

ORIGINAL ARTICLE

# Relationship between growth hormone 1 genetic polymorphism and susceptibility to colorectal cancer

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The aim of this study was to evaluate the relationship between smoking, alcohol drinking and genetic polymorphism of the growth hormone 1 gene (*GH1*) T1663A with reference to colorectal cancer. We conducted a case-control study with 315 cases of colorectal cancer and 438 population-based controls in the Jiangsu Province, China. *GH1* T1663A genotypes were identified using PCR-RFLP (restriction fragment length polymorphism) methods. Information on smoking and drinking was collected using a questionnaire. Odds ratios (ORs) were estimated with an unconditional logistic model. The distribution of T/T and A/A genotypes was significantly different between controls and cases ( $\chi^2_{MH}=3.877$ ,  $P=0.049$ ). Compared with the *GH1* T/T genotype, the A/A genotype was at a decreased risk of developing colorectal cancer (sex-, age-, body mass index-, smoking- and alcohol drinking-adjusted OR=0.56, 95% confidence interval: 0.34–0.90). Smoking was not associated with the risk of colorectal cancer, whereas alcohol drinking was associated with an increased risk of colorectal cancer. Among nonsmokers or nondrinkers, individuals who had the *GH1* A/A genotype were at a decreased risk of developing colorectal cancer compared with individuals who had the *GH1* T allele. These results show that the *GH1* T1663A A/A genotype can decrease the risk for colorectal cancer.

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## INTRODUCTION

The growth hormone (GH) gene is associated with altered GH production. By binding to its receptor, GH stimulates the production of insulin-like growth factor-I (IGF-I) and its binding protein IGFBP-3, resulting in the regulation of cell proliferation, differentiation and apoptosis. IGF-1 is an important mitogen required for progression through the cell cycle. The GH/IGF-I axis has a clearly established role in somatic growth regulation, and may also contribute to neoplastic growth for several common cancers.<sup>1–3</sup> Some epidemiological studies have investigated the role of IGFs and IGFBPs in the etiology of cancers of the breast, colon, rectum, prostate and lung, as well as of childhood leukemia, and have provided reasonably consistent support for increased risk of solid tumors in association with relatively high levels of IGF-I, decreased risk of solid tumors and childhood leukemia in association with relatively high levels of IGFBP-3, as well as increased risk of breast cancer in association with a high ratio of IGF-I to IGFBP-3.<sup>1</sup> IGF-I mediates many of the physiological effects of GH, and plasma levels of IGF-I are associated with risk for colorectal cancer in healthy individuals.<sup>4–6</sup> Patients with acromegaly, a disease characterized by abnormally high levels of GH secretion, are at an elevated risk for colorectal cancer.<sup>7–9</sup> GH is the main determinant of

circulating levels of IGF-I and its main binding protein, IGFBP-3.<sup>1</sup> The *GH1* gene is polymorphic and a substitution polymorphism (T1663A) is considered to be associated with GH production.<sup>10</sup> To investigate possible relationships between *GH1* T1663A polymorphisms (rs2665802) and environmental factors (habitual smoking and alcohol drinking) for risk of colorectal cancers, we conducted a population-based case-control study in the Jiangsu Province of China.

## MATERIALS AND METHODS

### Study subjects

We recruited colorectal cancer cases using data obtained from the Cancer Registry in Huian and Jintan cities of the Jiangsu Province of China, and also recruited cases from patients who visited Jiangsu Provincial Cancer Hospital from these cities between August 2000 and September 2002. Cases were histopathologically diagnosed as having primary colorectal cancer. Physicians at the hospital or families of patients asked eligible cases to participate in our study, and doctors or nurses interviewed the subjects and collected their blood samples after obtaining informed consent. Population-based controls were selected from healthy residents in eight villages or towns of Huian and Jintan cities. Doctors of the public health center randomly selected one or two controls for each case, after matching for ethnicity, sex and age within 2 years of each case using the records of residents at the local governmental office,

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and then asked eligible residents for their participation, and conducted interviews and collected blood samples in the same manner. A total of 10 patients and 33 residents refused to participate in our study, but the final response rate was 97% for cases and 93% for controls. The ethical committee of the Jiangsu Provincial Institute of Cancer Research approved this study.

