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EDITORIAL

Genetic predisposition to *Helicobacter pylori*-induced gastric precancerous conditions

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Abstract

Gastric cancer is the most common malignancy of the gastrointestinal tract in East Asian populations and the second most frequent cause of cancer-related mortality in the world. While previous studies have investigated the genetic factors involved in gastric carcinogenesis, there still exist relatively few studies that have investigated the genetic traits associated with the risk of gastric precancerous conditions. In this paper we will review the biology and genetic polymorphisms involved in the genesis of gastric precancerous conditions reported to date and discuss the future prospects of this field of study. The associations of gastric precancerous conditions with polymorphisms in the cytotoxin-associated gene A-related genes (e.g. *PTPN11* G/A at intron 3, rs2301756), those in the genes involved in host immunity against *Helicobacter pylori* (*H. pylori*) infection (e.g. *TLR4* +3725G/C, rs11536889) or polymorphisms of the genes essential for the development/ differentiation of the gastric epithelial cells (e.g. *RUNX3* T/A polymorphism at intron 3, rs760805) have been reported to date. Genetic epidemiological studies of the associations between *H. pylori*-induced gastric precancerous conditions and other gene polymorphisms in these pathways as well as polymorphisms of the genes involved in other pathways like oxidative DNA damage repair pathways would provide useful evidence for the individualized prevention of these *H. pylori*-induced gastric precancerous conditions.

Key words: *Helicobacter pylori*; Gastric cancer; Single nucleotide polymorphisms; Genetic predisposition to disease; Gastric precancerous conditions

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INTRODUCTION

Gastric cancer is the most common malignancy of the gastrointestinal tract in East Asian populations and the second most frequent cause of cancer-related mortality in the world[1,2]. *Helicobacter pylori* (*H. pylori*) infection has been established as a major risk factor for developing gastric cancer and its precursor lesions by numerous epidemiological studies[3,4]. More than 50% of the world population is infected with this bacterium[5]. Most case-control and cohort studies have shown that the risk of patients with *H. pylori* infection for developing gastric cancer is from two- to six-fold[6]. Moreover, some of the trials on *H. pylori* eradication revealed that cure of its infection reduces the

development of gastric cancer in high risk populations[7,8]. Meanwhile, accumulated evidence indicates that there are three steps in gastric carcinogenesis: *H. pylori* infection, development of gastric precancerous conditions and carcinogenesis[9] (Figure 1). Severe gastric atrophy (GA) and corpus-predominant gastritis, intestinal metaplasia (IM) and dysplasia are well recognized as predominant predispositions to gastric cancer[10,11]. The extent of these gastric damages due to *H. pylori* infection seems to vary from one subject to another, suggesting the existence of some genetic factors that play important roles in determining the long-term outcome of *H. pylori* infection. While previous studies have investigated the genetic factors involved in gastric carcinogenesis[12,13] or *H. pylori* infection[14], the number of the reports that examined the roles of genetic factors in each step of gastric carcinogenesis was limited. Especially, few studies investigated the genetic traits associated with the risk of gastric precancerous conditions which would potentially be of significance for the prevention of gastric cancer itself. In this paper we will review the biology and genetic polymorphisms involved in the genesis of gastric precancerous conditions reported to date and discuss the future prospects in this field of study.

EPIDEMIOLOGY OF GASTRIC ATROPHY

GA is supposed to be a result of inflammation induced by *H. pylori* infection[15-21]. In epidemiologic studies, serum pepsinogens (PGs) have been used as a marker of GA[22] because it is easily available with a less invasive method. Several lifestyle factors like salty food intake[23], low light-colored vegetable intake[15,21], low vitamin C[16] and high starch intake[24] have been shown to be risk factors for GA among subjects with and without *H. pylori* infection. A recent study with 1071 *H. pylori*-infected Japanese revealed that those who take too much rice, miso soup, cod roe, and cuttlefish, representative Japanese traditional food, were at higher risk of developing GA[25]. Frequent rice intake was shown to increase the risk of atrophic gastritis in another study with 291 *H. pylori*-infected Japanese Brazilians[26]. A double-blinded randomized controlled intervention study in Japan demonstrated that 500 mg of vitamin C supplementation for 5 years prevented the decrease in average PGI/II ratio slightly without reduction of *H. pylori* seropositive percentage[27].

GENES AND POLYMORPHISMS INVOLVED IN GASTRIC PRECANCEROUS CONDITIONS

Identifying candidate genes for genetic predisposition to a gastric precancerous condition is a major challenge that stems from a profound understanding of the etiology of this condition. *p53* mutations are shown to be detected during the stages of GA and metaplasia[28]. The deregulation of the "hummingbird phenotype" induction in the gastric epithelial cells through cytotoxin-associated gene A (CagA)-src homology 2 domain-containing protein tyrosine phosphatase-2 (SHP-2) interaction[29] or the disruption of epithelial cell polarity through the CagA-Partitioning-defective 1 (PAR1) interaction[30] might possibly play important roles in the formation of gastric precancerous conditions. Also, like in other gastrointestinal carcinogenesis, TGF-β signaling and the subsequent inflammatory process might play essential roles in the genesis of these conditions[31]. These hypothetical biological mechanisms underlying the genesis of gastric precancerous conditions need to be investigated further in future research.

