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Functional *FEN1* genetic variants contribute to risk of hepatocellular carcinoma, esophageal cancer, gastric cancer and colorectal cancer

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As a DNA repair protein, Flap endonuclease 1 (FEN1) plays crucial parts in preventing carcinogenesis. Two functional germ line variants (–69G > A and 4150G > T) in the *FEN1* gene have been associated with DNA damage levels in coke oven workers and lung cancer risk in general populations. However, the role of these genetic variants on gastrointestinal cancer susceptibility is unknown. Therefore, we evaluated the association between these polymorphisms and gastrointestinal cancer risk in two independent case–control cohorts consisted of a total of 1850 gastrointestinal cancer (hepatocellular carcinoma, esophageal cancer, gastric cancer and colorectal cancer) patients and 2222 healthy controls. The impact of these variations on *FEN1* expression was also examined using liver, esophagus, stomach and colon normal tissues. It was found that the *FEN1* –69GG genotypes were significantly correlated to increased risk for developing gastrointestinal cancer compared with the –69AA genotype in both cohorts [Jinan cohort: odds ratios (OR) = 2.14, 95% confidence interval (CI) = 1.47–2.80, $P = 1.0 \times 10^{-6}$; Huaian cohort: OR = 1.93, 95% CI = 1.37–2.50, $P = 0.5 \times 10^{-6}$]. Similar results were observed for 4150G > T polymorphism. In the combined meta-analyses, OR for –69GG or 4150GG genotype was 2.02 (95% CI = 1.59–2.45) or 1.86 (95% CI = 1.45–2.28) compared with –69AA or 4150TT genotype. *In vivo FEN1* messenger RNA expression analyses showed that the –69G or 4150G allele carriers had ∼2-fold decreased *FEN1* expression in gastrointestinal tissues compared with –69A or 4150T carriers, indicating that lower *FEN1* expression may lead to higher risk for malignant transformation of gastrointestinal cells. Our results highlight *FEN1* as an important gene in human gastrointestinal oncogenesis and genetic polymorphisms in *FEN1* confer susceptibility to gastrointestinal cancers.

Introduction

As a structure-specific nuclease, Flap endonuclease 1 (FEN1) is involved in efficient 5′-flap removal during long-patch base excision repair and the maturation of Okazaki fragments in DNA replication (1–3). Additionally, FEN1 also acts as a 5′ exonuclease (4) and a gap-dependent endonuclease (5,6), which can be stimulated to promote apoptotic DNA fragmentation after apoptotic stimuli. Due to its pivotal role in DNA repair and other multiple DNA metabolic pathways, FEN1 can serve as a key enzyme in maintaining genomic stability and protecting against carcinogenesis (1–6). It was reported that the functional impairment of yeast RAD27 (the homolog of mammalian FEN1) leads to a remarkable increase in the rate of spontaneous mutations (6–8). Haploinsufficient *FEN1* can result in genome instability and carcinogenesis in mice (9). It has also been shown that *FEN1* mutations resulting in reduced nuclease activity occur in human cancer cells and 70% of mice knocked-in the mutated *FEN1* developed tumors in multiple organs (10). Therefore, abnormal expression of FEN1 resulting from naturally occurring genetic variants may contribute to cancer susceptibility.

Previously, two single-nucleotide polymorphisms (SNPs), –69G > A (rs174538, in the gene promoter region) and 4150G > T (rs4246215, in gene 3′-untranslated region), were identified after thoroughly re-sequencing the *FEN1* locus in 30 Chinese Han healthy volunteers (11). Electrophoretic mobility-shift assays and luciferase assays showed that –69G > A SNP causes increased promoter activity, which is most likely to be due to a higher binding affinity of the G allele with some unknown transcriptional inhibitors. In addition, the 4150G > T SNP is also associated with differential levels of *FEN1* RNA expression. Real-time analysis of *FEN1* RNA in lung tissues also suggested that both –69G > A and 4150G > T SNPs could influence gene expression *in vivo*. These SNPs were reproducibly associated with lung cancer risk in two independent cohorts (Beijing cohort: 1013 lung cancer patients and 1131 controls; Wuhan cohort: 827 lung cancer patients and 827 controls).

