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Cytotoxin-associated gene-A-seropositivity and *Interleukin-1* polymorphisms influence adverse cardiovascular events

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ABSTRACT

Aims: Although the bacterial virulent factor of cytotoxin-associated gene-A (CagA)-seropositivity and the host genetic factors of *interleukin (IL)-1* polymorphisms have been suggested to influence *Helicobacter pylori (HP)* -related diseases, the underlying mechanisms of the association between *HP* infection and acute coronary syndrome (ACS) remain unknown.

Methods and results: Among 341 consecutive ACS patients, the clinical outcomes after ACS included composite cardiovascular events within the 2-year follow-up period.

A significantly higher probability of primary outcomes was observed in *HP* positive patients than in *HP* negative patients. There were no significant differences in the rate of cardiovascular events between *HP* positive and *HP* negative patients in the absence of an *IL*-polymorphism, while there were significant differences in the presence of an *IL*-polymorphism. There were significant differences in the rate of cardiovascular events among CagA positive, CagA negative/ *HP* positive and CagA negative/*HP* negative patients. Moreover, via immunohistochemical staining, aortic CagA positive cells were confirmed in the vasa vasorum in CagA positive patients, whereas they could not be identified in CagA negative patients.

Conclusions: The bacterial virulence factor CagA and host genetic *IL-1* polymorphisms influence the incidence of adverse cardiovascular events, possibly through infection of atherosclerotic lesions.

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1. Introduction

Chronic bacterial and viral infection with chronic inflammation is an important determinant in the development of atherosclerosis [1], and the role of chronic infection and inflammation has been examined in its relation to the pathogenesis of atherosclerotic cardiovascular disease [2,3]. *Helicobacter pylori (H. pylori)* infection is the most common chronic bacterial infection, and a recent *meta*-analysis suggested a relationship between *H. pylori* infection and the risk of myocardial infarction (MI) [4]. However, the mechanisms underlying the association between *H. pylori* and cardiovascular diseases have not been completely clarified. Bacterial virulence, host genetic, and environmental factors affect the course and severity of *H. pylori*-related diseases.

Some *H. pylori* strains possess cytotoxin-associated gene-A (CagA), which encodes a hydrophilic, surface-exposed protein that produces cytokines and causes inflammation of the gastric mucosa [5], and CagA protein is an oncoprotein of significance that can induce malignant neoplasms in mammals by hit-and-run

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mechanisms [6]. Past studies have shown a significant association between CagA seropositivity and risk of atherosclerosis in tissues such as carotid arteries and coronary arteries [7].

A previous study reported that *interleukin (IL)-1* polymorphisms were significantly associated with *H. pylori*-related gastric inflammation, atrophy, and carcinogenesis [8], and we have found that *H. pylori*-infected patients with these *IL-1* polymorphisms also showed an increased level of inflammation and increased risk of MI [9,10], and smoking patients with *H. pylori* infection and *IL-1* polymorphisms had a significantly increased risk of cardiovascular events after acute coronary syndrome (ACS) [11]. Moreover, it was recently revealed that the CagA positive strain of *H. pylori* was involved in the pathogenesis of ACS recurrence [12]. Furthermore, it was recently reported that the CagA protein is transported from the blood to distant organs [13], but it is not known whether this protein is localized in atherosclerotic lesions.

The aims of the present study were to clarify 1) the role of the *H. pylori* virulence factor CagA and host *IL-1* polymorphisms in cardiovascular events and 2) localization of the CagA protein in atherosclerotic lesions.

2. Methods

2.1. Study population

The study included patients with consecutive coronary artery disease (CAD) who were treated at Kumamoto University Hospital between January 2009 and December 2014 (n = 873). Study flowchart is shown in Fig. 1. Of these patients, we excluded stable CAD cases (n = 514) and subjects whose serum samples were not stored. The other exclusion criteria included malignant diseases (n = 21), other inflammatory diseases (n = 4), other severe complications (n = 9), and status after receiving *H. pylori* eradication treatment. Of the remaining 341 ACS patients, 330 patients with prognostic data were enrolled. Then, we divided the enrolled patients into 2 groups: 130*H. pylori* positive patients and 200*H. pylori* negative patients (Supplemental Fig. 1).

