Letter to the Editor

The rs2227481 C>T Polymorphism in the *IL22* Gene Promoter Significantly Reduces the Risk of Liver, Lung, and Gastric Cancer in a Han Chinese Population*

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Cancer is a serious threat to public health and the economy worldwide. Statistical data from the World Health Organization (WHO) in 2018 have demonstrated that lung cancer is the most commonly diagnosed cancer (11.6% of the total cases) and the leading cause of cancer death (18.4% of the total cancer deaths), closely followed by gastric cancer (5.7% and 8.2%), and liver cancer (4.7% and 8.2%)[1]. The situation is similar for these three types of cancer in the Hubei province of China[2]. Interleukin-22 (IL22), a member of the IL-10 family, is regarded as a link between inflammation and cancer. Recent studies have shown that IL22 acts as a tumor promoter and may promote carcinogenesis rather than function in antitumor immunity[3]. Interestingly, evidence of IL22 involvement in carcinogenesis manifests as dysregulation of IL22 expression in patients with many common cancers, including those of the stomach, liver, lung, stoma gut, and skin[4]. Therefore, it is hypothesized that individuals carrying certain genetic variants in the *IL22* gene are vulnerable to the challenge of dysimmunity and are more susceptible to cancer. To test this hypothesis, we adopted a two-stage case-control approach to explore the potential contribution of *IL22* gene promoter polymorphisms to cancer susceptibility in a Chinese Han population of Hubei province, and further elucidated the specific mechanism underlying the contribution of associated polymorphisms to cancer susceptibility.

A total of 1,490 cancer patients (liver/lung/gastric cancer: 480/550/460) and 800 healthy controls were enrolled in this study. All cancer patients were confirmed histopathologically and recruited from Hubei Cancer Hospital and Wuhan Xinzhou District People's Hospital. Normal controls were selected from cancer-free individuals who visited Wuhan Xinzhou District People's Hospital for regular physical examinations. All participants were biologically unrelated Han Chinese living in the Hubei province of China, and signed informed consent was obtained from all participants. Ethics approval for this study was granted by the ethics committee of Wuhan University of Technology.

To focus on single nucleotide polymorphisms (SNPs) in the *IL22* gene promoter region, we first retrieved the SNP list within 2,000 bp upstream of the *IL22* gene in the NCBI dbSNP website (https://www.ncbi.nlm.nih.gov/snp). The potentially functional SNPs were screened out from the list using SNPinfo software (https://snpinfo.niehs.nih.gov/). Then, the SNPs in the second step, which were further confirmed using Alibaba2 software (http://gene-regulation.com/pub/programs/alibaba2/index.html?), were selected for genotyping in our experimental cohort using Sanger sequencing (Supplementary Table S1, available in www.besjournal.com). The sequencing diagrams of the selected SNPs are shown in Supplementary Figure S1 (available in www.besjournal.com).

After identifying an association between the rs2227481 polymorphism and cancer risk, we performed a functional study to elucidate the

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molecular mechanisms underlying the contribution of rs2227481 to cancer susceptibility. A dual luciferase assay was conducted to test the effect of rs2227481 on *IL22* transcriptional activity. Quantitative real-time RT-PCR was performed to examine the effects of the rs2227473 genotype on IL22 mRNA expression. Surface plasma resonance (SPR) analysis and chromatin immunoprecipitation (ChIP) assays were conducted to test the POU2F3 (Oct-11) binding capacity of the rs2227481 alleles. These assays have been described in our previous study[5], and the primers used are presented in Supplementary Table S2, available in www.besjournal.com.

All statistical analyses were performed using SPSS software (version 15.0; SPSS Inc., Chicago, IL). Differences in age, sex, smoking status, and alcohol status between cancer patients and normal controls were compared using a two-sided χ^2 test. Other differences were evaluated using the Student's *t*-test. Data are expressed as mean ± standard deviation (SD) from at least three independent experiments. The association between *IL22* gene polymorphisms and cancer risk was estimated using logistic regression analysis. The level of significance was set at $P < 0.05$, and Bonferroni correction was applied for multiple comparisons ($P < 0.008$, 0.05/6).

The characteristics of the participants are shown in Table 1. Regular smoking was defined as smoking at least one cigarette per day on average over one year or having quit smoking for less than one year. Regular drinking was defined as drinking at least 100 mL of alcohol per day on average over one year or having quit drinking for less than one year. The distributions of age, sex, smoking status, and drinking status were not significantly different between cancer patients and normal controls ($P > 0.05$). These results suggest that cancer patients and normal controls could be used for comparison for study purposes.

In stage I, the 13 selected *IL-22* gene promoter polymorphisms were genotyped in 300 cancer patients and 150 normal controls. Of the 13 SNPs, rs2227487, rs2227486, rs2227482, and rs2227474 did not show polymorphism. Among the remaining nine SNPs, only rs2227481 was significantly associated with the risk of liver, lung, and gastric cancer (Table 2). Specifically, the T allele was associated with a decreased risk of liver, lung, and gastric cancer susceptibility at *P*-values of 0.007, 0.001, and 0.003, respectively, with *OR* (95% *CI*) of 0.55 (0.35–0.85), 0.46 (0.29–0.72), and 0.51 (0.33–0.80), respectively, and TT+CT genotypes were associated with a lower risk of lung cancer than the CC genotype ($P = 0.003$, $OR = 0.44$, 95% $CI = 0.26$–0.75). Since there was no significant association between the other eight SNPs (rs2227485, rs2227484, rs2227483, rs2227511, rs2227479, rs2227478, rs2227473, and rs2227472) and cancer risk, they were eliminated in the stage II study (Supplementary Table S3, available in www.besjournal.com).