However, their roles on gastrointestinal cancer susceptibility and impact on *FEN1* expression in gastrointestinal tissues are still unknown. Therefore, we specifically examined whether these two polymorphisms are involved in development of four gastrointestinal cancers, including hepatocellular carcinoma (HCC), esophageal cancer (EC), gastric cancer (GC) and colorectal cancer (CRC), in two independent case–control cohorts from Jinan city (Shandong Province) and Huaian city (Jiangsu Province). To validate the biological function of these two SNPs *in vivo*, we also examined the association between *FEN1* genotypes and *FEN1* messenger RNA (mRNA) expression levels in liver, esophagus, stomach and colorectal tissues.

Materials and methods

Study cohorts

This study consisted of two case–control cohorts (i) Jinan cohort: 1022 patients with gastrointestinal cancers (410 HCC cases, 266 EC cases, 220 GC cases and 126 CRC cases) from Shandong Cancer Hospital, Shandong Academy of Medical Sciences (Jinan, Shandong Province, China) and sex- and age-matched (±5 years) 1221 controls. Patients were recruited between June 2009 and May 2011 at Shandong Cancer Hospital. All patients had not been treated by any medical treatments. The diagnosis of EC, GC and CRC patients were histologically confirmed. For HCC, diagnosis of patients was confirmed by a pathological examination combined with positive imaging (magnetic resonance imaging and/or computerized tomography). Control subjects were randomly selected from a pool of 4200 individuals from a community cancer-screening program for early detection of cancer conducted in Jinan city during the same time period as the patients were collected. (ii) Huaian cohort: 828 patients with gastrointestinal cancers (237 HCC cases, 289 EC cases, 192 GC cases and 110 CRC cases) from Huaian No. 2 Hospital (Huaian, Jiangsu Province, China) and sex- and age-matched (±5 years) 1001 controls. Patients were consecutively recruited between January 2009 and June 2011 at Huaian No. 2 Hospital. Controls were cancer-free individuals selected from a community cancer-screening program (2500 individuals) for early detection of cancer conducted in Huaian city during the same time period as the patients were collected.

Abbreviations: CI, confidence interval; CRC, colorectal cancer; EC, esophageal cancer; FEN1, Flap endonuclease 1; GC, gastric cancer; HCC, hepatocellular carcinoma; mRNA, messenger RNA; OR, odds ratio; PCR, polymerase chain reaction; SNP, single-nucleotide polymorphism.

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Individuals who smoked one cigarette per day for over 1 year were considered as smokers. Subjects were considered as alcohol drinkers, if they drank at least once per week. All participants were negative for antibodies to hepatitis C virus, hepatitis D virus or HIV since we excluded all participants with hepatitis C virus, hepatitis D virus or HIV infection. Twenty liver normal tissues, 15 esophagus normal tissues, 12 stomach normal tissues and 13 colorectal normal tissues adjacent to the tumors were obtained from surgically removed specimens of patients in Huaian No. 2 Hospital. The normal tissues sampled at least 2 cm away from the margin of the tumor. All subjects were ethnic Han Chinese. At recruitment, informed consent was obtained from each subject. This study was approved by the institutional Review Boards.

Polymorphism genotyping

FEN1 –69G > A and 4150G > T genotypes were examined using polymerase chain reaction (PCR)-based restriction fragment length polymorphism as previously reported (11). In PCR–restriction fragment length polymorphism genotyping, the primers used for amplifying DNA segments containing the either *FEN1* –69G > A or 4150G > T sites were 5′-ggaggttccaggagcgctcta-3′/5′-ttctccaccgcttgtccc-3′ or 5′-tatgtcaggctcaaaccac-3′/5′-cagccagtaatcagt-cacaa-3′, respectively. Restriction enzyme SalI (New England Biolabs) or Alw26I (Fermentas) was used to distinguish the—69G > A or 4150G > T genotypes, respectively. A 15% random sample was reciprocally tested by direct sequencing, and the reproducibility was 99.8%.

Real-time analysis of FEN1 mRNA

SYBR-Green real-time quantity PCR method was used to examine *FEN1* mRNA levels in normal tissues as described previously (11). In brief, total RNA was isolated and converted to complementary DNA using an oligo$(dT)_{15}$ primer and Superscript II (Invitrogen). Relative gene expression quantitation for *FEN1* and β-*actin* as an internal reference gene was carried out using the ABI 7500 real-time PCR system in triplicates. The primers used for *FEN1* were 5′-ctgtggacctcatccagaagca-3′ and 5′-ccagcacctcaggttccaaga-3′ and for β-*actin* were 5′-ggcggcaccaccatgtaccct-3′ and 5′-aggggccggactcgtcatact-3′.

