The relationship between gene polymorphism and gastric cancer development - *How is salt sensitivity associated with gastric cancer development?*

Authors:

Tomoyuki Shibata, MD PhD

Department of Gastroenterology, Fujita Health University School of Medicine

Addess: 1-98 Dengakugakubo, Kutsukake-cho, Toyoake, Aichi, 470-1192, Japan

Address for correspondence:

Tomoyuki Shibata, 1-98 Dengakugakubo, Kutsukake-cho, Toyoake, Aichi, 470-1192, Japan

Phone: +81-562-93-9240,

Fax: +81-562-93-8300,

Email: shibat03@fujita-hu.ac.jp

Key Words: *Helicobacter pylori*, cytotoxin-associated gene A, gastric cancer, salt sensitivity, angiotensinogen, G-protein β 3 subunit, genetic polymorphism

Abstract

Since the discovery of *Helicobacter pylori*, the relationships between *Helicobacter pylori* infection and several diseases have been identified.

Especially, *Helicobacter pylori* has been investigated as a risk factor for gastric cancer, and some toxins (cytotoxin-associated gene A and vacuolating cytotoxin A) produced by *Helicobacter pylori* were identified. Cytotoxin-associated gene A has been continuously investigated as a promoter of gastric carcinogenesis. Although toxins are an important factor for gastric carcinogenesis, host factor or other risk factors for gastric carcinogenesis are also important to explain the incident rate of gastric carcinogenesis in *Helicobacter pylori* infectious patients. Many genetic polymorphisms were picked up as candidates for gastric cancer susceptibility. Salt was firstly demonstrated as a risk factor of gastric cancer in 1950s, and salt was revealed as a promoter for gastric carcinogenesis in some experimental examinations. Salt sensitivity genes (angiotensinogen, α- and β-adducin, aldosterone synthase gene, the G-protein β 3 subunit) have been also investigated as a risk factor for hypertension and gastric carcinogenesis. More studies will be needed to clarify these relationships.

1. Introduction

Since the discovery of *Helicobacter pylori* (*H. pylori*) approximately 30 years ago, the relationships between *H. pylori* and several diseases have been identified[1]. In particular, three major cohort studies showed an association between *H. pylori* and gastric cancer, thereby attracting a lot of attention from previously skeptical gastroenterologists[2-4]. Soon after these studies were published, the International Agency for Research on Cancer (IARC) defined *H. pylori* as a class I carcinogen, thus confirming its association with gastric cancer [5].

This review summarized studies that proved that *H. pylori* is a risk factor for gastric cancer as well as studies that investigated how salt intake, another proposed risk factor for gastric cancer, contributes to gastric cancer, with the focus on host factors in the form of genetic polymorphisms.

2. Helicobacter infection and gastric cancer

With regards to the carcinogenicity of *H. pylori*, while undeniable, the next question to answer is what factors (causative mechanism) actually lead to the development of gastric cancer. Toxins produced by *H. pylori* that are more harmful than ammonia have been found, and these toxins activate intracellular signal transduction pathways by directly injecting into the cells, causing mutations, inducing vacuole-like membrane vesicles in the cytoplasm and altering the cytoskeleton[6-10]. Among these toxic proteins, CagA (Cytotoxin-associated gene A) protein was extensively investigated by researchers from early days, and virulent genetic pattern was clarified. The potent toxin CagA, called the East Asian type, harbors an EPIYA-D site; it is so named because it is derived from a bacterial strain common in East Asia, particularly in Japan and Korea[11]. Regarding CagA in particular, the question of why regional differences occur in infection and gastric cancer onset rates has been elucidated to some extent. Despite this association, the potent toxic strain of *H. pylori* that infects people does not necessarily cause gastric cancer in all patients whom it infects. One underlying cause of this is thought to be the host factors[12].

3. Host factors for gastric carcinogenesis

Since the 2000s, when the Human Genome Project was completed, differences in individual genotypes have gradually been identified. Available single-nucleotide polymorphism (SNP) data revealed racial differences in susceptibility to gastric cancer[12, 13]. SNPs and epigenomes were factors underlying human individuality. The development of sequencing methods and genome-wide analysis enabled researchers to gradually identify the factors that determined susceptibility to certain diseases. However, the genetic polymorphisms that indicate clear susceptibility to oncogenesis remain unknown. In contrast, whole-genome analysis has revealed the types of mutations associated with the histological types of gastric cancer[14].