For that rs2227483, rs2227482, rs2227481, rs2227511, rs2227479, and rs2227478 were simultaneously genotyped, we obtained the genotype data of these six SNPs in stage II (1,190 cancer patients and 650 normal controls). Of note, rs2227481, but not other SNPs, was still significantly associated with the risk of liver, lung, and gastric cancer in stage II as well as in stage I+II (Supplementary Table S4, available in www.besjournal.com). As shown in Table 2, the rs2227481

Table 1. Characteristics of participants in the present study

Group	Age, *n* (%)		Gender, *n* (%)		Smoking status, *n* (%)		Drinking status, *n* (%)		*P*-value[1]			
	≤ 60 years	> 60 years	Male	Female	Ever	Never	Ever	Never				
Liver cancer patients (*n* = 480)	280 (58.3)	200 (41.7)	343 (71.5)	137 (28.5)	140 (29.2)	340 (70.8)	158 (32.9)	322 (67.1)	0.154	0.517	0.237	0.217
Lung cancer patients (*n* = 550)	306 (55.6)	244 (44.4)	373 (67.9)	177 (32.1)	150 (27.3)	400 (72.7)	170 (31.0)	380 (69.0)	0.615	0.451	0.639	0.613
Gastric cancer patients (*n* = 460)	252 (54.8)	208 (45.2)	323 (70.3)	137 (29.7)	132 (28.8)	328 (71.2)	148 (32.1)	312 (67.9)	0.855	0.862	0.323	0.344
Normal controls (*n* = 800)	434 (54.3)	366 (45.7)	558 (69.7)	242 (30.3)	209 (26.1)	591 (73.9)	237 (29.6)	563 (70.4)				

Note. [1]Two-sided χ^2 test for the distributions of age (1st column), gender (2nd column), smoking status (3rd column), and drinking status (4th column) between liver/lung/gastric cancer patients and normal controls.

T allele conferred a reduced risk of liver, lung, and gastric cancer at *P*-values of 0.005, 0.006, and 0.004, respectively, with *OR* (95% *CI*) of 0.74 (0.60–0.92), 0.76 (0.62–0.92), and 0.73 (0.59–0.90), respectively, and CT+TT genotypes were associated with a lower risk of gastric cancer than the CC genotype (*P* = 0.004, *OR* = 0.68, 95% *CI* = 0.53–0.89). The pooled data (I+II) revealed an even more significant association between the rs2227481 T allele and lower risk of liver, lung, and gastric cancer, with *P*-values less than 0.001. Moreover, the rs2227481 T allele genotypes were significantly associated with a lower susceptibility to liver cancer (TT *vs*. CC, TT *vs*. CC+CT, and TT+CT *vs*. CC), lung cancer (TT *vs*. CC, TT *vs*. CC+CT, and TT+CT *vs*. CC), and gastric cancer (TT *vs*. CC, CT *vs*. CC, and TT+CT *vs*. CC).

Our two-stage case-control study consistently showed that the rs2227481 C>T polymorphism was significantly associated with a reduced risk of liver, lung, and gastric cancer. The interesting question is why rs2227481 affects individual susceptibility to cancer. In this study, we examined the IL22 mRNA levels in clinical liver, lung, and gastric cancer tissue samples with different rs2227481 genotypes and found that IL22 was upregulated in CC samples compared to combined CT and TT samples (Figure 1A–C). These results suggest that maintenance of an inadequate amount of IL22 mRNA may promote the development of liver, lung, and gastric cancers. Evidence from *in vitro* studies and xenograft models strongly supports our hypothesis. Tumor-infiltrating cells of hepatocellular carcinoma (HCC) are enriched in $IL22^+$ cells, and IL22 expression is positively correlated with the progression and staging of HCC[6]. Similarly, IL22 was found to be elevated in lung cancer tissues compared to matched peritumoral tissues, and overexpression of IL22 protected lung cancer cell lines from serum starvation-induced and chemotherapeutic drug-induced apoptosis[7]. Of note, IL22 is a more unambiguously pro-tumor cytokine in gastric cancer. Gastric cancer patients

Table 2. The association between *IL22* gene rs2227481 polymorphism and risk of liver, lung, and gastric cancer

Groups	*n*	Allele and genotype (frequency, %)					Logistic regression analysis [*P*, *OR* (95% *CI*)][1]					
		T	C	TT	CT	CC	T *vs*. C	TT *vs*. CC	TT *vs*. CT	CT *vs*. CC	TT+CT *vs*. CC	TT *vs*. CT+CC
Stage I												
Liver cancer	100	36 (18.0)	164 (82.0)	1 (1.0)	34 (34.0)	65 (65.0)	0.007, 0.55 (0.35–0.85)	0.027, 0.10 (0.01–0.77)	0.076, 0.15 (0.02–1.22)	0.103, 0.64 (0.38–1.09)	0.026, 0.55 (0.33–0.93)	0.040, 0.12 (0.02–0.91)
Lung cancer	100	31 (15.5)	169 (84.5)	1 (1.0)	29 (29.0)	70 (70.0)	0.001, 0.46 (0.29–0.72)	0.023, 0.09 (0.01–0.71)	0.105, 0.18 (0.02–1.44)	0.015, 0.51 (0.29–0.88)	0.003, 0.44 (0.26–0.75)	0.040, 0.12 (0.02–0.91)
Gastric cancer	100	34 (17.0)	166 (83.0)	1 (1.0)	32 (32.0)	67 (67.0)	0.003, 0.51 (0.33–0.80)	0.025, 0.10 (0.01–0.75)	0.086, 0.16 (0.02–1.30)	0.051, 0.59 (0.34–1.00)	0.011, 0.51 (0.30–0.86)	0.040, 0.12 (0.02–0.91)
Normal controls	150	86 (28.7)	214 (71.3)	12 (8.0)	62 (41.3)	76 (50.7)						
Stage II												
Liver cancer	380	166 (21.8)	594 (78.2)	17 (4.5)	132 (34.7)	231 (60.8)	0.005, 0.74 (0.60–0.92)	0.029, 0.52 (0.29–0.93)	0.233, 0.70 (0.39–1.26)	0.037, 0.75 (0.58–0.98)	0.136, 1.20 (0.95–1.52)	0.066, 0.59 (0.33–1.04)
Lung cancer	450	200 (22.2)	700 (77.8)	18 (4.0)	164 (36.4)	268 (59.6)	0.006, 0.76 (0.62–0.92)	0.011, 0.48 (0.27–0.84)	0.077, 0.60 (0.33–1.06)	0.092, 0.81 (0.63–1.04)	0.023, 0.75 (0.59–0.96)	0.022, 0.52 (0.30–0.91)
Gastric cancer	360	155 (21.5)	565 (78.5)	18 (5.0)	119 (33.1)	223 (61.9)	0.004, 0.73 (0.59–0.90)	0.056, 0.58 (0.33–1.01)	0.503, 0.82 (0.46–1.47)	0.012, 0.70 (0.53–0.92)	0.004, 0.68 (0.53–0.89)	0.144, 0.66 (0.38–1.15)
Normal controls	650	356 (27.4)	944 (72.6)	48 (7.4)	260 (40.0)	342 (52.6)						
Stage I+II (pooled data)												
Liver cancer	480	202 (21.0)	758 (79.0)	18 (3.8)	166 (34.5)	296 (61.7)	< 0.001, 0.70 (0.58–0.84)	0.002, 0.42 (0.25–0.73)	0.058, 0.58 (0.33–1.02)	0.009, 0.73 (0.57–0.93)	0.001, 0.68 (0.54–0.86)	0.008, 0.48 (0.28–0.82)
Lung cancer	550	231 (21.0)	869 (79.0)	19 (3.5)	193 (35.0)	338 (61.5)	< 0.001, 0.70 (0.58–0.84)	0.001, 0.39 (0.23–0.67)	0.022, 0.53 (0.31–0.91)	0.010, 0.74 (0.59–0.93)	0.001, 0.69 (0.55–0.86)	0.002, 0.44 (0.26–0.75)
Gastric cancer	460	189 (20.5)	731 (79.5)	19 (4.1)	151 (32.8)	290 (63.1)	< 0.001, 0.68 (0.56–0.82)	0.004, 0.46 (0.27–0.78)	0.163. 0.68 (0.39–1.17)	0.002, 0.68 (0.53–0.86)	0.000, 0.64 (0.51–0.81)	0.019, 0.53 (0.31–0.90)
Normal controls	800	442 (27.6)	1158 (72.4)	60 (7.5)	322 (40.3)	418 (52.2)						

