RESEARCH COMMUNICATION

The G-protein β3 Polymorphism is Associated with Diffuse Type Gastric Cancer in Japanese

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Abstract

From epidemiological evidence a high salt diet is a risk factor for gastric cancer, independent of *Helicobacter pylori* infection, and animal studies have shown that salt promotes carcinogenesis. The G-protein β 3(GNB3) C825T polymorphism has been linked with hypertension, salt sensitivity and multiple diseases. Our aim in this study was to clarify any association of the GNB3 C825T polymorphism with gastric cancer risk in Japanese. We examined 161 patients with gastric cancer and 183 control subjects. All underwent stomach biopsy by endoscopic procedures, and extracted DNA was genotyped using a primer pair including the GNB3 C825T polymorphism area by PCR-based restriction fragment length polymorphism (PCR-RFLP). Logistic-regression analysis was performed to assess the impact of the genetic polymorphism. Overall comparison of genotype frequency showed the CT genotype in control patients to be relatively infrequent, but no statistically significant differences were found. However, on comparison of subtypes of gastric cancer (intestinal and diffuse), a significantly increased risk of diffuse type of gastric cancer was foudn for the TT genotype (odds ratio compared to CC, 3.1, 95%CI 1.1-8.6, p=0.03). In conclusion, the TT genotype of GNB3 was associated with diffuse type of gastric cancer. The mechanism of the GNB3 polymorphism interaction with gastric cancer development needs to be clarified by future study.

Keywords: G-protein β3 - gastric cancer - PCR-RFLP - polymorphism

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Introduction

Gastric cancer is a still considerable public health problem in current world. While the incidence and mortality rates of gastric cancer have decreased in recent years, gastric cancer is the second cause of cancer death around the world (Murray and Lopez, 1997; Parkin et al., 2005). *Helicobacter pylori* (*H. pylori*) was designated as a causative pathogen for gastric carcinogenesis (1994). Inflammation may be a key factor in the process of carcinogenesis from chronic gastritis induced by *H. pylori* (Correa, 1992). However, there is a discrepancy that only a small percentage of infected patients actually develop gastric cancer. This fact suggests that some genetic factors may play an important role in the development of gastric cancer in long term outcome of *H. pylori* infection.

Epidemiologically, it has been reported that a high salt diet was one of risk factors for gastric carcinogenesis other than *H. pylori* infection (Weisburger, 2004; Tsugane, 2005; Shikata et al., 2006). The mechanisms of salt intake to gastric carcinogenesis are still unknown, however, animal model was also develop gastric cancer under high salt intake surroundings (Nozaki et al., 2002). The study showed salt itself did not have initiation effect for gastric carcinogenesis, but promoted carcinogenesis in stomach (Nozaki et al., 2002).

Heterotrimeric G-proteins play an important role in the stimulus-response coupling of membrane receptors linked to intracellular membrane receptors (Gollasch et al., 1993, Kleuss et al., 1991; 1992; 1993). It has been investigated about the potential role of these signal transduction systems in the development of several diseases that affect smooth muscle such as vascular disorder (Penn and Benovic, 2008). G-proteins are composed of different α, β, and γ subunits isoforms, and the β and γ subunits forming a functional monomer(Olate and Allende, 1991). On receptor activation, both α and β subunits dissociate from the receptor and in turn modulate a large variety of intracellular effecter systems(Johnson et al., 1991; Wieland and Mittmann, 2003). Thus, G-protein dysfunction could potentially block intracellular signal transduction.

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G-protein β3 (GNB3) C825T polymorphism have been interested in the association of hypertension as salt sensitivity gene and many variety diseases(Hegele et al., 1999, Kato et al., 1998, Klintschar et al., 2005, Morrison et al., 2001). This polymorphism give rise to three possible genotypes (i.e., CC, TC, and TT), and was reported to be associated with gene activity (Baumgart et al., 1999).

Our aim of this study was to clarify the association of GNB3 C825T polymorphism as a salt sensitivity gene and gastric cancer risk in Japanese.