Statistics

The associations between *FEN1* genotypes and risk of gastrointestinal cancers were estimated by odds ratios (ORs) and their 95% confidence intervals (CIs) computed by logistic regression models. Student's *t*-test was used to assess differences in *FEN1* transcript abundance with different genotypes. All ORs were adjusted for age, sex, smoking, drinking or hepatitis B virus infection status, where it was appropriate. In the meta-analyses, association between *FEN1* polymorphisms and gastrointestinal cancer risk was re-calculated using crude ORs together with their corresponding 95% CIs. If the *P* value of the heterogeneity test was ≥0.05, a fixed effect model (the Mantel–Haenszel method) was performed to calculate the combined OR (12), which assumed the same homogeneity of effect size across all studies. If the *P* value of the heterogeneity test was <0.05, it showed that the between-study heterogeneity was statistically significant. A random effects mode (the DerSimonian and Laird method) was used to calculate the combined OR (13). A *P* value of <0.05 was used as the criterion of statistical significance, and all statistical tests were two-sided. All analyses were performed using SAS 9.0 (SAS Institute) and Stata 11.0 (StataCorp LP).

Results

The subject characteristics are shown in Table I. All observed genotype frequencies in both controls and patients conform to Hardy–Weinberg equilibrium. The allelic frequencies for the –69A and 4150T were 0.364 and 0.360 among 1221 (1220 for 4150T) healthy controls in Jinan cohort and 0.458 and 0.457 among 1001 control

Table I. Distribution of selected characteristics among gastrointestinal cancer patients and controls

Variable	HCC			EC			GC			CRC		
	Cases, *n* (%)	Controls, *n* (%)	P^a	Cases, *n* (%)	Controls, *n* (%)	P^a	Cases, *n* (%)	Controls, *n* (%)	P^a	Cases, *n* (%)	Controls, *n* (%)	P^a
Jinan cohort												
	n = 410	*n* = 423		*n* = 266	*n* = 386		*n* = 220	*n* = 250		*n* = 126	*n* = 162	
Age (years)			0.351			0.351			0.399			0.612
≤56	200 (48.8)	220 (52.0)		130 (48.9)	203 (52.6)		112 (50.9)	137 (54.8)		67 (53.2)	91 (56.2)	
>56	210 (51.2)	203 (48.0)		136 (51.1)	183 (47.4)		108 (49.1)	113 (45.2)		59 (46.8)	71 (43.8)	
Sex			0.275			0.615			0.416			0.783
Male	322 (78.5)	345 (81.6)		197 (74.1)	279 (72.3)		164 (74.5)	178 (71.2)		79 (62.7)	99 (61.1)	
Female	88 (21.5)	78 (18.4)		69 (25.9)	107 (27.7)		56 (25.5)	72 (28.8)		47 (37.3)	63 (38.9)	
Smoking status			<0.001			<0.001			<0.001			0.047
No	147 (35.9)	201 (47.5)		72 (27.1)	185 (47.9)		72 (32.7)	131 (52.4)		49 (38.9)	82 (50.6)	
Yes	263 (64.1)	222 (52.5)		194 (72.9)	201 (52.1)		148 (67.3)	119 (47.6)		77 (61.1)	80 (49.4)	
Drinking status						0.002						
No	NA	NA		150 (56.4)	170 (44.1)		NA	NA		NA	NA	
Yes				116 (43.6)	216 (55.9)							
HBsAg			<0.001									
Positive	310 (75.7)	38 (8.9)		NA	NA		NA	NA		NA	NA	
Negative	100 (24.3)	385 (91.1)										
Huaian cohort												
	n = 237	*n* = 315		*n* = 289	*n* = 337		*n* = 192	*n* = 204		*n* = 110	*n* = 145	
Age (years)			0.702			0.897			0.209			0.619
≤59	121 (51.1)	166 (52.7)		155 (53.6)	179 (53.1)		99 (51.6)	118 (57.8)		58 (52.7)	81 (55.9)	
>59	116 (48.9)	149 (47.3)		134 (46.4)	158 (46.9)		93 (48.4)	86 (42.2)		52 (47.3)	64 (44.1)	
Sex			0.587			0.616			0.481			0.992
Male	189 (79.7)	257 (81.6)		204 (70.6)	244 (72.4)		138 (71.9)	153 (75.0)		72 (65.5)	95 (65.5)	
Female	48 (20.3)	58 (18.4)		85 (29.4)	93 (27.6)		54 (28.1)	51 (25.0)		38 (34.5)	50 (34.5)	
Smoking status			<0.001			<0.001			<0.001			0.002
No	83 (35.0)	177 (56.2)		67 (23.2)	169 (50.1)		51 (26.6)	114 (55.9)		37 (33.6)	77 (53.1)	
Yes	154 (65.0)	138 (43.8)		222 (76.8)	168 (49.9)		141 (73.4)	90 (44.1)		73 (66.4)	68 (46.9)	
Drinking status												
No	NA	NA		NA	NA		NA	NA		NA	NA	
Yes												
HBsAg			<0.001									
Positive	184 (77.5)	31 (9.7)		NA	NA		NA	NA		NA	NA	
Negative	53 (22.5)	284 (90.3)										