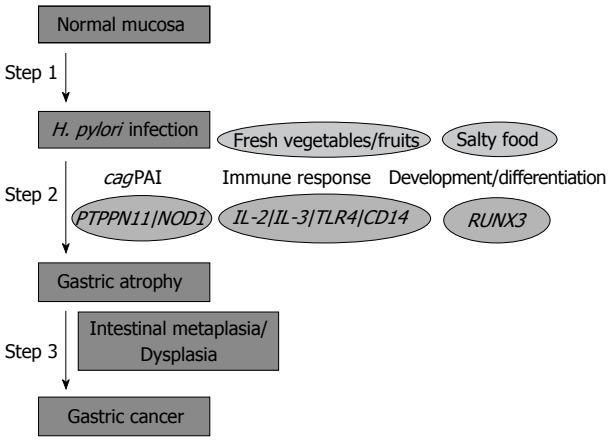

Figure 1 Steps in *Helicobacter pylori*-related gastric cancer. *H. pylori*: *Helicobacter pylori*; NOD1: Nucleotide-binding oligomerization domain protein 1; PTPN11: Protein-tyrosine phosphatase non receptor-type 11; RUNX3: Runt-related gene 3; *cag*PAI: *cag* pathogenicity island.

Although biological mechanisms involved in the genesis of gastric precancerous conditions remain largely unclear, they seem to involve both direct effects by the virulence factors of *H. pylori* and indirect effects derived from pro-inflammatory immune response by the host[28].

Cag pathogenicity island-related genes and their polymorphisms

The former effects of *H. pylori* virulence factors may include those induced by *H. pylori* induced virulence factor CagA[32-34]. CagA is a 120 to 145-kDa *H. pylori* protein encoded by the *cagA* gene[35,36] which is localized at one end of the *cag* pathogenicity island (*cag*PAI), a 40-kb DNA segment considered to be horizontally transfected to the *H. pylori* genome[37,38]. CagA is delivered from *H. pylori* bacterium into host cell cytoplasms through the type Ⅳ secretion system[39] and undergoes tyrosine phosphorylation[40]. In the injected gastric epithelial cells, CagA induces cellular spreading and elongation, called the hummingbird phenotype, which is thought to play important roles in *H. pylori*-induced gastric carcinogenesis. In this CagA-dependent morphologic transformation of gastric epithelial cells, a key molecule SHP-2 is required[41]. Binding of tyrosine phosphorylated CagA to the SH2 domains of SHP-2 causes a conformational change in SHP-2 itself that leads to aberrantly activated SHP-2 phosphatase. SHP-2 plays a major role in intracellular signaling provoked by various growth factors, hormones or cytokines and is widely expressed in both embryonic and adult tissues[30,42]. SHP-2

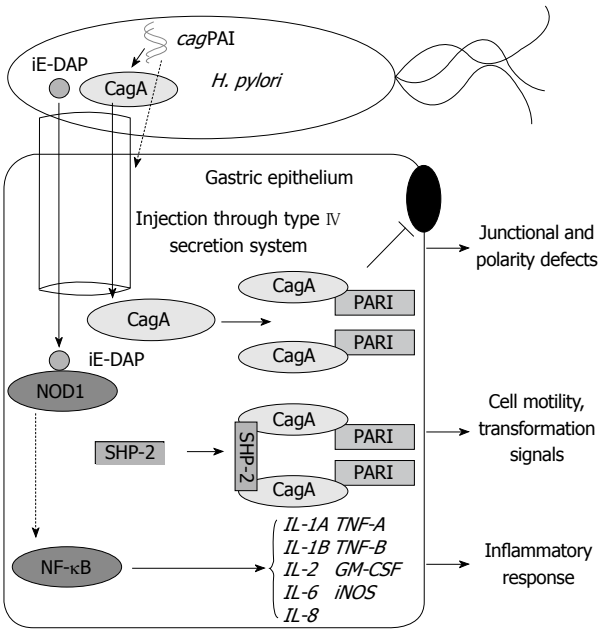

Figure 2 Changes in the gastric epithelial cells due to the activation of Helicobacter pylori cag pathogenicity island-related molecules. *H. pylori*: *Helicobacter pylori*; iE-DAP: γ-D-glutamyl-meso-diaminopimelic acid; iNOS: Inducible nitric oxide synthase; NF-κB, Nuclear factor κB; NOD1: Nucleotide-binding oligomerization domain protein 1; PAR1: Partitioning-defective-1; SHP-2: Src homology 2 domain-containing protein tyrosine phosphatase-2; *cag*PAI: *cag* pathogenicity island.

is required for full activation of the Ras-MAP kinase cascade in response to growth factor-receptor interaction and plays an important role in cell morphogenesis as well as cell motility[43] which might partly explain the mechanism for the formation of hummingbird phenotype.

Meanwhile, CagA is shown to disrupt the tight junctions and causes loss of epithelial apical-basolateral polarity through the specific interaction of CagA with partitioning-defective-1 (PAR1)/ microtubule affinity-regulating kinase-2 (MARK2)[30,34] (Figure 2). PAR1b is localized to the basolateral membrane in normal polarized epithelial cells while atypical protein kinase C (aPKC) complex is localized specifically to the apical membrane. When CagA is delivered and injected into normal polarized gastric epithelial cells, CagA inhibits the kinase activity of PAR1b by binding directly to its kinase domain which subsequently leads to junctional and polarity defects followed by the disorganization of the epithelial monolayer[30]. PAR1b exists as a homodimer in the cells and two CagA proteins bind to a PAR1b dimer, also essential for stable CagA-SHP2 interaction.