### Environmental factors

Smoking and drinking habits were covered in our questionnaire. Each subject was asked whether he/she had ever smoked at least one cigarette per day for  $\geq 6$  months. If he/she answered yes, he/she was further asked about the age at which he/she started to smoke cigarettes regularly, the average number of cigarettes smoked per day and the number of years he/she smoked. If the subject had quit smoking at least 1 year ago, the age at which he/she stopped smoking was recorded. Each subject was asked whether he/she had ever drunk alcoholic beverages at least once a month for  $\geq 1$  year. If his/her answer was yes, he/she was asked to provide the age at which he/she started to drink regularly, as well as the frequency and usual amount of liquor, beer and grape wine consumed separately every time. If the subject had quit his/her drinking habit at least 1 year ago, the age at which he/she stopped drinking was recorded. Consumption of ethanol every month was calculated according to 40 g per 100 g of liquor, 3.5 g per 100 g of beer and 12 g per 100 g of grape wine.

### DNA extraction and GH1 genotyping

Whole blood was collected into EDTA-coated tubes and centrifuged for 15 min, and the buffy coat layer was isolated. Genomic DNA was extracted from 200  $\mu$ l of buffy coat using a Qiagen QIAamp DNA Blood Mini Kit (QIAGEN Companies, Germany). The PCR assay used to detect the T-to-A variant at position 1663 (T1663A) in intron 4 of the *GH1* gene was a two-step method because of the close homologies that exist between *GH1* and other related genes in the *GH* cluster.<sup>10</sup> The primers for the first amplification were F4 (5'-GGCTGACCCAGGAGTCC-3') and R1 (5'-AGAAGGACACCTAGTCAGACA-3'). Reactions were carried out in a total volume of 25  $\mu$ l containing 12.5 pmol of each primer, 2.5  $\mu$ l 4 $\times$ dNTPs, 1.5  $\mu$ l 10 $\times$ buffer, 1 IU Tag polymerase and 1  $\mu$ l genomic DNA. PCR conditions were 95 °C for 5 min, followed by 30 cycles of 95 °C for 1 min, 62 °C for 1 min and 72 °C for 30 s, with a final extension at 72 °C for 10 min. A volume of 3  $\mu$ l of this product was further amplified with primers *GH1*F2 (5'-GAGAAACACTGCTGCCCTCTTTT AGACG-3') and *GH1*R2 (5'-AAGAGAAGGAGAGGCCAAGC-3') to produce a 180-bp product. The PCR product was subjected to *Aat*II enzyme digestion in 37 °C for 3 h, and samples were then analyzed by electrophoresis in 3% agarose gels. The T allele was digested with *Aat*II to fragments of 149 and 31 bp, whereas the A allele was not digested with *Aat*II.

### Statistical analysis

The body mass index (BMI) of subjects was calculated as weight (kg)/height ( $m^2$ ). The Mantel-Haenszel  $\chi^2$ -test was used to compare frequencies, and the *t*-test was used to compare means between cases and controls. The strength of associations between colorectal cancer and polymorphisms of *GH1* was measured as odds ratios (ORs). ORs and their 95% confidence intervals were obtained using unconditional logistic regression analysis. We calculated adjusted ORs for age (continuous), sex, BMI (continuous), smoking and drinking habits. To investigate gene-environment interactions, we also calculated (stratified analysis) ORs according to combinations of *GH1* genotypes and habits of smoking and drinking, with *GH1* T allele carriers as reference. The procedure LOGISTIC from the statistical package SAS (SAS Institute Inc., USA) was used for the calculations. The probability of Hardy-Weinberg equilibrium was assessed by the  $\chi^2$ -test.

## RESULTS

A total of 190 male and 125 female cases with colorectal cancer, and 222 male and 216 female controls (Table 1), were included in this study. The proportional distribution of females in controls was significantly higher than that in colorectal cases. The mean age and BMI did not significantly differ between cases and controls. The proportional distributions of smokers and drinkers were significantly higher in colorectal cancer cases than in controls.

