations are required to validate the contributions of these lncRNA SNPs to gastric cancer risk assessment. Our data represent an advance in biomedical science as it shows that combinations of SNPs in two common ncRNAs have enhanced links to gastric cancer, a view that supports their use as diagnostic markers.

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