4. Salt and gastric cancer risk

Are there any other risk factors for gastric cancer? In addition to host and bacterial factors, smoking has been found to be an environmental risk factor for gastric cancer[15, 16]. Salt intake has also been believed to be a risk factor for gastric cancer from some time. Subsequent to the report by Sato et al. that indicated an association between salt and gastric cancer, various studies have been conducted to further define this relationship[17]. An epidemiological study discovered a high incidence of gastric cancer in regions where large amounts of foods preserved in salt are consumed. Furthermore, the decrease in gastric cancer incidence in the United States was assumed to be due to the popularization of household refrigerators, which led to a decrease in the consumption of salted foods[18, 19]. An epidemiological study conducted by Tsugane et al. demonstrated that high salt intake may exacerbate *H. pylori* infection [20].

This leaves us with the question of whether high salt intake is actually associated with gastric cancer onset. Charnley et al. reported that after feeding a high-salt diet to rats, subsequent flow cytometric analysis of the gastric mucosa revealed an increase in the number of cells at the S-phase of the cell cycle, leading to the conclusion that susceptibility to sensitization to mutagens causes gastric cancer[21]. Furihata et al.

conducted a similar study on rats and demonstrated the direct clastogenicity of salt, which promotes mucosal proliferation and induces DNA synthesis as well as regeneration of the mucosa, thereby contributing to gastric cancer onset.[22]. Tatematsu et al. produced rat gastric cancer models using N-methyl-N-nitro-N-nitrosoguanidine (MNNG) or 4-nitroquinoline-1-oxide (4-NQO), and they showed that salt induced gastric carcinogenesis[23]. Furthermore, they demonstrated that the effect of salt in gastric carcinogenesis was as promoter not as initiator through the same experimental models. Similar results were reported by Nozaki et al. and Kato et al[24, 25]. Nozaki et al. designed the various experimental models these are combined N-methyl-N-nitrosourea (MNU), *H. pylori* infection and high salt food. As a result of these investigations, they concluded that gastric cancer was developed under the combined condition of the mixture of salt, MMNUU and *H. pylori* infection. Kato et al. also reported that the promoter effect of gastric carcinogenesis depends on the concentration of salt. Fox et al. used mouse models, and they showed high salt intake induced the spread of *H. pylori* colonies and the progression of gastritis[26].

From these results as described above, it was revealed that salt had the progression effect of *H. pylori* infection, however, the mechanism of this effect is still unclear. As one of hypothesis, a study showed that one of the toxins of *H. pylori*, CagA expression was increased with salt condition[27]. The enhancement of the toxin may favor for the *H. pylori* infection status.

5. The polymorphisms of salt sensitivity genes

Are there some sort of relationships between salt consumption and the host sensitivity? The initial analysis about the association between salt consumption and the host sensitivity was the analysis about hypertensive patients. Cardiac researchers are very interested in finding a genetic association between salt sensitivity and hypertension. Angiotensinogen (AGT) is a salt sensitivity gene implicated in this process and is one of the most important elements of the rennin-angiotensin system (RAS). AGT and RAS are involved in

vascular tone, cardiovascular remodeling, salt and water balance[28]. Recently, there has been strong interest in the AGT M235T polymorphism and its association with hypertension and salt sensitivity[29, 30]. This polymorphism was previously reported to be associated with plasma AGT levels[30]. The T235 allele varies widely in frequency, occurring in 35%–45% of whites, 75%–80% of Asians and African Americans, and 90% of Africans[31, 32]. These distribution differences led to a hypothesis that the T235 allele, which was associated with higher angiotensinogen expression and greater sodium reabsorption, was adaptive in the tropical, sodium-poor environment of sub-Saharan Africa but was selected against as modern humans radiated out of Africa into other environments.