**Table 1 Background characteristics of colorectal cancer cases and their controls**

Subject characteristics	Controls (n=438)		Cases (n=315)		$\chi^2_{MH}$	P-value
<b>Gender</b>						
Male	222	50.7	190	60.3	6.85	0.010 <sup>a</sup>
Female	216	49.3	125	39.7		
<b>Age (years)</b>						
<40	42	9.6	44	14.0	9.21	0.056 <sup>a</sup>
40-49	75	17.1	54	17.1		
50-59	149	34.0	88	27.9		
60-69	131	29.9	85	29.0		
>70	41	9.4	44	14.0		
Mean age $\pm$ s.d.	55.7 $\pm$ 11.0		55.3 $\pm$ 12.7			0.617 <sup>b</sup>
<b>BMI (<math>kg\ m^{-2}</math>)</b>						
$\leq 22.9$	222	50.7	161	51.1	0.11	0.945 <sup>a</sup>
23-29.9	205	46.8	145	46.0		
$\geq 30$	11	2.5	9	2.9		
Mean BMI $\pm$ s.d.	23.2 $\pm$ 3.0		23.1 $\pm$ 3.2			0.565 <sup>b</sup>
<b>Smoking status</b>						
Nonsmoker	283	64.6	176	55.9	7.86	0.020 <sup>a</sup>
Current smoker	138	31.5	116	36.8		
Former smoker	17	3.9	23	7.3		
<b>Smoking index (pack-years)</b>						
0	283	64.6	176	55.9	6.65	0.036 <sup>a</sup>
1-19	68	15.5	54	17.1		
$\geq 20$	87	19.9	85	27.0		
<b>Drinking status</b>						
Nondrinker	329	75.1	190	60.3	20.97	0.001 <sup>a</sup>
Current drinker	98	22.4	104	33.0		
Former drinker	11	2.5	21	6.7		
<b>GH-1 T1663A genotypes</b>						
T/T	167	38.1	133	42.2	3.91	0.142 <sup>a</sup>
T/A	201	45.9	147	46.7		
A/A	70	16.0	35	11.1		

Abbreviation: BMI, body mass index.

<sup>a</sup>Mantel-Haenszel  $\chi^2$ -test.

<sup>b</sup>*t*-test.

The distributions of *GH1* T/T, T/A and A/A genotypes were 38.1, 45.9 and 16.0% in controls, and 42.2, 46.7 and 11.1% in colorectal cases, respectively (Table 1). The proportional distribution of T/T and A/A genotypes was significantly different between controls and colorectal cancer cases ( $\chi^2_{MH}=3.877$ ,  $P=0.049$ ), but that of three groups was not significantly different ( $\chi^2_{MH}=3.907$ ,  $df=2$ ,  $P=0.142$ ). The frequencies of the A variant of the *GH1* allele were 39% for controls and 34% for colorectal cancer cases, and were in Hardy-Weinberg equilibrium ( $\chi^2=0.532$  and  $\chi^2=0.350$ ,  $P$ -value  $> 0.05$ ). It shows that subjects from the population are representative. After adjusting for sex, age, BMI and habitual smoking and drinking, decreased OR for colorectal cancer (0.56, 95% confidence interval=0.34-0.90) was observed in individuals with the *GH1* A/A genotype, when compared with the *GH1* T/T genotype. Similar ORs were observed in stratified analyses for males and females and for colon and rectal cancer, although they were not statistically significant (Table 2).

Table 3 shows the relationship of smoking and alcohol drinking to colorectal cancer. There was no statistically significant association

**Table 2 GH1 genotypes and risk of colorectal cancer**

Genotype	Controls	Colorectum	OR (95% CI)	Colon	OR (95% CI)	Rectum	OR (95% CI)
<i>Total<sup>a</sup></i>							
T/T	167	133	1.00	50	1.00	83	1.00
T/A	201	147	0.89 (0.65–1.22)	44	0.69 (0.43–1.09)	103	1.02 (0.71–1.47)
A/A	70	35	0.56 (0.34–0.90)	11	0.50 (0.24–1.03)	24	0.61 (0.35–1.07)
<i>Male<sup>b</sup></i>							
T/T	85	77	1.00	31	1.00	46	1.00
T/A	103	94	0.96 (0.62–1.47)	28	0.64 (0.35–1.18)	66	1.21 (0.74–1.97)
A/A	34	19	0.55 (0.28–1.07)	6	0.43 (0.16–1.16)	13	0.63 (0.29–1.38)
<i>Female<sup>b</sup></i>							
T/T	82	56	1.00	19	1.00	37	1.00
T/A	98	53	0.79 (0.49–1.28)	16	0.69 (0.33–1.46)	37	0.85 (0.49–1.47)
A/A	36	16	0.58 (0.29–1.18)	5	0.51 (0.17–1.56)	11	0.64 (0.29–1.43)

Abbreviations: BMI, body mass index; CI, confidence interval; OR, odds ratio.