Materials and Methods

Cases and Controls

This study included patients who received upper gastrointestinal endoscopy at the Fujita Health University Hospital in Japan. Consecutively enrolled subjects were screened for gastric cancer by upper gastrointestinal endoscopy followed by a barium X-ray examination. Cases were 161 Japanese patients (111 men, and 50 women, average age 64.3±12.4 years) who were diagnosed with primary gastric cancer, and controls were 174 patients (88 men, and 86 women, average age 60.4±13.3 years) without gastric cancer who also underwent upper gastrointestinal endoscopy. Gastric cancer was diagnosed histologically at the Pathology Division of our hospital, and cancers was classified according to Lauren' s classification (Lauren, 1965). The information of cancer staging and anatomic location were also obtained for the evaluations of clinico-pathological features. Patients with malignancies in other organs were excluded.

The Ethical Committee of the School of Medicine at Fujita Health University approved the protocol. Written informed consent was obtained from each subject.

DNA Extraction

Gastric biopsy specimens were taken from the non-cancerous mucosa in the antrum and the greater curvature of the stomach, using an upper gastrointestinal scope. Some parts of each specimen were fixed in 10% buffered formalin and embedded in paraffin, while the other parts were immediately frozen and stored at -80°C until DNA extraction. All histological diagnoses were made at the Division of Pathology of our hospital. The genomic DNA was extracted from stored samples at -80°C using proteinase K and DNA extraction kits (Quiagen, Valencia, CA).

Polymorphism Analysis of GNB3 Gene

The genotype for the GNB3 C825T polymorphism was determined using PCR-based restriction fragment length polymorphisms (PCR-RFLP). We used primers that included the GNB3 C825T polymorphism area. Subsequently, the identification was done after PCR-amplification, using the following primers 5'-TGACCCACTTGCCACCCGTGC-3'and 5'-GCAGCAGCCACCGCTGGC-3'. PCR was carried out with 0.1μg of genomic DNA in a volume of 20μL. The DNA was denatured at 95°C for 5 minutes, followed by 30 cycles at 95°C for 45 seconds, 64°C for 45 seconds, and 72°C for 45 seconds, with a final extension at 72°C for 7 minutes. The PCR reactions were done using Blend Taq (Toyobo Co., Ltd., Osaka, Japan). The amplified PCR products were digested overnight with 5 units of BseDI (Fermentas, Ontario, Canada) at 55°C. Subsequently, the digested products were analyzed on 3% agarose gels. These gels were stained with ethidium bromide (0.5 μg/mL), and the genotypes were determined by analyses of different bands. The presence of a BseDI site was indicated by the cleavage of the 268 bp amplified product to yield fragments of 152 and 116 bp. Genotyping was confirmed by direct sequencing in a few randomly selected samples.

Detection of H. pylori

H. pylori infection status was determined by microscopic examination, urea breath test, or serum anti-HP antibody titers. Infection was diagnosed when at least one of these tests was positive.

Statistical Analysis

Hardy-Weinberg equilibrium of the GNB3 gene allele in the controls and gastric cancer patients were assessed by c^2 statistics. Clinical characteristics between patients with or without gastric cancer, and differences in gastritis scores between C/C genotype and T/T were examined by the Mann-Whitney U test. Logistic-regression analysis was used to estimate odds ratios (OR) and 95% confidence intervals (Camilleri et al.) for the genotypes, with adjustment for age, sex, and H. pylori infection status. A p value <0.05 was considered statistically significant.

Results

Characteristics of the subjects

The characteristics of the cases and controls are summarized in Table 1. Average age and H. pylori infection rate was higher in gastric cancer patients than in controls ($p<0.05$).

Distribution of the GNB3 genotypes

Table 2 shows the genotype frequencies of GNB3 in patients with gastric cancer and the control. The C825T polymorphism of GNB3 was typed in all 335 subjects. Among cases, the distribution of genotypes was as follows: CC=33, CT=90 and TT=38. Among controls, the distribution was as follows: CC=42, CT=84 and TT=48 (Table 2). The frequency of GNB3 polymorphism in the controls and gastric cancer patients did not deviate significantly from those expected under the Hardy-Weinberg equilibrium (p=0.13, 0.66 respectively).