Note. [1]The *OR* (95% *CI*) and the corresponding *P*-value were calculated using logistic regression analysis, and adjusted for age, gender, smoking, and drinking status.

had higher circulating frequencies of IL22-producing T cells compared to healthy controls, which positively correlated with tumor stage and negatively correlated with patient survival[8].

Next, we used the Alibaba2 software to predict that the rs2227481 C>T polymorphism creates a transcription factor binding site for POU2F3 (Oct-11) (Figure 1D). POU2F3, a member of the POU domain family, acts to both stimulate and repress transcription in a general and cell type-specific mode[9]. Interestingly, the SPR analysis revealed that, compared with the C allele oligonucleotide probe, the T allele oligonucleotide probe had a higher binding affinity to HEK293 nuclear proteins or purified recombinant POU2F3 protein (Figure 1E). Moreover, the ChIP assay results demonstrated that

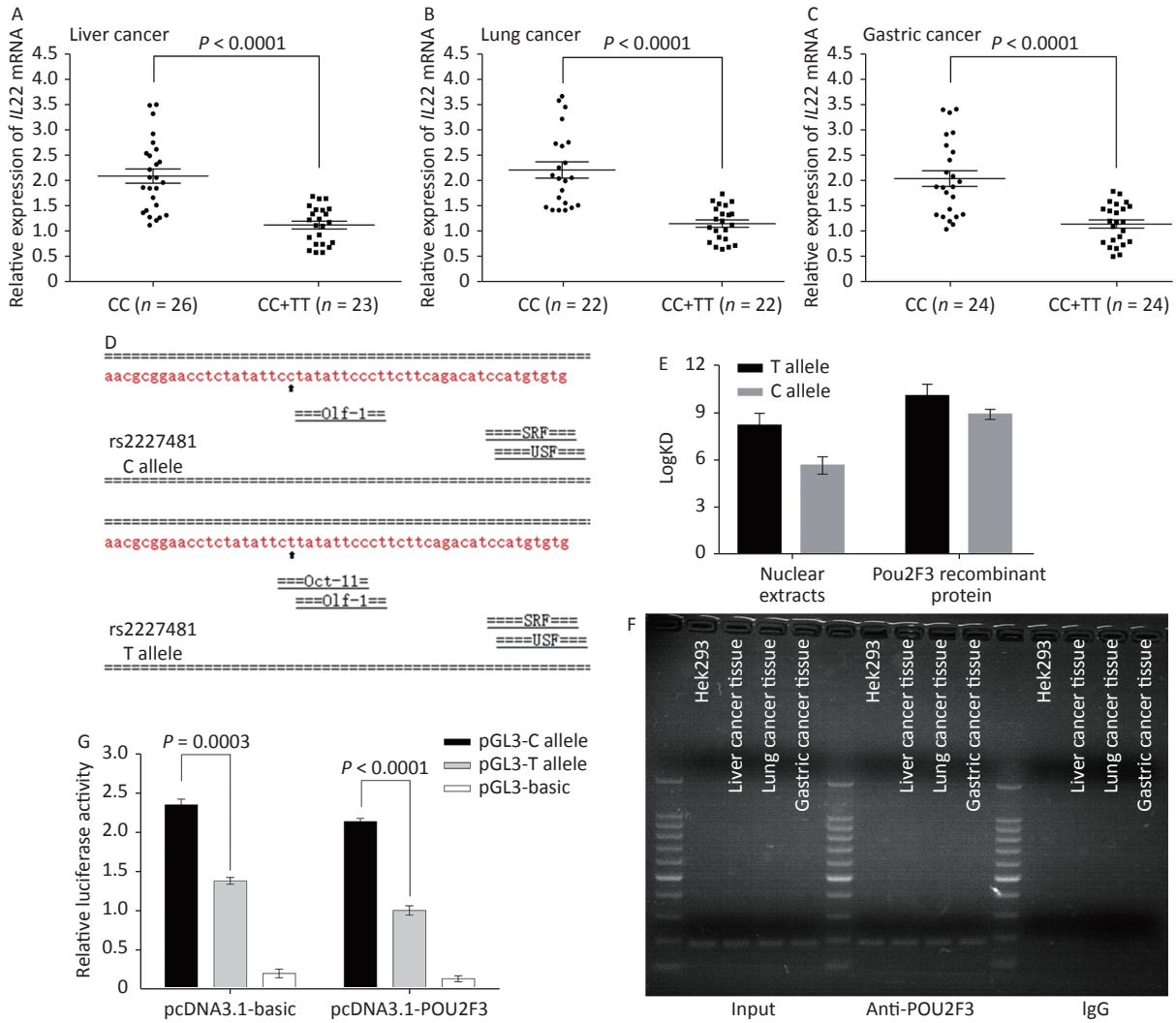

Figure 1. The rs2227481 C>T polymorphism decreases *IL22* gene expression by increasing the binding affinity of the transcription repressor POU2F3. Quantitative real-time RT-PCR analysis of *in vivo* IL22 mRNA levels in 49 liver cancer tissue samples (A), 44 lung cancer tissue samples (B), and 48 gastric cancer tissue samples (C) with different genotypes. (D) Bioinformatics analysis predicted transcription factors for the rs2227481 C>T polymorphism. (E) SPR analysis comparing the binding affinity of HEK293 nuclear extracts or POU2F3 recombinant protein to DNA probes containing either the rs2227481 T or C alleles. (F) ChIP assays using HEK293 cells and one liver cancer tissue sample. The presence of POU2F3 binding to *IL22* gene promoter was verified by PCR. (G) A luciferase construct containing either the C or T allele of rs2227481 was co-transfected with pcDNA3.1-basic vector or pcDNA3.1-POU2F3 expression vector in HEK293 cells.