This association between salt sensitivity genes and gastric cancer risk also attract the interest of investigators, and Shibata et al. performed a case-control study with the intention to clarify this relationship[33]. They studied 399 Japanese patients included gastric cancer patients, and they were divided into two groups for assessment of AGT polymorphism. There were 197 gastric cancer (GC) patients, and there were 202 patients with no evidence of GC who served as the control group. The AGT M235T polymorphism was investigated by PCR-based restriction fragment length polymorphism (PCR-RFLP) analysis. The genotype distribution was not significantly different between GC and control patients (Table 1). There is another study reporting the relation between AGT polymorphism and gastric cancer risk[34]. Although in this study, there was a significant relationship between AGT-20 C allele and *H. pylori* related gastric cancer risk, the authors did not mention about the salt sensitivity.

In addition to the polymorphisms of AGT, candidates of salt sensitivity genes were α- and β-Adducin, aldosterone synthase gene (CYP11B2), the G-protein β 3 subunit (GNB3). There is one study about the association between the polymorphism of GNB3 C825T and gastric cancer risk[35]. Cases were 161 Japanese patients (111 men, and 50 women) who were diagnosed with primary gastric cancer, and controls were 174 patients (88 men, and 86 women)

without gastric cancer. The genotype for the GNB3 C825T polymorphism was determined using PCR-RFLP. In overall analysis, there was no significant difference between case and control group, however, there was a significantly increased risk of diffuse type of gastric cancer in TT genotype (Table 2).

Studies about the polymorphism of salt sensitivity genes and gastric cancer risk do not exist other than in our reports. The reason is not clear, however, the researchers may think the role of salt is not so important than *H. pylori* infection upon gastric carcinogenesis. Even so, future study is needed to clear the relationship between salt sensitivity and gastric cancer development.

6. Summary and conclusions

In this review, study history about associated factors with gastric carcinogenesis, especially about salt in both experimental studies and cohort studies was described. Salt is necessary for the maintenance of human homeostasis, however, excessive salt intake (around over 10g/day) may induce various harmful events in humans. Salt alone seems not to initiate gastric cancer, whereas gastric cancer development is found after *H. pylori* eradication. Therefore there is a possibility that excessive intake of salt may be involved with this event. The continued study about the mechanism of promoter effect of salt upon gastric carcinogenesis will be needed.

Acknowledgement

I thank all members of department of gastroenterology and endoscopy unit in Fujita Health University for helping taking samples.

References

1. Marshall BJ, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. *Lancet (London, England)*. 1984; **1**: 1311-5.

2. Forman D, Newell DG, Fullerton F, Yarnell JW, Stacey AR, Wald N, *et al.* Association between infection with Helicobacter pylori and risk of gastric cancer: evidence from a prospective investigation. *Bmj*. 1991; **302**: 1302-5.

3. Nomura A, Stemmermann GN, Chyou PH, Kato I, Perez-Perez GI, Blaser MJ. Helicobacter pylori infection and gastric carcinoma among Japanese Americans in Hawaii. *N Engl J Med*. 1991; **325**: 1132-6.

4. Parsonnet J, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N, *et al.* Helicobacter pylori infection and the risk of gastric carcinoma. *N Engl J Med*. 1991; **325**: 1127-31.

5. Schistosomes, liver flukes and Helicobacter pylori. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Lyon, 7-14 June 1994. *IARC Monogr Eval Carcinog Risks Hum*. 1994; **61**: 1-241.

6. Blaser MJ, Perez-Perez GI, Kleanthous H, Cover TL, Peek RM, Chyou PH, *et al.* Infection with Helicobacter pylori strains possessing cagA is associated with an increased risk of developing adenocarcinoma of the stomach. *Cancer Res*. 1995; **55**: 2111-5.

7. Crabtree JE, Farmery SM. Helicobacter pylori and gastric mucosal cytokines: evidence that CagA-positive strains are more virulent. *Laboratory investigation; a journal of technical methods and pathology*. 1995; **73**: 742-5.

8. Tsutsumi R, Takahashi A, Azuma T, Higashi H, Hatakeyama M. Focal adhesion kinase is a substrate and downstream effector of SHP-2 complexed with Helicobacter pylori CagA. *Molecular and cellular biology*. 2006; **26**: 261-76.