Recently, a cytosolic pattern recognition receptor, nucleotide-binding oligomerization domain protein 1 (NOD1), was found to respond to peptidoglycan delivered by *H. pylori cag*PAI[44]. NOD1 is known to sense the essential γ-D-glutamyl-meso-diaminopimelic acid (iE-DAP) dipeptide which is uniquely contained in peptidoglycan of all gram negative and certain gram-positive bacteria[45].

As the precise relationship of gastric precancerous conditions like GA and IM with these *cag*PAI-associated molecules is largely left unknown, further investigations are required to clarify the roles of these *cag*PAI-related molecules in the genesis of gastric precancerous conditions.

The studies on the associations between genotypes and GA among the infected were relatively limited (Table 1)[46-59]. In the majority of these studies, serum PGs were measured for the diagnosis of gastric mucosal atrophy where gastric mucosal atrophy was grouped into "none" (PG Ⅰ >70 ng/mL or PG Ⅰ /PG Ⅱ > 3), "mild" (PG Ⅰ ≤ 70 ng/mL and PG Ⅰ /PG Ⅱ ≤ 3, excluding "severe" cases) or "severe" (PG Ⅰ ≤ 30 ng/mL and PG Ⅰ /PG Ⅱ ≤ 2)[46,47,50,52,54-56,58], while the diagnosis of GA was done based on the endoscopic findings in the rest of the studies[48,49,51,53,58,60].

***Protein tyrosine phosphatase, non-receptor type, 11* G/A at intron 3 (rs2301756):** The protein tyrosine phosphatase, non-receptor type, 11 (*PTPN11*) G/A polymorphism at intron 3 (rs2301756) is a G-to-A single nucleotide substitution at 223 bp upstream of exon 4 in the *PTPN11* gene encoding SHP-2 at chromosome 12q24.1. The biological function of this polymorphism has not yet been reported. The first dataset showed that one (11.1%) out of 9 infected individuals with the *AA* genotype had GA while 134 (56.1%) among 239 infected with the *G* allele had atrophy[60]. Our recent report of 1636 non-cancer Japanese subjects demonstrated that the risk of severe GA was significantly reduced for those with at least one *A* allele of this *PTPN11* G/A polymorphism at intron 3 (OR = 0.62, 95%CI: 0.42-0.90), confirming the association of this *PTPN11* gene polymorphism with the risk of gastric precancerous conditions in *H. pylori*-infected subjects[46]. If the polymorphism is functional or linked to a functional one, the association can be biologically explained by the difference in the strength of signal transduction through the CagA-SHP2 complex. According to the NCBI dbSNP, the frequencies of the *G* allele of rs2301756, high risk allele for GA, is 0.802 among 1484 Japanese and 0.917 among 48 Chinese while it was 0.348 among 46 African American and 0.064 among 46 Caucasians, indicating that Japanese and Chinese become high risk ethnic groups through CagA-positive *H. pylori* infection if the hypothesis that the *G* allele confers stronger signals *via* the CagA-SHP2 interaction is true.

***Nucleotide-binding oligomerization domain protein 1* G796A (E266K):** A recent report revealed that the carriage of the *NOD1* G796A mutation increases the susceptibility for GA strikingly: OR = 34.2 in *NOD1* 796*AA* and OR = 13.35 in *NOD1* 796*GA* compared to subjects with *NOD1* 796*GG*[48].

Immune related genes and their polymorphisms

For the latter effects of pro-inflammatory immune response by the hosts, TLR4 recognizes lipopolysaccharide (LPS) of gram-negative bacteria and is proved to play important roles in *H. pylori* infection through the interaction of macrophage/monocyte TLR4 with *H. pylori* LPS[61,62].

Table 1 Polymorphisms reported on the associations with gastric atrophy among Helicobacter pylori seropositives as well as odds ratio and/or gastric atrophy percent.