NA, information not available.
[a]Two-sided χ^2 test.

subjects in Huaian cohort. The distribution of allelic frequencies of both SNPs were significantly different between Jinan and Huaian Chinese populations ($P < 0.0001$). Linkage disequilibrium analysis showed that these two SNPs are in strong linkage, with $D' = 0.95$ and $r^2 = 0.86$ in Jinan cohort and $D' = 0.97$ and $r^2 = 0.97$ in Huaian cohort.

In either Jinan cohort or Huaian cohort, carriers of *FEN1* –69GG genotype showed significantly and consistently elevated risks to develop HCC, EC and GC compared with –69AA carriers (for HCC: $OR_{Jinan} = 2.25$, 95% CI = 1.30–3.45, $P = 0.002$ and $OR_{Huaian} = 1.92$, 95% CI = 1.21–3.15, $P = 0.010$; for EC: $OR_{Jinan} = 1.93$, 95% CI = 1.14–3.35, $P = 0.014$ and $OR_{Huaian} = 2.11$, 95% CI = 1.29–3.35, $P = 0.002$; for GC: $OR_{Jinan} = 2.33$, 95% CI = 1.19–4.59, $P = 0.008$ and $OR_{Huaian} = 1.99$, 95% CI = 1.07–3.71, $P = 0.026$) (Table II and Figure 1). However, no association between this SNP and CRC risk was observed (all $P > 0.05$) (Table II and Figure 1). Logistic regression analyses also revealed that individuals with *FEN1* –69GA and 4150GT genotypes were significantly associated with increased risk of HCC or EC in Huaian cohort (–69GA: $OR_{HCC} = 1.65$, 95% CI = 1.04–2.64, $P = 0.043$; $OR_{EC} = 1.72$, 95% CI = 1.09–2.68, $P = 0.020$; 4150GT: $OR_{HCC} = 1.64$, 95% CI = 1.02–2.65, $P = 0.043$; $OR_{EC} = 1.65$, 95% CI = 1.04–2.60, $P = 0.033$) (Tables II and III and Figure 1B). In Jinan cohort, individuals with *FEN1* –69GA genotype were only significantly associated with increased risk of HCC (OR = 1.78, 95% CI = 1.15–

Table II. Genotype frequencies of *FEN1* –69G > A among cases and controls and their association with the risk of gastrointestinal cancers