9. Leunk RD, Johnson PT, David BC, Kraft WG, Morgan DR. Cytotoxic activity in broth-culture filtrates of Campylobacter pylori. *Journal of medical microbiology*. 1988; **26**: 93-9.

10. de Bernard M, Arico B, Papini E, Rizzuto R, Grandi G, Rappuoli R, *et al.* Helicobacter pylori toxin VacA induces vacuole formation by acting in the cell cytosol. *Molecular microbiology*. 1997; **26**: 665-74.

11. Higashi H, Tsutsumi R, Fujita A, Yamazaki S, Asaka M, Azuma T, *et al.* Biological activity of the Helicobacter pylori virulence factor CagA is determined by variation in the tyrosine phosphorylation sites. *Proceedings of the National Academy of Sciences of the United States of America*. 2002; **99**: 14428-33.

12. El-Omar EM, Carrington M,

Chow WH, McColl KE, Bream JH, Young HA, *et al.* Interleukin-1 polymorphisms associated with increased risk of gastric cancer. *Nature*. 2000; **404**: 398-402.

13. Sugimoto M, Furuta T, Shirai N, Nakamura A, Xiao F, Kajimura M, *et al.* Different effects of polymorphisms of tumor necrosis factor-alpha and interleukin-1 beta on development of peptic ulcer and gastric cancer. *Journal of gastroenterology and hepatology*. 2007; **22**: 51-9.

14. Kakiuchi M, Nishizawa T, Ueda H, Gotoh K, Tanaka A, Hayashi A, *et al.* Recurrent gain-of-function mutations of RHOA in diffuse-type gastric carcinoma. *Nat Genet*. 2014; **46**: 583-7.

15. Gonzalez CA, Pera G, Agudo A, Palli D, Krogh V, Vineis P, *et al.* Smoking and the risk of gastric cancer in the European Prospective Investigation Into Cancer and Nutrition (EPIC). *Int J Cancer*. 2003; **107**: 629-34.

16. Nishino Y, Inoue M, Tsuji I, Wakai K, Nagata C, Mizoue T, *et al.* Tobacco smoking and gastric cancer risk: an evaluation based on a systematic review of epidemiologic evidence among the Japanese population. *Japanese journal of clinical oncology*. 2006; **36**: 800-7.

17. Sato T FT, Suzuki T, Takayanagi J, Murakami T, Shiotsuki N, Tanaka R, Tsuji R. The relation between gastric cancer mortality rate and salted food intake in several places in Japan. *Bull Inst Public Health*. 1959; **8**: 187-98.

18. La Vecchia C, Negri E, D'Avanzo B, Franceschi S. Electric refrigerator use and gastric cancer risk. *British journal of cancer*. 1990; **62**: 136-7.

19. Boeing H, Frentzel-Beyme R. Regional risk factors for stomach cancer in the FRG. *Environmental health perspectives*. 1991; **94**: 83-9.

20. Tsugane S, Tei Y, Takahashi T, Watanabe S, Sugano K. Salty food intake and risk of Helicobacter pylori infection. *Jpn J Cancer Res*. 1994; **85**: 474-8.

21. Charnley G, Tannenbaum SR. Flow cytometric analysis of the effect of sodium chloride on gastric cancer risk in the rat. *Cancer Res*. 1985; **45**: 5608-16.

22. Furihata C, Ohta H, Katsuyama T. Cause and effect between concentration-dependent tissue damage and temporary cell proliferation in rat stomach mucosa by NaCl, a stomach tumor promoter. *Carcinogenesis*. 1996; **17**: 401-6.

23. Tatematsu M, Takahashi M, Fukushima S, Hananouchi M, Shirai T. Effects in rats of sodium chloride on experimental gastric cancers induced by N-methyl-N-nitro-N-nitrosoguanidine or 4-nitroquinoline-1-oxide. *J Natl Cancer Inst*. 1975; **55**: 101-6.

24. Nozaki K, Shimizu N, Inada K, Tsukamoto T, Inoue M, Kumagai T, *et al.* Synergistic promoting effects of Helicobacter pylori infection and high-salt diet on gastric carcinogenesis in Mongolian gerbils. *Jpn J Cancer Res*.