Polymorphism	Function	Rs No.	Subjects [Ref.]	OR and/or GA%
IL-1B C-31T	5'UTR	rs1143627	253 Japanese[104]	CC (54%),CT (52%),TT (56%)
IL-1B C-31T	5'UTR	rs1143627	455 Jpn.Brazil.[59]	CC, CT: 0.61,TT: 0.58 CC (36%), CT (31%), TT (21%)
IL-1B C-31T	5'UTR	rs1143627	1328Venezuelan[94]	TT, CT: 1.01, CC: 0.91
IL-2 T-330G	5'UTR	rs2069762	244 Japanese[52]	GG ,TG: 1.64, TT: 2.78 [a]GG (38%), TG (50%), TT (62%)
IL-4 C-33T	5'UTR	rs2070874	249 Japanese[52]	CC, CT: 2.47, TT: 1.80 CC (38%), CT (60%), TT (53%)
IL-4 T-590C	5'UTR	rs2243250	1301 Venezuelan[53]	TT, CT: 0.82, CC: 0.81
IL-4R C-3223T	5'UTR	rs2057768	1301 Venezuelan[53]	CC, CT: 0.97, TT: 1.01
IL-4R A398G	Non-synonymous (Ile50Val)	rs1805010	1301 Venezuelan[53]	AA, AG: 1.14, GG: 1.52[a]
IL-6 G-174C	5'UTR	rs1800795	1315 Venezuelan[94]	GG, CG: 0.98, CC: 0.57
IL-8 T-251A	5'UTR	rs4073	1347 Venezuelan[94]	TT, AT: 0.98, AA: 1.07
IL-10 G-1082A	5'UTR	rs1800896	1301 Venezuelan[53]	GG, AG: 1.05, AA: 1.14
IL-13 C-1111T	5'UTR	rs1800925	248 Japanese[52]	CC, CT+TT: 0.41 [a]CC (59%), CT+TT (45%)
TNF-A T-1031C	5'UTR	rs4647198	455 Jpn.Brazil.[105]	CC (29%),TC (33%), TT (34%)
TNF-A C-857T	5'UTR	rs1799724	456 Jpn.Brazil.[105]	CC (32%), CT (36%), TT (39%)
TNF-A-1031&-857	5'UTR	rs1799724	455 Jpn.Brazil.[105]	CC&CC (29%), TT&CC (33%), TC&CT (43%), TT&TT (39%)
TNF-A G-308A	5'UTR	rs1800629	1327 Venezuelan[106]	GG, AG+AA: 1.27
MCP1 G-2518A	5'UTR	rs1024611	1311 Venezuelan[94]	AA, AG: 1.02, GG: 1.18
PTPN11 G/A at intron3	Intron	rs2301756	248 Japanese[46]	GG, GA: 0.70, AA: 0.09 [a]GG (59%),GA (49%),AA (11%)
PTPN11 G/A at intron3	Intron	rs2301756	979 Japanese[47][b]	GG, GA+AA: 0.62[c] [a]GG (22%),GA+AA (15%)[c]
NOD1 G796A	Non-synonymous (Glu266Lys)	rs2075820	150 Turks[48][e]	GG, GA: 13.35[a], AA: 34.2[a]
TLR4 A+896G	Non-synonymous (Asp299Gly)	rs4986790	103 Caucasians[49]	AA, AG: 11.0* AA (36%), AG (87%)[d]
TLR4 A+896G	Non-synonymous (Asp299Gly)	rs4986790	717 Venezuelan[94]	GlyGly/AspGly, AspAsp: 1.53
TLR4 G+3725C	3'UTR	rs11536889	980 Japanese[50][b]	GG, GC+CC: 1.33 GG(18%), GC+CC (22%)[c]
CD14 C-159T(C-260T)	5'UTR	rs2569190	717 Venezuelan[94]	CC, CT+TT: 1.06
PRKCH rs3783799 G/A	Intron	rs3783799	1638 Japanese[54]	GG, GA: 0.99, AA: 2.37[a]
iNOS C150T	Non-synonymous (Ser608Leu)	rs2297518	250 Japanese[55]	CC, CT+TT: 0.75
RUNX3 T/A at intron3	Intron	rs760805	938 Japanese[56][b]	TT, TA: 1.51[a], AA:1.59[a] TT(46%), TA (56%), AA (56%)
HSP70-2 A/B (A1267G)	Synonymous (Gln351Gln)	rs1061581	137 Japanese[57]	BB (45%), AA+AB (67%)[c]
FAS G-1377A	5'UTR	rs2234767	109 Taiwanese[85]	No association
FAS A-670G	5'UTR	rs1800682	109 Taiwanese[85]	No association
FASL T-844C	5'UTR	rs763110	109 Taiwanese[85]	TT, TC+CC: 9.4[a]
PGC Ins/Del	Ins/Del	(unknown)	86 Chinese[68]	DD (90%), others (50%)[a]
NQO1 C609T	Non-synonymous (Pro187Ser)	rs1800682	396 Japanese[95]	TT, CT: 1.25, CC: 1.23
GSTM1	Ins/Del	(unknown)	396 Japanese[95]	Null, Present: 1.35
GSTT1	Ins/Del	rs71748309	396 Japanese[95]	Null, Present: 0.87
ACE Ins/Del	Ins/Del	rs1799752	271 Japanese[107]	II, ID: 1.12, DD: 0.99
RANTES C-471T	5'UTR	rs2107538	344 Germans[108]	No association
SDHC JST173800 C/G	3'UTR	rs3813632	249 Japanese[109]	CC, GC: 1.26, GG: 0.51 CC (53%), GC (59%), GG (38%)
IFNGR1 G-611A	5'UTR	rs1327474	805 Portuguese[110]	GG, GA: 1.2, AA: 1.2
IFNGR1 C-56T	5'UTR	rs2234711	814 Portuguese[110]	CC, CT: 1.4, TT: 1.3

[a]Statistically significant ($P < 0.05$); [b]Number of *H. pylori* infected subjects (*H. pylori* seropositive subjects or subjects with gastric atrophy); [c]OR for/percentages of subjects with severe gastric atrophy; [d]There was no subject with GG genotype; [e]CagA-positive subjects, *H. pylori*: *Helicobacter pylori*; GA: Gastric atrophy; OR: Odds ratio.