2.2. Ethics statement, clinical parameters, and laboratory assays, Genotyping, immunohistochemical staining of gastric tissues and aortic aneurysms, clinical outcomes, statistical analysis

Detailed ethics statement, clinical parameters, and laboratory assays, genotyping, immunohistochemical staining of gastric tissues and aortic aneurysms, clinical outcomes, and statistical analysis are available in the Supplementary Material.

3. Results

3.1. Clinical characteristics of the study patients

Detailed clinical characteristics of the study patients is available in the Supplementary Material.

3.2. Clinical outcomes

During the follow-up period (median 347 days), 37 (11.4%) of the patients experienced an adverse cardiovascular event (17.3% of the *H. pylori* positive patients and 7.6% of the *H. pylori* negative patients). Kaplan-Meier analysis demonstrated a significantly higher probability of adverse outcomes in the *H. pylori* positive patients than in *H. pylori* negative patients (log-rank test, P = 0.011; Fig. 1A).

3.3. Cardiovascular event rates between H. Pylori positive and negative patients according to the presence of IL-1 polymorphisms

We divided the patients into two groups according to presence (n = 228) or absence (n = 97) of *IL-1* polymorphisms. There were no statistically significant differences in the clinical characteristics of the patients between the groups based on the presence or absence of *IL-1* polymorphisms (Supplemental Table 1). Fig. 1B demonstrates that there were no significant differences in the rates of cardiovascular events between *H. pylori* positive and *H. pylori* negative patients in the absence of an *IL-1* polymorphism (p = 0.60), while there were significant differences in the presence of an *IL-1* polymorphism (p = 0.011, Fig. 1C).

3.4. Cardiovascular event rates between H. Pylori positive and negative patients according to the presence of IL- polymorphisms

We divided the subjects into the following 3 groups: CagA positive (n = 91), CagA negative/ *H. pylori* positive (n = 46) and CagA negative/ *H. pylori* negative (n = 193) according to their CagA and *H. pylori* seropositivity. Fig. 1D demonstrates that there were significant differences in the rates of cardiovascular events among the 3 groups (p = 0.041).

3.5. Multivariate analysis on clinical outcomes and cox proportional hazard analyses depending on H. Pylori and CagA seropositivity

Detailed multivariate analysis on clinical outcomes and cox proportional hazard analyses depending on *H. pylori* and CagA seropositivity are available in the Supplementary Material.

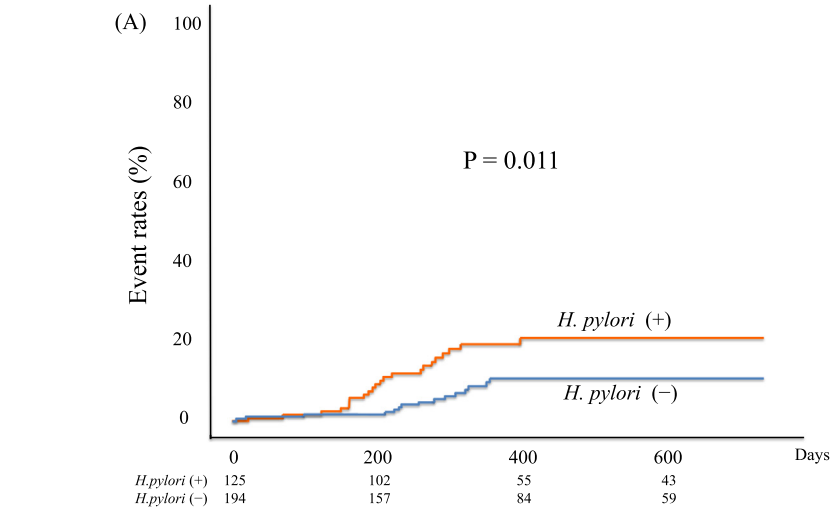
3.6. Immunohistochemical staining of CagA protein in atherosclerotic lesions