Cancer types	Genotypes	Jinan cohort				Huaian cohort			
		Cases, *n* (%)	Controls, *n* (%)	OR[a] (95% CI)	*P*	Cases, *n* (%)	Controls, *n* (%)	OR[a] (95% CI)	*P*
HCC		*n* = 410	*n* = 423			*n* = 237	*n* = 315		
	AA	34 (8.3)	64 (15.2)	1.00 (Reference)		34 (14.2)	70 (22.1)	1.00 (Reference)	
	GA	173 (42.2)	185 (43.7)	1.78 (1.15–2.73)	0.013	117 (49.3)	149 (47.3)	1.65 (1.04–2.64)	0.043
	GG	203 (49.5)	174 (41.1)	2.25 (1.30–3.45)	0.002	87 (36.5)	96 (30.6)	1.92 (1.21–3.15)	0.010
P_{trend}[b]				0.001				0.021	
EC		*n* = 266	*n* = 386			*n* = 289	*n* = 337		
	AA	24 (9.0)	55 (14.3)	1.00 (Reference)		38 (13.2)	73 (21.8)	1.00 (Reference)	
	GA	105 (39.5)	168 (43.5)	1.42 (0.81–2.43)	0.186	144 (49.7)	163 (48.5)	1.72 (1.09–2.68)	0.020
	GG	137 (51.5)	163 (42.2)	1.93 (1.14–3.35)	0.014	107 (37.1)	100 (29.7)	2.11 (1.29–3.35)	0.002
P_{trend}[b]				0.008				0.005	
GC		*n* = 220	*n* = 250			*n* = 192	*n* = 204		
	AA	16 (7.3)	34 (13.6)	1.00 (Reference)		25 (12.8)	42 (20.6)	1.00 (Reference)	
	GA	86 (39.1)	108 (43.2)	1.72 (0.89–3.37)	0.113	96 (50.2)	101 (49.5)	1.63 (0.89–2.90)	0.102
	GG	118 (53.6)	108 (43.2)	2.33 (1.19–4.59)	0.008	71 (37.0)	61 (29.9)	1.99 (1.07–3.71)	0.026
P_{trend}[b]				0.007				0.035	
CRC		*n* = 126	*n* = 162			*n* = 110	*n* = 145		
	AA	11 (8.7)	24 (14.8)	1.00 (Reference)		17 (15.5)	30 (21.0)	1.00 (Reference)	
	GA	51 (40.5)	73 (45.1)	1.55 (0.64–3.66)	0.291	53 (48.6)	71 (48.7)	1.35 (0.70–2.72)	0.430
	GG	64 (50.8)	65 (40.1)	2.17 (0.98–5.09)	0.052	40 (35.9)	44 (30.3)	1.60 (0.79–3.44)	0.228
P_{trend}[b]				0.038				0.206	

[a]Data were calculated by logistic regression with adjustment for age, sex and smoking.
[b]Test for trend of odds was two sided and based on likelihood ratio test assuming a multiplicative model.

Table III. Genotype frequencies of *FEN1* 4150G > T among cases and controls and their association with the risk of gastrointestinal cancers

Cancer types	Genotypes	Jinan cohort				Huaian cohort			
		Cases, *n* (%)	Controls, *n* (%)	OR[a] (95% CI)	*P*	Cases, *n* (%)	Controls, *n* (%)	OR[a] (95% CI)	*P*
HCC		*n* = 411	*n* = 423			*n* = 237	*n* = 315		
	TT	39 (9.5)	60 (14.2)	1.00 (Reference)		34 (14.4)	69 (21.9)	1.00 (Reference)	
	GT	177 (43.1)	187 (44.2)	1.45 (0.91–2.34)	0.105	118 (49.7)	148 (47.1)	1.64 (1.02–2.65)	0.043
	GG	195 (47.4)	176 (41.6)	1.71 (1.04–2.75)	0.020	85 (35.9)	98 (31.0)	1.79 (1.07–3.04)	0.023
P_{trend}[b]				0.025				0.042	
EC		*n* = 249	*n* = 386			*n* = 289	*n* = 337		
	TT	20 (8.0)	53 (14.1)	1.00 (Reference)		38 (13.0)	72 (21.5)	1.00 (Reference)	
	GT	114 (45.8)	172 (45.6)	1.79 (1.01–3.14)	0.046	141 (48.8)	164 (48.6)	1.65 (1.04–2.60)	0.033
	GG	115 (46.2)	161 (42.7)	1.92 (1.10–3.41)	0.021	110 (38.2)	101 (29.9)	2.07 (1.26–3.39)	0.002
P_{trend}[b]				0.062				0.004	
Gastric cancer		*n* = 210	*n* = 250			*n* = 192	*n* = 204		
	TT	17 (8.1)	33 (13.2)	1.00 (Reference)		25 (13.1)	43 (21.1)	1.00 (Reference)	
	GT	82 (39.0)	110 (44.0)	1.46 (0.78–2.89)	0.265	95 (49.5)	102 (50.1)	1.62 (0.89–2.92)	0.101
	GG	111 (52.9)	107 (42.8)	2.07 (1.03–3.99)	0.028	72 (37.4)	59 (28.8)	2.12 (1.13–3.98)	0.013
P_{trend}[b]				0.016				0.017	
CRC		*n* = 119	*n* = 161			*n* = 110	*n* = 145		
	TT	11 (9.2)	22 (13.7)	1.00 (Reference)		16 (14.7)	30 (21.0)	1.00 (Reference)	
	GT	47 (39.5)	74 (46.0)	1.28 (0.56–3.05)	0.558	55 (49.8)	71 (48.7)	1.35 (0.65–2.74)	0.431
	GG	61 (51.3)	65 (40.3)	1.89 (0.82–4.50)	0.118	39 (35.5)	44 (30.3)	1.58 (0.77–3.44)	0.228
P_{trend}[b]				0.062				0.202	