The initial recognition of LPS and subsequent signaling by TLR4 is supported by several accessory proteins: LPS first binds to lipopolysaccharide-binding protein (LBP) which works as an opsonin for CD14 which then acts as a catalyst for the binding of LPS to MD-2[63]. Then the signal induced by LPS/MD-2/TLR4 complex is transmitted through myeloid differentiation factor 88 (MyD88), interleukin (IL)-1 receptor associated kinase (IRAK), tumor necrosis factor (TNF) receptor-associated factor 6 (TRAF6) and inhibitory κB kinase (IKK) to nuclear factor (NF)-κB, leading to the production of pro-inflammatory cytokines such as IL-1A, IL-1B, IL-6 or TNF-A[64] (Figure 3). Meanwhile, the human immune system is also balanced by the anti-inflammatory cytokines like IL-10, IL-4 or IL-13 which are controlled by regulatory T cells[65]. In these inflammatory processes, increased expression of inducible nitric oxide synthase (iNOS) is shown to play important roles in the production of oxygen radicals whereas overexpression of cyclooxygenase-2 (COX-2) is demonstrated to contribute to the proliferation of the gastric epithelium through the up-regulation of cell-cycles as well as to the propagation of gastric inflammation *via* the prostaglandin pathways[66]. The induction of iNOS is also supposed to be modulated by the activity of protein kinase C-eta (PRKCH) *via* the phosphorylation of NF-κB or activator protein-1 (AP-1)[67,68].

Oxidative DNA damage is also supposed to play important roles in the pathogenesis of *H. pylori*-induced gastric mucosal damage where 8-OHdG is a potential sensitive marker of DNA oxidation[69]. The damaged bases in DNA

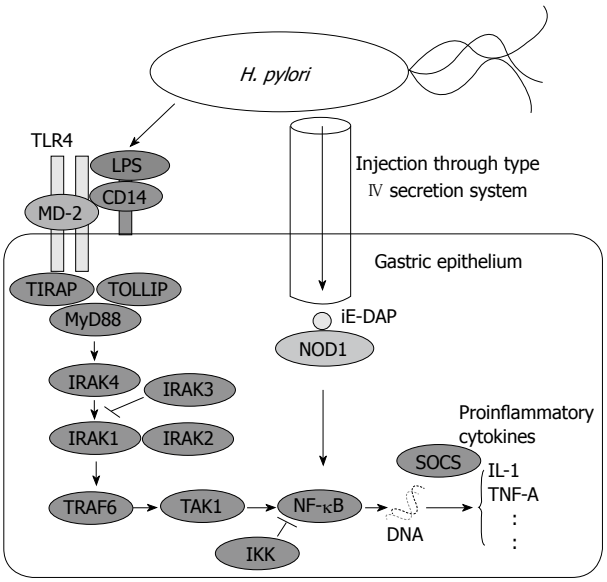

Figure 3 Signal pathways from Helicobacter pylori to cytokine gene expression through innate immunity. *H. pylori*: *Helicobacter pylori*; IKK: Inhibitory κB kinase; iE-DAP: γ-D-glutamyl-meso-diaminopimelic acid; IRAK: Interleukin 1 receptor-associated kinase; MyD88: Myeloid differentiation factor 88; NF-κB, Nuclear factor κB; NOD1: Nucleotide-binding oligomerization domain protein 1; SOCS: Suppressor of cytokine signaling; TOLLIP: Toll-interacting protein; TIRAP: TIR domain-containing adaptor protein.

are mainly repaired by the base excision repair (BER) system; the accumulation of 8-Hydroxy-2'-deoxyguanosine (8-OHdG) or 2-hydroxyadenine (2-OH-A) in DNA is prevented by the co-operation of mutT human homolog-1 (MTH1), 8-hydroxyguanine DNA glycosylase (OGG1) and mutY human homolog (MUTYH)[70]. The number of studies that investigated the contribution of these molecules involved in the inflammatory response, such as innate immune response, oxygen radical production, oxidative DNA damage repair processes, together with cell-cycle regulation and/or cell proliferation in the genesis of *H. pylori*-induced gastric precancerous conditions, is also limited, requiring further biological investigations in the near future.

***TLR4* polymorphisms (+896 A/G, rs4986790; +3725G/C, rs11536889):** One study in Caucasians showed that the *TLR4* +896 A/G polymorphism was associated with the risk of GA where the *TLR4* +896 *G* carriers had an 11-fold increased risk of GA with hypochlorhydria[49]. A subsequent Japanese study also clarified the possible association between another genetic variation in *TLR4* gene, the *TLR4* +3725G/C polymorphism (rs11536889) and the risk of severe GA in Japanese[50], suggesting the significance of genetic variations in host innate immunity due to *TLR4* polymorphisms also in East Asian populations.

***CD14* C-159T polymorphism:** There is one single nucleotide polymorphism in the promoter region of the *CD14* gene, CD14 C-159T polymorphism, which is critical for CD14 expression[71]. A recent study by one Japanese group demonstrated that *CD14* promoter -159*TT* and *T* carrier were associated with lower risk of GA in *H. pylori*-infected subjects who were 61 years or older[51].

***IL-2* T-330G and *IL-13* C-1111T polymorphisms:** *IL-2* T-330G polymorphism was demonstrated to be a functional polymorphism[72] with higher IL-2 production in *GG* genotype than in *TT* genotype[73]. Those with *TT* genotype were shown to be at a higher risk of GA[52], less frequent in Asians (38% out of 29 individuals) than in Caucasians (51% out of 199 individuals)[74].

IL-13 gene in chromosome 5q31 has several polymorphisms; at least 3 polymorphisms at the promoter region, 2 polymorphisms at intron 1, Arg130Gln and 4 polymorphisms at 3' UTR of exon 4 have been reported[75]. The -1111*TT* genotype was shown to harbor increased binding ability of nuclear proteins and was also reported to be associated with asthma[75,76]. As for the risk of GA, -1111*TT* was found to be a low risk genotype[52]. The biological mechanism involved was not yet clarified.