the IL22 promoter fragment with the rs2227481 site was occupied by POU2F3 (Figure 1F). The co-transfection experiment showed that ectopic POU2F3 expression generally decreased the luciferase activities of the plasmids containing the rs2227481 C allele or T allele, and the rs2227481 polymorphism amplified the promoter function disparity (Figure 1G). Taken together, our results demonstrate that POU2F3 acts as a transcription repressor of the *IL22* gene, and compared with the C allele, the rs2227481 T allele increases the binding affinity of POU2F3 to the *IL22* gene promoter, which finally contributes to the decreased IL22 expression level, thereby reducing cancer susceptibility.

The present study is the first to demonstrate a significant association between the *IL22* rs2227481 C>T polymorphism and a reduced risk of liver, lung, and gastric cancer in a Han Chinese population. Meanwhile, it was also revealed that the *IL22* gene rs2227481 C>T polymorphism decreases IL22 expression by increasing the binding affinity of the transcriptional suppressor POU2F3. Our findings emphasize and reinforce the role of *IL22* in the carcinogenesis of liver, lung, and gastric cancer, and the results reported here may initiate a novel strategy for the prediction and prevention of liver, lung, and gastric cancer. However, further confirmatory studies with cohort expansion are needed in other ethnic groups and Chinese populations from other regions.

The authors declare that there are no conflicts of interest.

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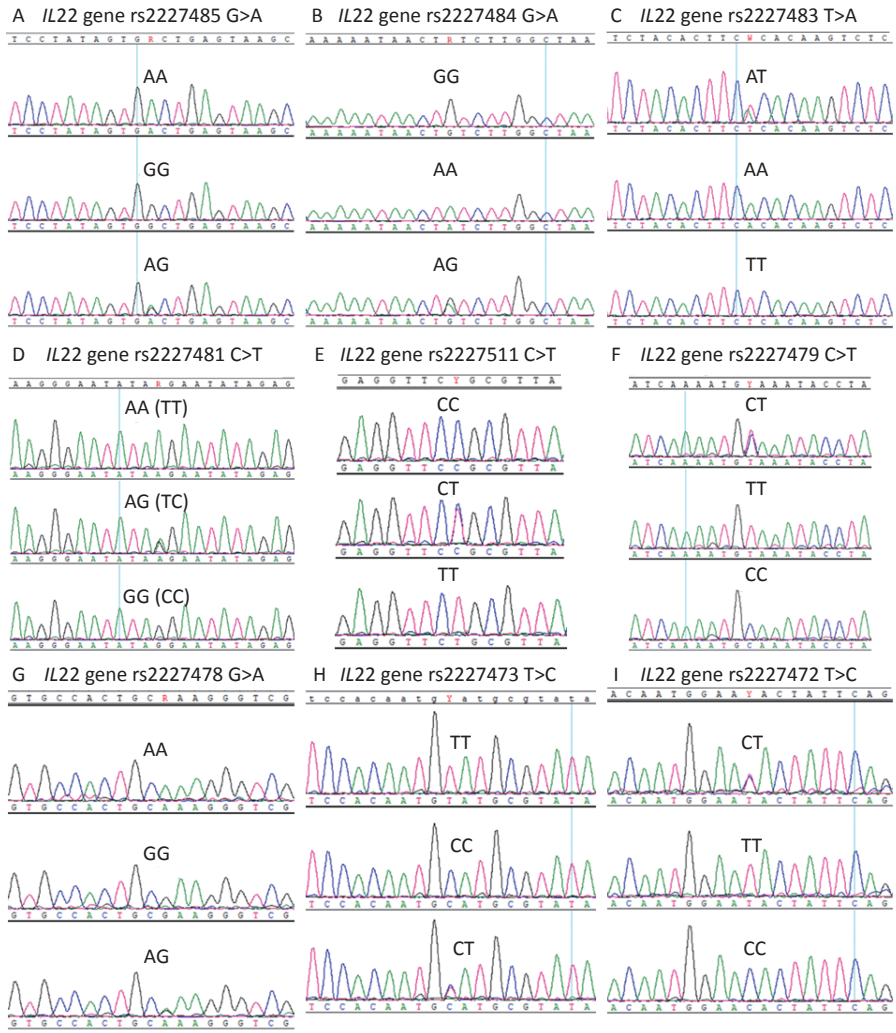

Supplementary Figure S1. The genotyping diagrams of *IL22* gene polymorphisms. (A) rs2227485, (B) rs2227484, (C) rs2227483, (D) rs2227481, (E) rs2227511, (F) rs2227479, (G) rs2227478, (H) rs2227473, and (I) rs2227472.

Supplementary Table S1. Detailed information of the *IL22* gene polymorphisms included in the present study

No.	SNP ID	Chromosome	Position	Allele	Region	Distance to TSS (bp)	Genotyping assay (Sanger sequencing)
1	rs2227487	12	66933859	C/A	promoter	311	Amplification primer: 5′-AGATTCTGCTTGTGACGG-3′ (Forward) 5′-ATAGTTGTTGTAGGATTATTTGG-3′ (Reverse) Sequencing primer: 5′-AGATTCTGCTTGTGACGG-3′
2	rs2227486	12	66933971	G/A	promoter	423	
3	rs2227485	12	66933980	A/G	promoter	432	
4	rs2227484	12	66934196	G/A	promoter	648	
5	rs2227483	12	66934443	A/T	promoter	895	Amplification primer: 5′-GGCAACCACCATTCTACTCTT-3′ (Forward) 5′-CACGGACTCACTTTCCTACCA-3′ (Reverse) Sequencing primer: 5′-TAAGTTTGTCAATACTAATGC-3′
6	rs2227482	12	66934487	T/G	promoter	939	
7	rs2227481	12	66934608	C/T	promoter	1,060	
8	rs2227511	12	66934623	C/T	promoter	1,075	
9	rs2227479	12	66934713	C/T	promoter	1,165	
10	rs2227478	12	66934889	A/G	promoter	1,341	
11	rs2227474	12	66935253	T/C	promoter	1705	Amplification primer: 5′-TGTAACAGAACACCGAAAT-3′ (Forward) 5′-TACCCAAAGGATACAACAT-3′ (Reverse) Sequencing primer: 5′-TACCCAAAGGATACAACAT-3′
12	rs2227473	12	66935305	C/T	promoter	1757	
13	rs2227472	12	66935400	T/C	promoter	1852	