In additional analyses, the associations between the GNB3 polymorphism and clinicopathological features of gastric cancer, such as tumor location, stage, Lauren's histological classification, lymphatic and venous invasion, lymph node metastasis, peritoneal dissemination and distant metastasis were evaluated. There was a significant increased risk of diffuse type of gastric cancer in TT genotype. The odds ratio of gastric cancer of TT polymorphism of GNB3 C825T referred to CC was 3.1 (95%CI 1.1-8.6), (p=0.03). In contrast in intestinal type of gastric cancer patients, the TT genotype was relatively infrequent compare to the CC genotype (Table 3).

Table 1. Characteristics of Gastric Cancer Patients and Control Patients

	GC	Controls	P
Number	161	174	
Males/females	111/50	88/86	N.S.
Average age (±SD)	64.3±12.4	60.4±13.3	<0.05[a]
HP positive rate (%)	91.3	57.5	<0.05[a]

GC: gastric cancer, HP: Helicobacter pylori; [a] GC vs. Controls, Mann-Whitney U test.

Table 2. GNB3 Polymorphism and GC Risk

Genotypes	patients with GC n (%)	control patients n (%)	OR (95%CI) CC vs TT	P
C/C	33 (20.4)	42 (24.1)	reference	
C/T	90 (55.9)	84 (48.3)	NA	0.097
T/T	38 (23.6)	48 (27.6)	1.24 (0.61-2.52)	0.550

GC: gastric cancer, CI: confident interval, NA: not analyzed

Table 3. Associaton between GNB3 Polymorphism and Clinicopathologic Characteristics of Gastric Cancer

Variables(n)	Genotype			CC vs. TT	
	C/C	C/T	T/T	OR(95%CI)	***P***
Paticnts Without GC (174)	42	84	48	Reference	
Tumor location					
Cardia (5)	0	3	2	ND	
Non-cardia (156)	33	87	36	1.26 (0.63-2.52)	0.523
Upper third (6)	0	6	0	ND	
Middle third (87)	19	50	18	1.24 (0.61-2.52)	0.550
Lower third (63)	14	31	18	1.26 (0.63-2.52)	0.523
Staging					
Early (74)	17	43	14	0.78 (0.31-.199)	0.600
Advanced (87)	16	47	24	1.71 (0.75-3.94)	0.204
Lauren's classification					
Intestinal type (89)	23	49	17	0.61 (0.26-1.46)	0.269
Diffuse type (65)	7	38	20	1.05 (0.29-3.73)	0.030
Mixed (7)	3	3	1	0.35 (0.02-6,60)	0.480
Lymphatic invasion					
Positive (75)	15	46	14	1.13 (0.44-2.88)	0.800
Negative (54)	14	26	14	0.91 (0.35-2.35)	0.844
Venous invasion					
Positive (39)	9	19	11	1.49 (0.48-4.56)	0.490
Negative (90)	20	53	17	0.84 (0.35-1.97)	0.683
Lymph node metastasis					
Positive (77)	12	44	21	2.07 (0.82-5.25)	0.125
Negative (84)	21	46	17	0.82 (0.35-1.93)	0.647
Peritoneal dissemination					
Positive (29)	6	15	8	1.32 (039-4.41)	0.655
Negative (132)	27	75	30	1.23 (0.57-2.62)	0.600
Distant metastasis					
Positive (18)	2	10	6	2.80 (0.52-15.3)	0.233
Negative (143)	31	80	32	1.10 (0.53-2.31)	0.769

All data adjusted for sex, age, and *H. pylori* infection status; *Significantly different at p<0.05 according to logistic-regression analysis; GC, gastric cancer; ND, not determined

Discussion

In this study, the homozygous GNB3 825-TT genotype was associated with an increased risk of diffuse type of gastric cancer in Japanese. As a polymorphism of salt sensitivity gene, GNB3 C825T was not related with intestinal type of gastric cancer. Although the precise mechanism of these relation remains to be elucidated, at least our findings could indicate that 825TT variant of GNB3 may influence the cellular response in gastric carcinogenesis different from intestinal metaplasia-adenoma-carcinoma sequence.