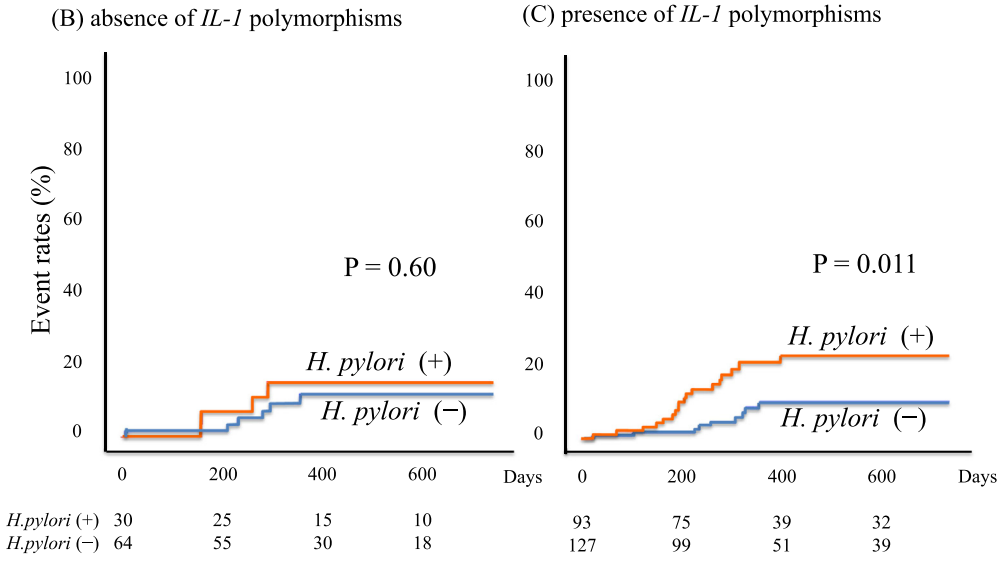
As shown in Fig. 2A, the CagA antibody that was used in the present study was confirmed by immunostaining in the gastric mucosal tissue of a CagA positive patient. Fig. 2C–F show representative photomicrographs of aortic aneurysm sections from CagA negative (Fig. 2C and 2D) and positive (Fig. 2E and F) patients that were stained with CagA antibodies. Aortic CagA positive cells were confirmed in the vasa vasorum of a Cag A positive patient (Fig. 2E and F), whereas they could not be identified in CagA negative patient (Fig. 2C and D).

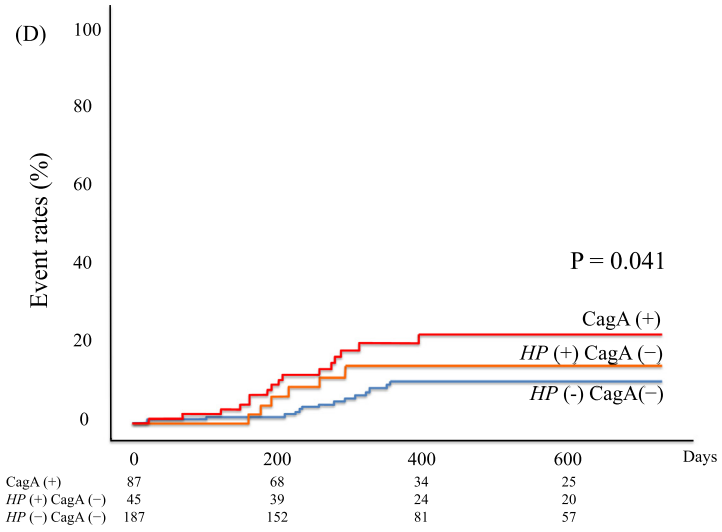
4. Discussions

To our knowledge, this is the first study to examine the role of CagA-seropositivity and host *IL-1* polymorphisms for their relationship to adverse cardiovascular events. The present study has three important findings. First, in condition of *IL-1* polymorphisms, *H. pylori* positive patients had a higher rate of cardiovascular events than *H. pylori* negative patients. Second, we observed an association of CagA positive *H. pylori* infection with increased cardiovascular events in ACS patients. Third, we confirmed the localization of CagA protein to atherosclerotic lesions in Cag A positive patients.

In vitro studies have shown that inflammatory mediators found in atheroma, such as IL-1β, tumor-necrosis factor (TNF)-α, and CD40 ligand (CD154), augment matrix metalloproteinases (MMPs) expression in mononuclear phagocytes and endothelial and smooth muscle cells 14]. Mast cells in the lesion may release the MMP inducer TNF-α well as serine proteinases that can activate latent MMP proenzymes [15,16]. These concepts were comprehensively reviewed [14]. Furthermore, recently, the Canakinumab Anti-inflammatory Thrombosis Outcome Study (CANTOS), trial







[17] revealed that cardiovascular events and cancer incidence and mortality were decreased by canakinumab, an anti-inflammatory drug used in patients with arteriosclerotic diseases. These results offer major evidence to support our results.