***IL-4R* C-332T (rs1805010) polymorphism:** One study of Venezuelan subjects revealed that those with homozygotes with the low activity allele (*GG*) of the A398G polymorphism in the *IL-4R* gene (rs1805010) had a modestly increased risk of GA (OR = 1.52, 95%CI: 1.05-2.21)[53], suggesting the role of genetic variability in the anti-inflammatory mediators in the genesis of *H. pylori*-induced gastric precancerous conditions.

***Inducible nitric oxide synthase* C150T (rs2297518) and *PRKCH* rs3783799 G/A polymorphisms:** PRKCH is shown to be involved in oxidative stress by activating iNOS and nitric oxide production[67]. The associations of the polymorphisms in these two genes [*iNOS* C150T (rs2297518) and *PRKCH* rs3783799 G/A polymorphisms] with the risk of GA were investigated in the Japanese population which revealed that those with *PRKCH* rs3783799 *AA* genotype were at significantly higher risk of severe GA (OR = 2.37, 95%CI: 1.11-5.05)[54] while there were no significant association between the *iNOS* C150T polymorphism and risk of GA[55].

Other miscellaneous genes and their polymorphisms

Recently, it was reported that the loss expression of *sonic hedgehog* (*Shh*), a regulatory gene essential for developmental patterning, and aberrant expressions of *caudal-type homeobox transcription factor 2* (*CDX2*), a master regulatory gene of intestinal development and differentiation, in *H. pylori*-induced atrophic gastritis are the early events correlated with the occurrence of IM which can be reversible by the eradication of *H. pylori*. In accordance with these findings, CDX2 expression has been demonstrated to be associated with intestinal phenotypes in gastric cancers[77].

Another important tumor suppressor gene in intestinal-type gastric cancer is *runt-related gene 3 (RUNX3)* encoding a subunit of polyomavirus enhancer binding protein 2[78], since expression of *RUNX3* is greatly reduced in

IMs in human stomachs[79] and *RUNX3*$^{-/-}$ mouse gastric epithelial cells have a potential to differentiate into CDX-2 positive intestinal type cells[80]. Li *et al*[78] and Levanon *et al*[81] reported that the gastric mucosa of *RUNX3* null mice showed hyperplasia, indicating that loss of *RUNX3* leads to gastric carcinogenesis in humans. Consistent with this, an analysis of *RUNX3* in human stomach cancer cell lines and primary human tumors revealed hemizygosity in 40% of the tumors examined and silencing by promoter hypermethylation in 60% of the tumors; this figure increased up to 90% in the advanced stage tumors. It is shown that the *RUNX3*$^{-/-}$ mouse gastric mucosa exhibits hyperplasias due to the stimulated proliferation and suppressed apoptosis in the cells, suggesting that *RUNX3* is an attractive candidate as a tumor suppressor of gastric cancer. The CpG island of *RUNX3* P2 promoter is hypermethylated in human and mouse gastric cancer cell lines and in primary human tumors[78,82], also suggesting the tumor suppressor function of *RUNX3* in the etiology of stomach cancer.

Heat-shock protein (*HSP*) 70 plays essential roles in cellular response to a variety of environmental stresses by acting as molecular chaperons in the folding of newly synthesized proteins in cells and assist in the folding of damaged proteins[83]. HSP expression in the gastric mucosa is shown to be attenuated by *H. pylori* infection and aspirin intake and one HSP inducer geranylgeranylacetone (GGA) reportedly protects gastric mucosa from iNOS induced by *H. pylori* infection[84], suggesting that HSP has important roles in protecting gastric mucosa against *H. pylori* or aspirin induced injuries. Gastric carcinogenesis can also be regarded as a multistep process that initiates with the disregulation of normal controls of apoptosis and cell proliferation in which FAS receptor-ligand system is shown to be a key regulator of apoptosis[85].

Pepsinogen C *(PGC)*, alternatively called pepsinogen II or gastricsin, an inactive precursor of pepsin C, is an aspartic protease specifically produced by the gastric chief cells, cardiac cells, pylori cells and Brunner's glands from late infant stages to the adulthood period. PGC is considered to be a differentiation marker of gastric epithelium whose changes in expression may reflect the severity of gastric mucosal damage[60].

***Runt-related gene 3* T/A polymorphism at intron 3 (rs760805):** Among *H. pylori* seropositive subjects, we found a significant association between *RUNX3* rs760805 polymorphism and the risk of GA with the age- and sex-adjusted OR of 1.51 (95%CI: 1.11-2.05, $P = 0.008$) in *TA*, 1.59 (95%CI: 1.08-2.33, $P = 0.019$) in *AA* and 1.53 (95%CI: 1.14-2.05, $P = 0.004$) in *TA+AA* compared with *TT* genotype[56]. This finding was in accordance with the recent biological report that *RUNX3* expression correlated with chief cell differentiation in human gastric cancers[86].

***Heat-shock protein 70-2* A/B (A1267G) polymorphism:** It is shown that the *AA* genotype of *HSP 70-2* A/B polymorphism (*PsiI* polymorphism, corresponding to A1267G polymorphism) had the highest level of mRNA expression compared with the other genotypes (*AB* or *BB*). Recently one Japanese group reported that the *BB* genotype of *HSP 70-2* gene is significantly associated with the reduced risk of severe GA in *H. pylori* infected older subjects[57], indicating the importance of this *HSP* polymorphism in the genesis of *H. pylori*-induced gastric precancerous conditions. In a recent study, polymorphisms in *HSP 70* genes along with *TNF* polymorphisms showed a significant severity-dose-response as risk markers from precancerous lesions to gastric cancer in Mexican population, presumably because of their association with the intense and sustained inflammatory response[87].