[a]Data were calculated by logistic regression with adjustment for age, sex, smoking, drinking and hepatitis B virus infection, where it was appropriate.
[b]Test for trend of odds was two-sided and based on likelihood ratio test assuming a multiplicative model.

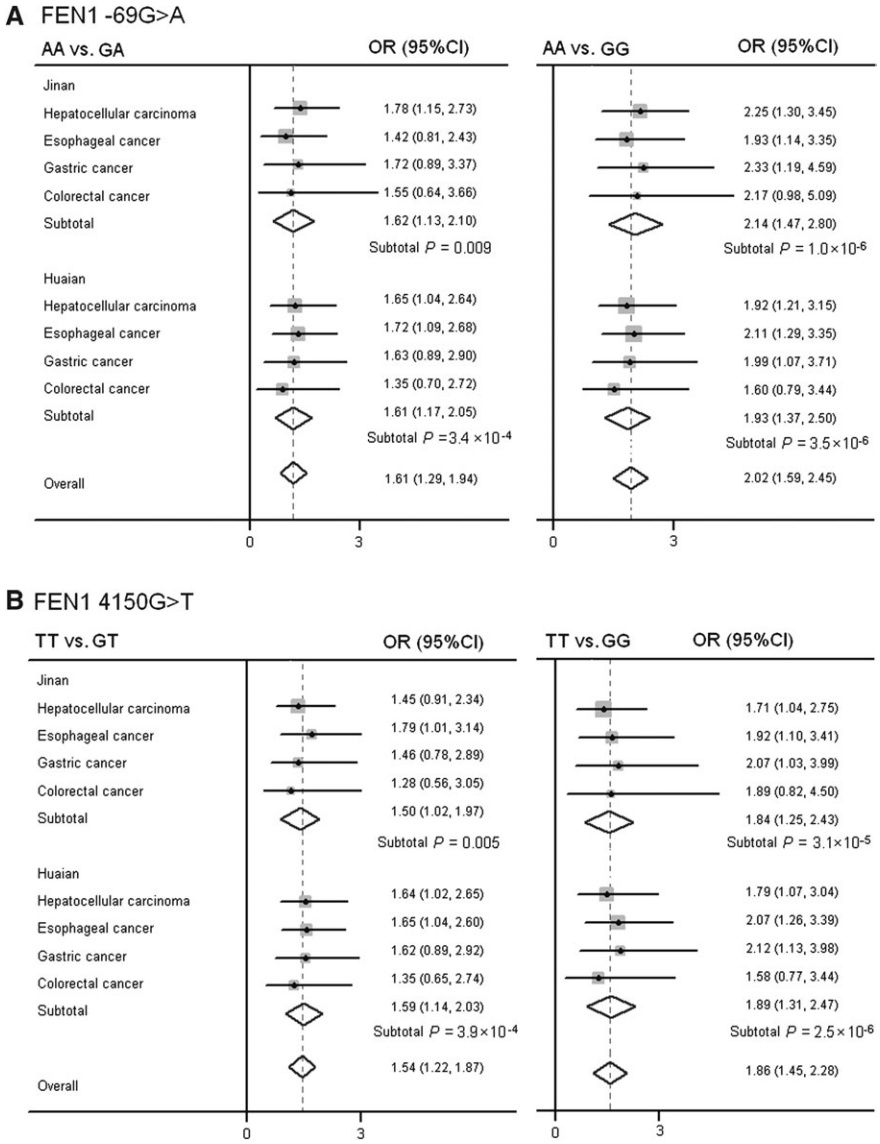

Fig. 1. Combined meta-analyses of associations between *FEN1* –69G > A or 4150G > T polymorphisms and risk of four gastrointestinal cancers (HCC, EC, GC and CRC) in Jinan and Huaian case–control cohorts. (**A**) Results of *FEN1* –69G > A polymorphism. (**B**) Results of *FEN1* 4150G > T polymorphism.