<sup>a</sup>ORs were adjusted for age, sex, BMI and status of smoking and alcohol drinking.<sup>b</sup>ORs were adjusted for age, BMI and status of smoking and alcohol drinking.**Table 3 Smoking and alcohol drinking with risk of colorectal cancer**

	Controls (n)	Cases (n)	OR	95% CI
<i>Smoking status<sup>a</sup></i>				
Nonsmoker	283	176	1.00	
Current smoker	138	116	0.99	0.68–1.46
Former smoker	17	23	1.38	0.66–2.89
Current and former	155	139	1.03	0.71–1.49
<i>Smoking index (pack-years)<sup>a</sup></i>				
0	283	176	1.00	
1–19	68	54	0.92	0.58–1.46
≥20	87	85	1.13	0.72–1.75
<i>Drinking status<sup>b</sup></i>				
Nondrinker	329	190	1.00	
Current drinker	98	104	1.68	1.14–2.50
Former drinker	11	21	3.04	1.37–6.72
Current and former	109	125	1.80	1.24–2.62

Abbreviations: CI, confidence interval; OR, odds ratio.

<sup>a</sup>ORs were adjusted for age, sex and alcohol drinking status.<sup>b</sup>ORs were adjusted for age, sex and smoking status.

between smoking habit and colorectal cancer risk, whereas alcohol drinking showed significantly increased ORs for colorectal cancer.

Table 4 shows data for interactions between *GH1* and habitual smoking and drinking for risk of colorectal cancer. The interactions between the *GH1* polymorphism and status of smoking and alcohol drinking were not statistically significant ( $P$  for smoking=0.4277 and  $P$  for drinking=0.1226). Compared with nonsmokers or nondrinkers with the *GH1* T allele, the *GH1* A/A genotype showed decreased ORs both in nonsmokers (age-, sex-, BMI- and alcohol drinking-adjusted OR=0.47, 95% confidence interval=0.25–0.88) and nondrinkers (age-, sex-, BMI- and smoking adjusted-OR=0.56, 95% confidence interval=0.32–0.98).

## DISCUSSION

In this study, we detected that a polymorphism in the human *GH1* gene might be associated with risk of colorectal cancer in Chinese, that is, the *GH1* 1663A/A genotype decreases the risk of colorectal cancer.

**Table 4 Interaction between GH1 genotype and status of smoking and alcohol drinking, and the odds ratios (ORs) for colorectal cancer**

	Genotype	Controls	Cases	OR (95% CI)
<i>Smoking status<sup>a</sup></i>				
Nonsmoker	T/T+T/A	238	161	1.00
Nonsmoker	A/A	45	15	0.47 (0.25–0.88)
Current and former	T/T+T/A	130	119	0.93 (0.62–1.40)
Current and former	A/A	25	20	0.72 (0.36–1.46)
<i>Smoking index (pack-years)<sup>a</sup></i>				
0	T/T+T/A	238	161	1.00
1–19	T/T+T/A	54	42	0.82 (0.49–1.37)
≥20	T/T+T/A	76	77	0.99 (0.78–1.26)
0	A/A	45	15	0.47 (0.25–0.88)
1–19	A/A	14	12	0.73 (0.30–1.77)
≥20	A/A	11	8	0.86 (0.52–1.41)
<i>Drinking status<sup>b</sup></i>				
Nondrinker	T/T+T/A	276	161	1.00
Nondrinker	A/A	53	19	0.56 (0.32–0.98)
Current and former	T/T+T/A	92	109	1.74 (1.16–2.62)
Current and former	A/A	17	16	1.37 (0.65–2.89)

Abbreviations: BMI, body mass index; CI, confidence interval.

<sup>a</sup>ORs were adjusted for age, sex, BMI and alcohol drinking status.<sup>b</sup>ORs were adjusted for age, sex, BMI and smoking status.