Supplementary Table S2. Information of the primers used in the functional analysis of rs2227481

Assays	Primer information
Dual luciferase assay	pGL3-C allele (reporter plasmid): *IL22* gene promoter fragment was amplified with 5′-CTAGCTAGCCACGTTATTGCAAATG-3′ (forward) and 5′-CCCAAGCTTTTAGAGCCCGGAGGGT-3′ (reverse), then the PCR products were inserted into the NheI and HindIII restriction sites of the pGL3-basic vector.pGL3-T allele (reporter plasmid): Site-directed mutagenesis of pGL3-C allele (reporter plasmid)
Quantitative real-time RT-PCR	*IL22* gene: 5′-TGACGACCAGAACATCCAGA-3′ (forward) 5′-AATCGCCTTGATCTCTCCAC-3′ (reverse) *GAPDH* gene: 5′-TGCACCACCAACTGCTTAGC-3′ (forward) 5′-GGCATGGACTGTGGTCATGAG-3′ (reverse).
SPR (surface plasma resonance) analysis	rs2227481 [C] probe: 5′-ACCTCTATATTCCTATATTCCCTTC-3′ (forward) 5′-GAAGGGAATATAGGAATATAGAGGT-3′ (reverse)rs2227481 [T] probe: 5′-ACCTCTATATTCTTATATTCCCTTC-3′ (forward) 5′-GAAGGGAATATAAGAATATAGAGGT-3′ (reverse)
ChIP (chromatin immunoprecipitation) assay	Identification of *IL22* gene fragment: 5′-TTTTTAAATAATTTGAAGGTA-3′ (forward) 5′-ATTTCCAAATAATCCTATAAC-3′ (reverse)

Supplementary Table S3. The association between *IL22* gene polymorphisms and cancer risk in stage I

Groups	*n*	Allele and genotype (frequency, %)					Logistic regression analysis [*P*, *OR* (95% *CI*)][1]					
		1	2	11	12	22	1 *vs*. 2	11 *vs*. 22	11 *vs*. 12	12 *vs*. 22	11+12 *vs*. 22	11 *vs*. 12+22
rs2227485 A/G (1-A, 2-G)												
Liver cancer	100	94 (47.0)	106 (53.0)	22 (22.0)	50 (50.0)	28 (28.0)	0.422, 0.86 (0.60–1.24)	0.422, 0.74 (0.36–1.53)	0.817, 0.93 (0.49–1.75)	0.477, 0.80 (0.44–1.48)	0.406, 0.78 (0.44–1.40)	0.627, 0.86 (0.47–1.57)
Lung cancer	100	103 (51.5)	97 (48.5)	26 (26.0)	51 (51.0)	23 (23.0)	0.855, 1.03 (0.72–1.48)	0.857, 1.07 (0.52–2.21)	0.818, 1.08 (0.58–1.99)	0.988, 1.00 (0.53–1.88)	0.951, 1.02 (0.56–1.86)	0.812, 1.07 (0.60–1.92)
Gastric cancer	100	95 (47.5)	105 (52.5)	23 (23.0)	49 (49.0)	28 (28.0)	0.488, 0.88 (0.62–1.26)	0.492, 0.78 (0.38–1.60)	0.974, 0.99 (0.53–1.86)	0.439, 0.79 (0.43–1.45)	0.406, 0.78 (0.44–1.40)	0.762, 0.91 (0.50–1.66)
Normal	150	152 (50.7)	148 (49.3)	37 (24.7)	78 (52.0)	35 (23.3)						
rs2227484 G/A (1-G, 2-A)												
Liver cancer	100	177 (88.5)	23 (11.5)	78 (78.0)	21 (21.0)	1 (1.0)	0.865, 1.05 (0.60–1.83)	0.810, 1.35 (0.12–15.09)	0.939, 1.03 (0.55–1.91)	0.829, 1.31 (0.11–15.41)	0.813, 1.34 (0.12–14.95)	0.901, 1.04 (0.57–1.91)
Lung cancer	100	180 (90.0)	20 (10.0)	81 (81.0)	18 (18.0)	1 (1.0)	0.488, 1.23 (0.69–2.19)	0.787, 1.40 (0.13–15.66)	0.510, 1.24 (0.65–2.36)	0.787, 1.40 (0.13–15.66)	0.813, 1.34 (0.12–14.95)	0.488, 1.25 (0.67–2.34)
Gastric cancer	100	179 (89.5)	21 (10.5)	80 (80.0)	19 (19.0)	1 (1.0)	0.605, 1.16 (0.66–2.06)	0.794, 1.38 (0.12–15.47)	0.644, 1.16 (0.62–2.19)	0.891, 1.19 (0.10–13.99)	0.813, 1.34 (0.12–14.95)	0.616, 1.17 (0.63–2.18)
Normal	150	264 (88.0)	36 (12.0)	116 (77.3)	32 (21.4)	2 (1.3)						
rs2227483 A/T (1-A, 2-T)												
Liver cancer	100	109 (54.5)	91 (45.5)	30 (30.0)	49 (49.0)	21 (21.0)	0.634, 1.09 (0.76–1.56)	0.656, 1.18 (0.57–2.43)	0.587, 1.18 (0.65–2.13)	1.000, 1.00 (0.52–1.92)	0.851, 1.06 (0.57–1.97)	0.565, 1.18 (0.67–2.06)
Lung cancer	100	111 (55.5)	89 (44.5)	31 (31.0)	49 (49.0)	20 (20.0)	0.487, 1.14 (0.79–1.63)	0.507, 1.28 (0.62–2.65)	0.513, 1.22 (0.68–2.20)	0.885, 1.05 (0.54–2.03)	0.705, 1.13 (0.61–2.11)	0.457, 1.24 (0.71–2.16)
Gastric cancer	100	107 (53.5)	93 (46.5)	29 (29.0)	49 (49.0)	22 (22.0)	0.798, 1.05 (0.73–1.50)	0.820, 1.09 (0.53–2.24)	0.669, 1.14 (0.63–2.07)	0.888, 0.96 (0.50–1.82)	1.000, 1.00 (0.54–1.84)	0.686, 1.12 (0.64–1.97)
Normal	150	157 (52.3)	143 (47.7)	40 (26.7)	77 (51.3)	33 (22.0)						
rs2227511 C/T (1-C, 2-T)												
Liver cancer	100	167 (83.5)	33 (16.5)	69 (69.0)	29 (29.0)	2 (2.0)	0.726, 0.92 (0.56–1.49)	0.671, 0.65 (0.09–4.73)	0.837, 0.94 (0.54–1.65)	0.719, 0.69 (0.09–5.19)	0.683, 0.66 (0.09–4.78)	0.778, 0.92 (0.53–1.60)
Lung cancer	100	168 (84.0)	32 (16.0)	70 (70.0)	28 (28.0)	2 (2.0)	0.841, 0.95 (0.58–1.55)	0.682, 0.66 (0.09–4.80)	0.974, 0.99 (0.56–1.74)	0.694, 0.67 (0.09–5.01)	0.683, 0.66 (0.09–4.78)	0.910, 0.97 (0.56–1.69)
Gastric cancer	100	170 (85.0)	30 (15.0)	71 (71.0)	28 (28.0)	1 (1.0)	0.919, 1.03 (0.62–1.69)	0.813, 1.34 (0.12–15.05)	0.987, 1.01 (0.57–1.77)	0.818, 1.33 (0.12–15.41)	0.813, 1.34 (0.12–14.95)	0.955, 1.02 (0.58–1.77)
Normal	150	254 (84.7)	46 (15.3)	106 (70.7)	42 (28.0)	2 (1.3)						