***FASL* T-844C polymorphism:** Lately, one study group in Taiwan investigated the relationship between precancerous gastric lesions and polymorphisms in the promoter regions of the death pathway genes *FAS* and *FASL* (*FAS* G-1377A, *FAS* A-670G and *FASL* T-844C) in 109 *H. pylori*-infected Taiwanese individuals and found that *FASL* -844 *C* allele significantly increased the risk of atrophy in the gastric corpus, with an adjusted OR of 5.0 (95%CI: 1.5-6.8)[85].

***Pepsinogen C* ins/del polymorphism:** A recent study among Chinese demonstrated that subjects with *PGC del/del* genotype were at significantly higher risk of atrophic gastritis (OR = 3.11; 95%CI: 1.44-6.71) and *H. pylori*-seropositive subjects with *PGC del/del* genotype had significantly elevated risk of atrophic gastritis (OR = 11.16; 95%CI: 1.37-90.84) with the interaction of 6.48[58], suggesting the positive link between *PGC* gene polymorphism and *H. pylori*-induced GA.

Genes and polymorphisms for advanced precancerous conditions

Advanced precancerous conditions like IM or dysplasia develops in some part of *H. pylori* infected subjects. One Chinese study demonstrated no significant differences in genotype frequencies of *CYP2E1, GSTM1, GSTP1, GSTT1, ALDH2* and *ODC* between those with mild chronic atrophic gastritis including 29.7% *H. pylori* negative subjects and those with deep IM or dysplasia with 20.2% of *H. pylori* negative subjects, but found significant interaction between *CYP2E1 Dra*I genotypes and smoking[88]. Another study in Germany revealed that carriers of both of *IL-1B -511T* and *IL-1RN 2rpt* alleles relative to subjects lacking *IL-1B -511T* or/and *IL-1RN 2rpt* alleles had significantly increased risk for the development of atrophic gastritis, IM and severe inflammation[89].

Recently, one Japanese study group investigated extensively into the genetic polymorphisms associated with the risk of gastric advanced precancerous conditions. They reported that the risk of IM among *H. pylori* seropositive individuals was significantly associated with the polymorphisms of *COMT* Val158Met[90], *cyclin D1* (*CCND1*) G870A[91], *p22PHOX* C242T[92], *VEGF* G1612A at 3'-UTR[93] and *HSP70-2* A1267G[57] while it was not associated with *VEGF* C936T at 3'-UTR[93]. A recent report by a Turkish group also revealed the significant association of risk of antral IM with G796A (E266K) polymorphism in

the *NOD1* gene encoding a cytosolic receptor to peptidoglycan delivered by *cag*PAI; the risk was strikingly increased in those with *AA* genotype (OR = 39.76) and also significantly increased in those with *GA* genotype (OR = 2.71)[48]. One group in the US investigated the associations of risk for *H. pylori*-induced gastric precancerous conditions with the polymorphisms in the genes involved in host-bacterial interaction, (*IL-1B* C-31T, *IL-6* G-174C, *IL-8* T-251A, *MCP-1* G-2518A and *TNF* G-308A), bacterial LPS signaling (*CD14* C-260T, *TLR4* Asp299Gly, *NOD2* del 3020 ins C and *NOD2* Gly908Arg) and anti-inflammatory cytokine signaling (*IL-10* G-1082A, *IL-4* T-590C, *IL-4R* C-3223T and *IL-4R* A398G) among Venezuelan subjects[53,94,95], in which they found the associations between risk of dysplasia and *IL-8* T-251A polymorphism (OR = 2.00 for *AA*, 1.33 for *AT*; *P* for trend = 0.02) or between that of IM and *CD14* C-260T (OR = 1.45 for *CT*, 1.45 for *TT*; *P* for trend = 0.025) or *IL-10* G-1082A (OR = 1.34 for *AG*, 1.50 for *AA*; *P* for trend = 0.055) polymorphisms. Another group in Taiwan investigated the associations of the risk of precancerous gastric lesions and polymorphisms in the promoter regions of the death pathway genes *FAS* and *FASL* (*FAS* G-1377A, *FAS* A-670G and *FASL* T-844C) in 109 *H. pylori*-infected Taiwanese individuals where they found significantly increased risk of IM in the antrum for those with *FAS* -1377 *A* allele with the adjusted OR of 0.3 (95%CI: 0.1-0.9)[85].

FUTURE PROSPECTS

Intriguing genes for future investigation

Although the *G* allele of *PTPN11* may be a part of the genetic traits to develop GA *via* signal transduction from CagA, there seems to be other genetic traits involved in this process. CagA binds several molecules, Grb2 which transduces the signal to Ras-MAP kinase pathway causing cell proliferation, c-Met hepatocyte growth factor (HGF) receptor which have a role of cell proliferation and motility, zona occludens-1 (ZO-1), a tight-junction protein, and PAR1/MARK kinase which has an essential role in epithelial cell polarity[30,96-99]. Although no studies have been conducted, functional polymorphisms of these molecules might also be possible candidates for the genetic traits of GA.