GH secretion is partially determined by polymorphisms in the *GH1* gene; Hasegawa *et al.*<sup>11</sup> observed that frequency of the A allele at A1663T in GH-insufficient children, normal short children and healthy normal-height adults was 57.0, 35.9 and 42.2%, respectively. The frequency of the polymorphism in the GH insufficiency group was significantly different from that in other groups. Six of eight patients with the severe type of GH insufficiency had the A/A genotype at A1663T, whereas two had the A/T genotype at A1663T (none had the T/T genotype). These results showed that the *GH1* 1663 A allele is associated with lower GH secretion. Le Marchand *et al.*<sup>10</sup> has also found that the *GH1* 1663 A/A genotype is associated with a lower ratio of plasma IGF-I/IGFBP-3 and a higher level of IGFBP-1, which are consistent with a lower GH secretion. The carcinogenic role of IGFs in cancer is supported by epidemiological studies, which have

found that high levels of circulating IGF-I and low levels of IGFBP-3 are associated with increased risk of several common cancers, including colorectal cancer, breast cancer, prostate cancer, ovarian cancer, lung cancer and childhood leukemia.<sup>1,10</sup> Le Marchand *et al.*<sup>10</sup> have observed that the human *GH1* gene T1663A polymorphism is associated with decreased risk of colorectal cancer in Caucasians and native Hawaiians. Khoury-Shakour *et al.*<sup>12</sup> found that the A allele of the *GH1* polymorphism is associated with reduced risk of colorectal cancer among physically inactive individuals, indicating an interaction between physical activity and the GH/IGF-I system. Our findings in this study are consistent with these previous results.

In stratified analyses, we found that both males and females with the *GH1* 1663A/A genotype have a decreased OR for colorectal, colon and rectal cancers, although it was not statistically significant.

In the previous study, we found that alcohol drinking is associated with increased risk of colorectal cancer, whereas smoking is not associated with risk of colorectal cancer.<sup>13,14</sup> In this study, we found that the protective effect of the *GH1* 1663A/A genotype for colorectal cancer was more notable in nonsmokers of cigarettes and nondrinkers of alcohol. The relationship between GH1 and habitual smoking and drinking is unclear, but *GH1* increases production of both IGF-1 and IGFBP-3, accounting in part for the relatively high correlation between plasma IGF-1 and IGFBP-3. Our result showed that habitual smoking and drinking may affect the role of *GH1*, or may affect the serum levels of IGF-1 and IGFBP-3. There are already some studies on the association between habitual smoking and alcohol drinking and the serum levels of IGF-I and IGFBP-3,<sup>15–21</sup> although those results are inconsistent. However, several studies have suggested that habits of smoking and alcohol drinking are associated with serum levels of IGF-1 and IGFBP-3. Kaklamani *et al.*<sup>15</sup> observed that serum levels of IGF-1 were positively associated with pack-year history of smoking, and serum levels of IGF-BP3 were independently and negatively associated with the number of cigarettes per day or pack-year history of smoking. Clinical studies also showed that high-nicotine cigarettes increase serum GH levels.<sup>20,21</sup> Gapstur *et al.* found that greater alcohol intake and, to a lesser extent, greater number of cigarettes smoked per day, were associated with lower serum IGF-I concentrations in both Black and White men. Smoking was also inversely associated with IGFBP-3, but only in White men.<sup>16</sup> Yu and Rohan<sup>1</sup> noted that the associations of alcohol and cigarette smoking with IGF-I and IGFBP-3 could be mutually confounding. In this study, we found no significant interaction between habitual smoking or drinking and the *GH1* T1663A polymorphism with regard to risk for colorectal cancer.

Finally, some limitations in this study require further discussion. The sample size in this study was not sufficient for stratified subgroup analyses, with consequent reduction in the magnitude of statistical power and increase in the potential for random error. Another possible problem is selection bias for controls, these being recruited by local health staff, although from the general population with a high response rate. The proportional distribution of females in controls was higher than that in colorectal cases, which may have caused a lower prevalence of smokers and alcohol drinkers in the present controls, although we adjusted for sex and age in all statistical analyses.

In summary, this study revealed a positive association between the *GH1* T1663A polymorphism and decreased risk of colorectal cancer, with a significant interaction between the *GH1* T1663A polymorphism and habitual smoking and alcohol drinking with regard to development of colorectal cancer. The data support the fact that colorectal cancer susceptibility with *GH1* polymorphisms may be altered by background environmental factors.

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