Continued

Groups	*n*	Allele and genotype (frequency, %)					Logistic regression analysis [*P*, *OR* (95% *CI*)][1]					
		1	2	11	12	22	1 *vs*. 2	11 *vs*. 22	11 *vs*. 12	12 *vs*. 22	11+12 *vs*. 22	11 *vs*. 12+22
rs2227479 C/T (1-C, 2-T)												
Liver cancer	100	182 (91.0)	18 (9.0)	82 (82.0)	18 (18.0)	0 (0)	0.595, 0.84 (0.44–1.60)	—	0.577, 0.83 (0.42–1.62)	—	—	0.577, 0.83 (0.42–1.62)
Lung cancer	100	186 (93.0)	14 (7.0)	86 (86.0)	14 (14.0)	0 (0)	0.780, 1.10 (0.55–2.20)	—	0.771, 1.11 (0.54–2.28)	—	—	0.771, 1.11 (0.54–2.28)
Gastric cancer	100	184 (92.0)	16 (8.0)	84 (84.0)	16 (16.0)	0 (0)	0.892, 0.96 (0.49–1.86)	—	0.887, 0.95 (0.47–1.91)	—	—	0.887, 0.95 (0.47–1.91)
Normal	150	277 (92.3)	23 (7.7)	127 (84.7)	23 (15.3)	0 (0)						
rs2227478 A/G (1-A, 2-G)												
Liver cancer	100	163 (81.5)	37 (18.5)	68 (68.0)	27 (27.0)	5 (5.0)	0.301, 0.78 (0.48–1.25)	0.192, 0.38 (0.09–1.63)	0.747, 0.91 (0.51–1.62)	0.255, 0.42 (0.09–1.89)	0.202, 0.39 (0.09–1.66)	0.498, 0.83 (0.48–1.43)
Lung cancer	100	165 (82.5)	35 (17.5)	70 (70.0)	25 (25.0)	5 (5.0)	0.455, 0.83 (0.51–1.35)	0.206, 0.39 (0.09–1.68)	0.970, 1.01 (0.56–1.82)	0.217, 0.39 (0.08–1.75)	0.202, 0.39 (0.09–1.66)	0.732, 0.91 (0.52–1.58)
Gastric cancer	100	165 (82.5)	35 (17.5)	69 (69.0)	27 (27.0)	4 (4.0)	0.455, 0.83 (0.51–1.35)	0.345, 0.48 (0.10–2.21)	0.785, 0.92 (0.52–1.64)	0.415, 0.52 (0.11–2.51)	0.357, 0.49 (0.11–2.24)	0.609, 0.87 (0.50–1.51)
Normal	150	255 (85.0)	45 (15.0)	108 (72.0)	39 (26.0)	3 (2.0)						
rs2227473 C/T (1-C, 2-T)												
Liver cancer	100	182 (91.0)	18 (9.0)	83 (83.0)	16 (16.0)	1 (1.0)	0.710, 1.12 (0.61–2.08)	0.791, 0.69 (0.04–11.12)	0.596, 1.20 (0.61–2.36)	0.699, 0.57 (0.03–9.77)	0.773, 0.66 (0.04–10.75)	0.641, 1.17 (0.60–2.27)
Lung cancer	100	181 (90.5)	19 (9.5)	82 (82.0)	17 (17.0)	1 (1.0)	0.854, 1.06 (0.58–1.94)	0.784, 0.68 (0.04–10.99)	0.746, 1.12 (0.57–2.17)	0.730, 0.61 (0.04–10.36)	0.773, 0.66 (0.04–10.75)	0.792, 1.09 (0.57–2.10)
Gastric cancer	100	179 (89.5)	21 (10.5)	80 (80.0)	19 (19.0)	1 (1.0)	0.856, 0.95 (0.53–1.71)	0.771, 0.66 (0.04–10.72)	0.937, 0.97 (0.51–1.86)	0.788, 0.68 (0.04–11.53)	0.773, 0.66 (0.04–10.75)	0.897, 0.96 (0.51–1.81)
Normal	150	270 (90.0)	30 (10.0)	121 (80.7)	28 (18.6)	1 (0.7)						
rs2227472 T/C (1-T, 2-C)												
Liver cancer	100	114 (57.0)	86 (43.0)	30 (30.0)	54 (54.0)	16 (16.0)	0.557, 1.11 (0.78–1.60)	0.473, 1.32 (0.62–2.83)	0.854, 0.95 (0.53–1.69)	0.349, 1.40 (0.70–2.80)	0.356, 1.37 (0.70–2.66)	0.910, 1.03 (0.59–1.80)
Lung cancer	100	112 (56.0)	88 (44.0)	33 (33.0)	46 (46.0)	21 (21)	0.714, 1.07 (0.75–1.53)	0.780, 1.11 (0.54–2.26)	0.498, 1.22 (0.68–2.19)	0.769, 0.91 (0.47–1.76)	0.949, 0.98 (0.53–1.83)	0.539, 1.19 (0.69–2.05)
Gastric cancer	100	113 (56.5)	87 (43.5)	32 (32.0)	49 (49.0)	1919.0)	0.633, 1.09 (0.76–1.57)	0.646, 1.19 (0.57–2.46)	0.717, 1.11 (0.62–1.99)	0.853, 1.07 (0.54–2.09)	0.747, 1.11 (0.59–2.10)	0.653, 1.13 (0.66–1.96)
Normal	150	163 (54.3)	137 (45.7)	44 (29.3)	75 (50.0)	31 (20.7)						

Note. [1]The *OR* (95% *CI*) and the corresponding *P*-value were calculated by logistic regression analysis, and adjusted for age, gender, smoking, and drinking status.