GNB3 825T allele variant was initially reported as a susceptible genotype associated with essential hypertension (Siffert et al., 1998). This association was postulated due to enhanced G-protein activation and increased cellular responses as a result of active splice variant of GNB3 C>T substitution (Siffert et al., 1998). Increased cellular response is associated with cellular Na+-H+ exchanger activity in these population(Delva et al., 1993). Polymorphism of GNB3 C825T is also associated with obesity, diabetes mellitus and atherosclerosis other than essential hypertension(Siffert et al., 1999a; b; Hegele et al., 1999; Wascher et al., 2003; Kiani et al., 2005; Grove et al., 2007; Daimon et al., 2008). The relation to obesity was hypothesized as the adipogenesis activity of G proteins (Su et al., 1993).

Studies about GNB3 C825T and carcinogenesis have

been reported recently in multiple type of cancer(Krippl et al., 2004; Eisenhardt et al., 2005; Sheu et al., 2007; Clar et al., 2008; Lehnerdt et al., 2008). In breast cancer patients 825TT genotype was associated with significant reduction of bone metastasis risk (Clar et al., 2008). In head and neck squamous cell carcinoma, significant decrease of relapse free and overall survival were observed in same genotype(Lehnerdt et al., 2008). Our study is the first report about the association of GNB3 825TT variant and diffuse type of gastric cancer development. Whereas, in bladder carcinoma patients, non-smokers revealed a shorter time to metastasis in 825T-allele carriers (Eisenhardt et al., 2005). And oncocytic thyroid tumors of follicular cell origin were significantly associated with the C allele of the GNB3 polymorphism (Sheu et al., 2007). These different results (different biological effect of this polymorphism) of studies including our study may be explained due to the different signal transduction route of GNB3 in different tumor cell type.

In this study, we focused on the function of GNB3 in gastric cancer development as a salt sensitivity gene. In experimental studies using animal model with H. pylori infection, salt is an important factor for intestinal metaplasia or carcinogenesis in animal gastric mucosa (Nozaki et al., 2002; Bergin et al., 2003). In carcinogenesis model, the majority of developmental cancer histology was differentiated type (Nozaki et al., 2002). In these studies, the precise role of high salt was not unveiled, however, these findings suggested the role of salt as an accelerator (promoter) in the process of cancer development. In our study, the polymorphism of GNB3 C825T was associated with diffuse type of gastric cancer in TT genotype. The association of undifferentiated type of gastric cancer and GNB3 polymorphism suggests that GNB3 variant involvement to another mechanism of gastric cancer development not to related to adenoma carcinoma sequence. One possible reason is the activation of MAP kinase through a signal transduction of Ras and Raf protein kinase associated with GNB3 (Moor et al., 2001; Rosskopf et al., 2003).

Interestingly, in recent studies, there were increasing reports about gastrointestinal dysfunction and GNB3 C825T polymorphism (Tahara et al., 2008; Van et al., 2008; Vries et al., 2009; Oshima et al., 2010). These association mechanisms were hypothesized by the differences of strength of signal transduction in pre-synaptic 5HT (serotonin) and NE (norepinephrin) release(Jankowski and Talley, 2009). In contrast of the results of upper gastrointestinal functional disorders and GNB3 polymorphism, no association between IBS and GNB3 has been detected in multiple studies(Andresen et al., 2006; Saito et al., 2007; Grudell et al., 2008). In the near future, more detailed association mechanism will be clarified.

In conclusion, the TT genotype of GNB3 was associated with diffuse type of gastric cancer. Nevertheless, the precise mechanism for these results remains to be determined, this polymorphism was possibly associated with not sequential gastric cancer development. One reason of these results might be salt sensitive effect of GNB3. The mechanism of GNB3 polymorphism and gastric cancer development is necessary to be clarified in future study. And in next step, the association of tumor metastasis and GNB3 polymorphism should be examined.

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