There seem to be other intriguing candidate molecules as possible susceptible factors. A recent study that examined the associations of 75 haplotype-tagging SNPs in genes in the TLR signaling pathway with pertussis toxin (PT)-IgG titers demonstrated that antibody response to pertussis vaccination was significantly associated with the polymorphisms in *CD14, TLR4, t*oll-interacting protein (*TOLLIP*), *TIR* domain-containing adaptor protein (*TIRAP*), *interleukin 1 receptor-associated kinase 3* (*IRAK3*), interleukin 1 receptor-associated kinase 4 (*IRAK4*), TIR domain-containing adaptor molecule 1 (*TICAM1*), and tumor necrosis factor ligand superfamily, member 4 (*TNFSF4*)[100]. Considering the crucial role of TLR4 pathways in the genesis of *H. pylori*-induced gastric cancer, it would be of interest to investigate the involvement of these polymorphisms in the three steps of *H. pylori*-induced gastric carcinogenesis. Meanwhile, although some previous studies have shown the essential roles of the polymorphisms of DNA BER genes (*OGG1*, *MUTYH* and *MTH1*) in *H. pylori*-related gastric carcinogenesis[101,102], few studies have investigated their roles in the genesis of *H. pylori*-induced gastric precancerous conditions. There are several other genes reported to be underlying the genesis of *H. pylori*-induced gastric precancerous conditions. Ornithine decarboxylase (ODC), the first and rate-limiting enzyme, is shown to be up-regulated by *H. pylori* with strong expression in atrophic and IM areas. In a recent Japanese study, CDX2 expression was observed in patients with chronic gastritis closely associated with IM while some other genes like mucin 1 (*MUC1*), *p27* or *p53* are also shown to be implicated in the genesis of IM[103]. The associations of these gene polymorphisms with the risk of *H. pylori*-induced gastric premalignant lesions have also not yet been examined. Further investigations are expected to investigate the significance of the polymorphisms of these genes in *H. pylori*-induced gastric precancerous conditions. Furthermore, haplotype analyses as a gene-specific approach to find novel functional polymorphisms in the genes involved or genome-wide association studies (GWAS) as a comprehensive approach to detect novel candidate gene polymorphisms strongly associated with disease risk should also be conducted to provide useful evidence for the individualized prevention of *H. pylori*-induced gastric precancerous conditions. By identifying the full genetic risk profile for *H. pylori*-induced gastric precancerous conditions, we will be able to target the population at risk and subsequently direct eradication therapy and closer follow-up to the affected individuals.

Recommendation for stepwise risk evaluation

Although a large number of studies have been reported concerning genetic traits associated with gastric cancer risk, few studies investigated which step of *H. pylori*-related gastric carcinogenesis (*H. pylori* infection, GA and gastric cancer) the genetic traits examined has effects on[9]. These stepwise evaluations of *H. pylori*-related gastric carcinogenesis provide us with more precise and detailed information about the genes involved in each step of *H. pylori* related gastric carcinogenesis which would help us establish the effective way of the individualized prevention against *H. pylori*-induced gastric cancer in the near future. We have already conducted this stepwise evaluation in previous reports[46,47,50,59] and expect that forthcoming studies by other groups would also be conducted in this way to improve the quality of the studies so that we can put the obtained results into practice for effective gastric cancer prevention.

It would also be relevant to claim that *H. pylori*-induced gastric precancerous conditions can be histologically subdivided into two lesions, GA and more advanced precancerous conditions of IM; thus the stepwise evaluation should be conducted with four steps, i.e. dividing the

third step of gastric carcinogenesis from GA into more precise categories of IM/dysplasia from GA and gastric carcinogenesis from IM/dysplasia as implicated in Figure 1. In addition, the investigation of the interaction between host genetic factors and dietary factors like salty food intake, low light-colored vegetable intake, low vitamin C and starch intake on the risk of *H. pylori*-induced gastric precancerous condition might provide clues for effective ways of individualized gastric cancer prevention. We think this is our future assignment and more profound collaborations between epidemiologists, pathologists, gastroenterologists and nutritionists would be required to accomplish these idealistic goals.

Other remaining questions

In addition to the points raised above, the roles of the candidate genes in the genesis of gastric precancerous conditions in each histological subtype of gastric cancer (diffuse type or intestinal type) need to be investigated. Also, as the underlying molecular biological mechanisms are largely unclear as mentioned earlier, molecular epidemiological studies should keep up with the advance of the biological research in the field of gastric precancerous conditions in the future.

CONCLUSION

While recent epidemiological studies revealed the important roles of polymorphisms in the *cag*PAI-related genes and genes involved in immune response or development/differentiation of gastric epithelial cells in the genesis of *H. pylori*-induced gastric precancerous conditions as discussed in this editorial review (Figure 1), the fields of genetic epidemiological study regarding *H. pylori*-induced gastric precancerous conditions are still left relatively uninvestigated in spite of its substantial significance for the prevention of gastric cancer. Association studies between the risk of *H. pylori*-induced gastric precancerous conditions and polymorphisms of other genes in *cag*PAI-related pathways, innate immunity or oxidative DNA damage repair pathways would potentially provide useful evidence for the individualized prevention of these *H. pylori*-induced gastric precancerous conditions.

Further investigation of the association of these polymorphisms with risk of *H. pylori*-induced gastric precancerous conditions together with the elucidation of the biological roles of these molecules would be required for the confirmation of recent evidence and realization of practical individualized prevention of *H. pylori*-induced gastric cancer in the near future.

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