Supplementary Table S4. The association between *IL22* gene polymorphisms and cancer risk in stage II and pooled data (I+II)

Groups	*n*	Allele and genotype (frequency, %)					Logistic Regression analysis [*P*, *OR* (95% *CI*)][1]					
		1	2	11	12	22	1 *vs*. 2	11 *vs*. 22	11 *vs*. 12	12 *vs*. 22	11+12 *vs*. 22	11 *vs*. 12+22
rs2227483 A/T (1-A, 2-T) in stage II												
Liver cancer	380	401 (52.8)	359 (47.2)	102 (26.9)	197 (51.8)	81 (21.3)	0.476, 0.94 (0.78–1.12)	0.570, 0.90 (0.63–1.29)	0.131, 0.80 (0.59–1.07)	0.453, 1.13 (0.82–1.57)	0.797, 1.04 (0.77–1.42)	0.182, 0.83 (0.62–1.09)
Lung cancer	450	475 (52.8)	425 (47.2)	123 (27.3)	229 (50.9)	98 (21.8)	0.457, 0.94 (0.79–1.11)	0.534, 0.90 (0.64–1.26)	0.180, 0.82 (0.62–1.09)	0.591, 1.09 (0.80–1.48)	0.930, 1.01 (0.76–1.36)	0.219, 0.85 (0.65–1.10)
Gastric cancer	360	364 (50.6)	356 (49.4)	90 (25.0)	184 (51.1)	86 (23.9)	0.099, 0.86 (0.72–1.03)	0.120, 0.75 (0.52–1.08)	0.069, 0.75 (0.55–1.02)	0.984, 1.00 (0.72–1.38)	0.492, 0.90 (0.66–1.22)	0.053, 0.75 (0.56–1.00)
Normal controls	650	707 (54.4)	593 (45.6)	200 (30.8)	307 (47.2)	143 (22.0)						
rs2227483 A/T (1-A, 2-T) in stage I+II												
Liver cancer	480	510 (53.1)	450 (46.9)	132 (27.5)	246 (51.2)	102 (21.3)	0.667, 0.97 (0.82–1.13)	0.751, 0.95 (0.69–1.31)	0.261, 0.86 (0.66–1.12)	0.501, 1.11 (0.83–1.48)	0.753, 1.05 (0.79–1.38)	0.340, 0.89 (0.69–1.14)
Lung cancer	550	586 (53.3)	514 (46.7)	154 (28.0)	278 (50.5)	118 (21.5)	0.710, 0.97 (0.83–1.13)	0.781, 0.96 (0.70–1.30)	0.353, 0.89 (0.69–1.14)	0.591, 1.08 (0.82–1.43)	0.811, 1.03 (0.79–1.34)	0.427, 0.91 (0.71–1.15)
Gastric cancer	460	471 (51.2)	449 (48.8)	119 (25.9)	233 (50.6)	108 (23.5)	0.175, 0.89 (0.76–1.05)	0.199, 0.81 (0.58–1.12)	0.148, 0.82 (0.62–1.07)	0.939, 0.99 (0.74–1.32)	0.545, 0.92 (0.70–1.21)	0.118, 0.81 (0.63–1.05)
Normal controls	800	864 (54.0)	736 (46.0)	240 (30.0)	384 (48.0)	176 (22.0)						
rs2227511 C/T (1-C, 2-T) in stage II												
Liver cancer	380	641 (84.3)	119 (15.7)	271 (71.3)	99 (26.1)	10 (2.6)	0.767, 1.04 (0.81–1.33)	0.769, 1.12 (0.52–2.45)	0.821, 1.03 (0.77–1.38)	0.838, 1.09 (0.49–2.43)	0.785, 1.11 (0.51–2.42)	0.771, 1.04 (0.79–1.38)
Lung cancer	450	778 (86.4)	122 (13.6)	337 (74.9)	104 (23.1)	9 (2.0)	0.095, 1.23 (0.97–1.56)	0.284, 1.55 (0.69–3.48)	0.159, 1.22 (0.92–1.62)	0.573, 1.27 (0.55–2.91)	0.342, 1.48 (0.66–3.29)	0.107, 1.25 (0.95–1.64)
Gastric cancer	360	615 (85.4)	105 (14.6)	264 (73.3)	87 (24.2)	9 (2.5)	0.352, 1.13 (0.88–1.46)	0.634, 1.22 (0.54–2.73)	0.371, 1.15 (0.85–1.55)	0.888, 1.06 (0.46–2.44)	0.695, 1.17 (0.53–2.62)	0.333, 1.15 (0.86–1.54)
Normal controls	650	1090 (83.8)	210 (16.2)	458 (70.5)	173 (26.6)	19 (2.9)						
rs2227511 C/T (1-C, 2-T) in stage I+II												
Liver cancer	480	808 (84.2)	152 (15.8)	340 (70.9)	128 (26.6)	12 (2.5)	0.911, 1.01 (0.81–1.26)	0.884, 1.06 (0.51–2.17)	0.924, 1.01 (0.78–1.31)	0.914, 1.04 (0.50–2.19)	0.891, 1.05 (0.51–2.16)	0.899, 1.02 (0.79–1.30)
Lung cancer	550	946 (86.0)	154 (14.0)	407 (73.9	132 (24.1)	11 (2.0)	0.155, 1.17 (0.94–1.45)	0.396, 1.38 (0.66–2.89)	0.229, 1.17 (0.91–1.50)	0.668, 1.18 (0.55–2.53)	0.457, 1.32 (0.63–2.77)	0.177, 1.18 (0.93–1.51)
Gastric cancer	460	785 (85.3)	135 (14.7)	335 (72.8)	115 (25.1)	10 (2.2)	0.376, 1.11 (0.88–1.39)	0.571, 1.25 (0.58–2.68)	0.436, 1.11 (0.85–1.45)	0.772, 1.12 (0.51–2.47)	0.619, 1.21 (0.57–2.60)	0.379, 1.12 (0.87–1.45)
Normal controls	800	1344 (84.0)	256 (16.0)	564 (70.5)	215 (26.9)	21 (2.6)						