2002; **93**: 1083-9.

25. Kato S, Tsukamoto T, Mizoshita T, Tanaka H, Kumagai T, Ota H, *et al.* High salt diets dose-dependently promote gastric chemical carcinogenesis in Helicobacter pylori-infected Mongolian gerbils associated with a shift in mucin production from glandular to surface mucous cells. *Int J Cancer*. 2006; **119**: 1558-66.

26. Fox JG, Dangler CA, Taylor NS, King A, Koh TJ, Wang TC. High-salt diet induces gastric epithelial hyperplasia and parietal cell loss, and enhances Helicobacter pylori colonization in C57BL/6 mice. *Cancer Res*. 1999; **59**: 4823-8.

27. Loh JT, Torres VJ, Cover TL. Regulation of Helicobacter pylori cagA expression in response to salt. *Cancer Res*. 2007; **67**: 4709-15.

28. MacGregor GA, Markandu ND, Roulston JE, Jones JC, Morton JJ. Maintenance of blood pressure by the renin-angiotensin system in normal man. *Nature*. 1981; **291**: 329-31.

29. Caulfield M, Lavender P, Farrall M, Munroe P, Lawson M, Turner P, *et al.* Linkage of the angiotensinogen gene to essential hypertension. *N Engl J Med*. 1994; **330**: 1629-33.

30. Jeunemaitre X, Soubrier F, Kotelevtsev YV, Lifton RP, Williams CS, Charru A, *et al.* Molecular basis of human hypertension: role of angiotensinogen. *Cell*. 1992; **71**: 169-80.

31. Corvol P, Jeunemaitre X, Charru A, Kotelevtsev Y, Soubrier F. Role of the renin-angiotensin system in blood pressure regulation and in human hypertension: new insights from molecular genetics. *Recent progress in hormone research*. 1995; **50**: 287-308.

32. Staessen JA, Kuznetsova T, Wang JG, Emelianov D, Vlietinck R, Fagard R. M235T angiotensinogen gene polymorphism and cardiovascular renal risk. *Journal of hypertension*. 1999; **17**: 9-17.

33. Shibata T, Tahara T, Arisawa T, Hirata I. Polymorphism of the salt sensitivity gene angiotensinogen and gastric cancer risk. *Mol Med Rep*. 2011; **4**: 723-6.

34. Sugimoto M, Furuta T, Shirai N, Kodaira C, Nishino M, Ikuma M, *et al.* Role of angiotensinogen gene polymorphism on Helicobacter pylori infection-related gastric cancer risk in Japanese. *Carcinogenesis*. 2007; **28**: 2036-40.

35. Shibata T, Tahara T, Yonemura J, Okubo M, Yoshioka D, Nakamura M, *et al.* The G-protein beta3 polymorphism is associated with diffuse type gastric cancer in Japanese. *Asian Pacific journal of cancer prevention : APJCP*. 2010; **11**: 1195-9.

Table 1. AGT M235T polymorphism and GC risk

genotypes	patients with GC *n* (%)	control patients *n* (%)	OR (95%C.I.) vs MM	*p*
MM	9 (4.4)	8 (3.8)	reference	
MT	57 (27.7)	60 (28.6)	1.02 (0.34-3.04)	0.975
TT	140 (67.9)	142 (67.6)	0.96 (0.35-2.64)	0.932
T carrier	197 (95.6)	202 (96.2)	0.97 (0.35-2.67)	0.952

GC: Gastric cancer; C.I.: confident interval.

Table 1 from ref. 35.

Table 2. GNB3 C825T polymorphism and GC risk

Variables(*n*)	genotype			CC vs. TT	
	C/C	C/T	T/T	OR(95%CI)	*p*
Patients 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(0.31-1.99)	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(0.39-4.41)	0.655
Negative (132)	27	75	30	1.23(0.57-2.62)	0.600
Distant metastasis					
Positive (18)	2	10	6	2.80(0.52-15.25)	0.233
Negative (143)	31	80	32	1.10(0.53-2.31)	0.796

NOTE: All data are 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

Table 2 from ref. 37