2.73, $P = 0.013$) and individuals with 4150GT genotype were only significantly associated with increased risk of EC (OR = 1.79, 95% CI = 1.01–3.14, $P = 0.046$) (Tables II and III and Figure 1A).

In the combined meta-analyses, we found that the –69GG genotype had a 2.14- or 1.93-fold increased risk for gastrointestinal cancer compared with the –69AA genotype in Jinan cohort or Huaian cohort (95% CI = 1.47–2.80, $P = 1.0 \times 10^{-6}$ or 95% CI = 1.37–2.50, $P = 3.5 \times 10^{-6}$). Interestingly, although the heterozygous –69GA genotype was not significantly associated with GC and CRC risks in both cohorts, pooled analyses also suggest that –69GA genotype had a OR of 1.62 (95% CI = 1.13–2.10, $P = 0.009$) or 1.61 (95% CI = 1.17–2.05, $P = 3.4 \times 10^{-4}$) in Jinan or Huaian cohort compared with AA genotype. Overall, results from 1850 gastrointestinal cancer cases and 2222 healthy controls showed that *FEN1* –69GG and GA genotypes were associated with increased gastrointestinal cancer risk compared with AA genotype (OR = 2.02, 95% CI = 1.59–2.45 or OR = 1.61, 95% CI = 1.29–1.94) (Figure 1A). Similar results were observed for 4150G > T polymorphism. The 4150GG and GT genotypes had a 1.86- and 1.54-fold increased risk compared with the 4150TT genotype (95% CI = 1.45–2.28 and 95% CI = 1.22–1.87) (Figure 1B). Although stratification analyses by age, sex or smoking status were also conducted, nothing interesting was observed (data not shown).

Haplotype analyses showed that $A_{-69}G_{4150}$, $G_{-69}G_{4150}$ and $G_{-69}T_{4150}$ haplotypes were associated with increased risk of developing gastrointestinal cancer in Jinan cohort, with the adjusted ORs being 1.38 (95% CI = 1.28–1.64; $P = 2.7 \times 10^{-6}$), 2.35 (95% CI 1.44–3.91; $P = 4.9 \times 10^{-4}$) and 4.06 (95% CI = 2.78–5.88; $P = 1.5 \times 10^{-14}$) compared with the $A_{-69}T_{4150}$ haplotype (Table IV). However, in Huaian cohort, only the $G_{-69}T_{4150}$ haplotype was associated with significantly increased risk of developing gastrointestinal cancer (OR = 1.29, 95% CI = 1.12–1.39) compared with the $A_{-69}T_{4150}$ haplotype (Table IV).

Since there is an allele-specific effect of –69G > A and 4150G > T SNPs on *FEN1* expression in lung tissues, we examined if similar phenomena could be observed in gastrointestinal tissues. As shown in Figure 2, we found that subjects with the –69AA genotype had significantly higher *FEN1* RNA levels (mean ± SE) than those with the –69GG and GA genotypes in liver, esophagus, stomach and colorectal normal tissues [liver: 0.217 ± 0.038 ($n = 5$) versus 0.099 ± 0.013 ($n = 15$), $P < 0.001$; esophagus: 0.242 ± 0.015 ($n = 4$) versus 0.108 ± 0.042 ($n = 11$), $P = 0.005$; stomach: 0.268 ± 0.060 ($n = 3$)

Table IV. Distribution of *FEN1* haplotypes frequencies among patients and controls and their association with gastrointestinal cancers

Haplotypes	No. of chromosomes (%)		OR[a] (95% CI)	P^b
	Patients	Controls		
Jinan cohort				
$A_{-69}T_{4150}$	532 (26.9)	849 (34.8)	1.00 (Reference)	
$A_{-69}G_{4150}$	1302 (65.8)	1520 (62.3)	1.38 (1.28–1.64)	2.7×10^{-6}
$G_{-69}G_{4150}$	42 (2.1)	29 (1.2)	2.35 (1.44–3.91)	4.9×10^{-4}
$G_{-69}T_{4150}$	103 (5.2)	41 (1.7)	4.06 (2.78–5.88)	1.5×10^{-14}
Huaian cohort				
$A_{-69}T_{4150}$	651 (39.3)	887 (44.3)	1.00 (Reference)	
$A_{-69}G_{4150}$	15 (0.9)	16 (0.7)	1.29 (0.61–2.71)	0.497
$G_{-69}G_{4150}$	12 (0.7)	14 (0.8)	1.21 (0.54–2.68)	0.693
$G_{-69}T_{4150}$	979 (59.1)	1085 (54.2)	1.29 (1.12–1.39)	0.001

[a]Adjusted for sex, age and smoking.
[b]After 1000 permutation tests.