Continued

Groups	*n*	Allele and genotype (frequency, %)					Logistic Regression analysis [*P*, *OR* (95% *CI*)][1]					
		1	2	11	12	22	1 *vs*. 2	11 *vs*. 22	11 *vs*. 12	12 *vs*. 22	11+12 *vs*. 22	11 *vs*. 12+22
rs2227479 C/T (1-C, 2-T) in stage II												
Liver cancer	380	722 (95.0)	38 (5.0)	342 (90.0)	38 (10.0)	0 (0)	0.195, 0.75 (0.49–1.16)	0.342, 4.21 (0.22–81.8)	0.316, 1.23 (0.82–1.86)	0.418, 3.43 (0.17–68.2)	0.350, 4.11 (0.21–79.9)	0.234, 1.28 (0.85–1.93)
Lung cancer	450	858 (95.3)	42 (4.7)	408 (90.7)	42 (9.3)	0 (0)	0.319, 0.81 (0.53–1.23)	0.286, 5.02 (0.26–97.5)	0.156, 1.33 (0.90–1.98)	0.382, 3.79 (0.19–75.1)	0.295, 4.87 (0.25–94.5)	0.107, 1.38 (0.93–2.05)
Gastric cancer	360	670 (93.1)	50 (6.9)	310 (86.1)	50 (13.9)	0 (0)	0.002, 0.53 (0.35–0.80)	0.376, 3.82 (0.20–74.1)	0.403, 0.85 (0.58–1.24)	0.323, 4.50 (0.23–89.0)	0.369, 3.90 (0.20–75.7)	0.518, 0.88 (0.60–1.29)
Normal controls	650	1216 (93.5)	84 (6.5)	569 (87.5)	78 (12.0)	3 (0.5)						
rs2227479 C/T (1-C, 2-T) in stage I+II												
Liver cancer	480	904 (94.2)	56 (5.8)	424 (88.3)	56 (11.7)	0 (0)	0.392, 1.16 (0.83–1.62)	0.338, 4.27 (0.22–82.8)	0.596, 1.10 (0.78–1.56)	0.371, 3.90 (0.20–76.8)	0.341, 4.22 (0.22–81.8)	0.485, 1.13 (0.80–1.60)
Lung cancer	550	1044 (94.9)	56 (5.1)	494 (89.8)	56 (10.2)	0 (0)	0.088, 1.34 (0.96–1.86)	0.289, 4.97 (0.26–96.4)	0.162, 1.28 (0.91–1.81)	0.371, 3.90 (0.20–76.8)	0.298, 4.83 (0.25–93.7)	0.116, 1.32 (0.93–1.86)
Gastric cancer	460	854 (92.8)	66 (7.2)	394 (85.7)	66 (14.3)	0 (0)	0.642, 0.93 (0.68–1.28)	0.363, 3.97 (0.20–77.0)	0.400, 0.87 (0.62–1.21)	0.316, 4.59 (0.23–90.2)	0.356, 4.04 (0.21–78.4)	0.500, 0.89 (0.64–1.24)
Normal controls	800	1493 (93.3)	107 (6.7)	696 (87.0)	101 (12.6)	3 (0.4)						
rs2227478 A/G (1-A, 2-G) in stage II												
Liver cancer	380	635 (83.6)	125 (16.4)	262 (69.0)	111 (29.2)	7 (1.8)	0.993, 1.00 (0.79–1.27)	0.266, 1.64 (0.69–3.93)	0.468, 0.90 (0.68–1.20)	0.188, 1.82 (0.75–4.45)	0.236, 1.69 (0.71–4.04)	0.684, 0.95 (0.72–1.24)
Lung cancer	450	746 (82.9)	154 (17.1)	304 (67.5)	138 (30.7)	8 (1.8)	0.688, 0.96 (0.76–1.20)	0.229, 1.67 (0.73–3.83)	0.201, 0.84 (0.64–1.10)	0.114, 1.98 (0.85–4.64)	0.184, 1.75 (0.77–4.02)	0.359, 0.89 (0.68–1.15)
Gastric cancer	360	605 (84.0)	115 (16.0)	257 (71.3)	91 (25.4)	12 (3.3)	0.775, 1.04 (0.81–1.33)	0.867, 0.94 (0.45–1.95)	0.621, 1.08 (0.80–1.45)	0.723, 0.87 (0.41–1.86)	0.824, 0.92 (0.45–1.91)	0.680, 1.06 (0.80–1.41)
Normal controls	650	1086 (83.5)	214 (16.5)	456 (70.1)	174 (26.8)	20 (3.1)						
rs2227478 A/G (1-A, 2-G) in stage I+II												
Liver cancer	480	798 (83.1)	162 (16.9)	330 (68.7)	138 (28.8)	12 (2.5)	0.650, 0.95 (0.77–1.18)	0.752, 1.12 (0.55–2.28)	0.431, 0.90 (0.70–1.16)	0.561, 1.24 (0.60–2.58)	0.691, 1.15 (0.57–2.34)	0.509, 0.92 (0.72–1.18)
Lung cancer	550	911 (82.8)	189 (17.2)	374 (68.0)	163 (29.6)	13 (2.4)	0.495, 0.93 (0.76–1.14)	0.651, 1.17 (0.59–2.35)	0.246, 0.87 (0.68–1.10)	0.403, 1.35 (0.67–2.75)	0.567, 1.22 (0.61–2.44)	0.327, 0.89 (0.70–1.13)
Gastric cancer	460	770 (83.7)	150 (16.3)	326 (70.9)	118 (25.6)	16 (3.5)	0.939, 0.99 (0.80–1.24)	0.578, 0.83 (0.43–1.60)	0.752, 1.04 (0.80–1.36)	0.509, 0.80 (0.41–1.57)	0.552, 0.82 (0.43–1.57)	0.890, 1.02 (0.79–1.31)
Normal controls	800	1341 (83.8)	259 (16.2)	564 (70.5)	213 (26.6)	23 (2.9)						

Note. [1] The *OR* (95% *CI*) and the corresponding *p*-value were calculated by logistic regression analysis, and adjusted for age, gender, smoking, and drinking status.