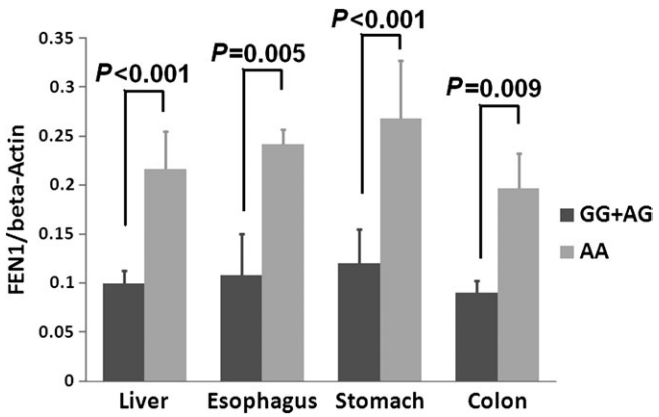

Fig. 2. *FEN1* mRNA expression in liver, esophagus, stomach and colorectal normal tissues grouped by *FEN1* –69G > A genotypes. Subjects with the –69AA genotype had significantly higher *FEN1* RNA levels (mean ± SE) than those with the –69GG and GA genotypes in liver, esophagus, stomach and colorectal normal tissues [liver: 0.217 ± 0.038 ($n = 5$) versus 0.099 ± 0.013 ($n = 15$), $P < 0.001$; esophagus: 0.242 ± 0.015 ($n = 4$) versus 0.108 ± 0.042 ($n = 11$), $P = 0.005$; stomach: 0.268 ± 0.060 ($n = 3$) versus 0.120 ± 0.035 ($n = 9$), $P < 0.001$ and colon and rectum: 0.198 ± 0.034 ($n = 3$) versus 0.091 ± 0.012 ($n = 10$); $P = 0.009$].

versus 0.120 ± 0.035 ($n = 9$), $P < 0.001$ and colon and rectum: 0.198 ± 0.034 ($n = 3$) versus 0.091 ± 0.012 ($n = 10$); $P = 0.009$]. Similar results were observed when the *FEN1* RNA levels were compared as a function of 4150G > T genotypes (data not shown).

Discussion

As an important tumor suppressor, depressed FEN1 expression may lead to malignant transformation of normal gastrointestinal cells (14). Interestingly, *FEN1* –69G and 4150G alleles, which are correlated to significantly decreased *FEN1* mRNA expression in normal gastrointestinal tissues, are associated with increased gastrointestinal cancer risks compared with –69A and 4150T alleles in two independent case–control cohorts. These results are consistent to our previous findings in lung cancer (11), indicating that these SNPs may be common cancer risk factors.

There is an obvious difference in the allelic and genotype frequencies of both –69G > A and 4150G > T SNPs in two Chinese populations included in the current study. After comparing genotype frequencies of normal healthy controls with our previous observation in Beijing and Wuhan populations, we believe that Jinan population is more similar to Beijing population, but Huaian population is similar to Wuhan population. These results also support our opinion that *FEN1* SNPs may be relatively novel, probably resulting from certain different evolutional pressures in different geographical areas. In spite of the difference between populations, these SNPs are consistently associated with risk of gastrointestinal cancers in two cohorts.

FEN1 –69G > A and 4150G > T SNPs were positively correlated to risk of HCC, EC and GC but not risk of CRC in the current study. However, *FEN1* mRNA expression results showed that these two genetic variants could also influence FEN1 expression *in vivo*, suggesting these two SNPs are also functional in colorectal cells. Negative results of the case–control studies may be due to the relative small sample size (126 patients in Jinan cohort and 110 in Huaian cohort). Therefore, these results on CRC warrant to be validated in other cohort in the future.

In summary, *FEN1* –69G > A and 4150G > T SNPs are common genetic risk factors for gastrointestinal cancers in Chinese populations. Given this fact, further efforts are warranted to explore whether *FEN1* genetic polymorphisms could be potentially useful for diagnosis of gastrointestinal cancers.

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