The relationship between *H. pylori* infection and atherosclerosis has previously been determined [18], and a recent study suggested a relationship between *H. pylori* infection and the risk of MI [4]. This association between *H. pylori* infection and atherosclerosis is

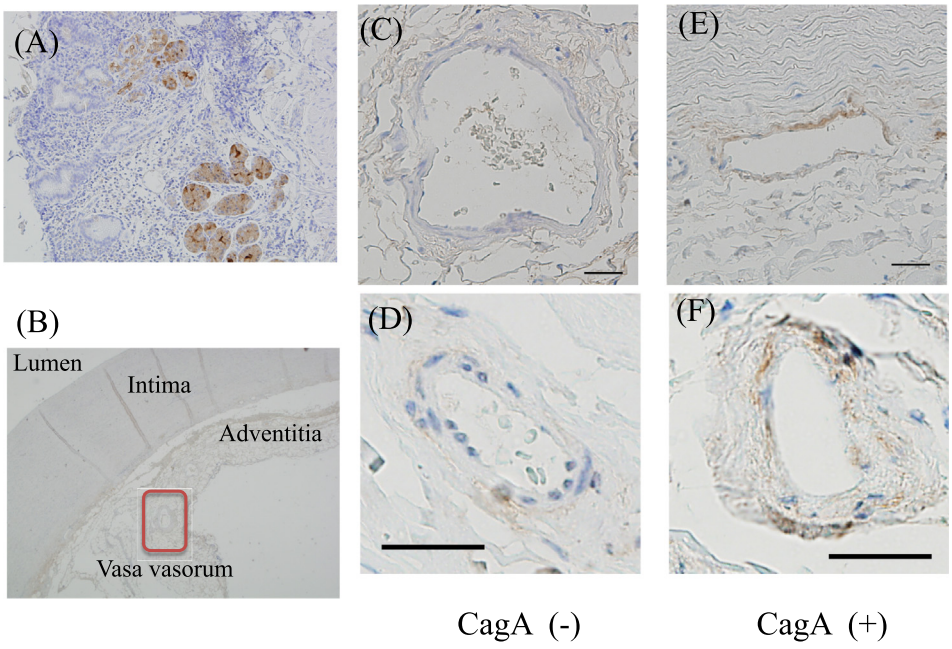


Fig. 2. Immunohistochemical staining of CagA protein. Fig. 2A indicates a representative photomicrograph of a gastric mucosal tissue section that was obtained from a CagA positive patient and stained with a CagA antibody. Fig. 2B indicates an aortic aneurysm section. The red rectangle demonstrates a representative vasa vasorum. Figures 5C–F indicate representative photomicrographs of vasa vasorum in aortic aneurysm sections that were obtained from CagA negative (Fig. 2C and D) and positive (Fig. 2E and F) patients and were stained with CagA antibodies. Scale bar:100 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

especially prevalent in patients with a CagA positive *H. pylori* infection, and previous studies have demonstrated that this specific type of infection might cause vulnerable plaques [19]. To date, there are insufficient data regarding the association between CagA positive *H. pylori* infection and atherosclerotic cardiovascular disease, and our present study is one of the first studies to provide a relationship between these two events since it shows that CagA-seropositivity significantly increases the risk of MI. A recent study reported that the anti-CagA antibodies react with antigens that are localized within coronary atherosclerotic plaques and exhibit immunohistochemical cross-reaction with the antigens that are expressed by the cells that are involved in the destabilization of plaques, such as fibroblasts and lymphocytes [7]. Additionally, there are several reports that CagA-seropositivity is associated with vascular atherosclerosis in tissues such as the coronary and carotid arteries [7]. Our previous study has shown that CagA-seropositive patients with *IL-1* polymorphisms had more severe endothelial dysfunction than patients without either of these factors [9]. According to the response-to-injury hypothesis of atherosclerosis, endothelial dysfunction is the first step in atherosclerosis, and previous studies have suggested that that *H. pylori* infection was associated with endothelial dysfunction and that this endothelial dysfunction might be reversible by eradicating *H. pylori*, according to findings of a prior study [20]. The cross-reaction of the anti-CagA antibody may possibly cause strong local inflammatory responses and the development of acute coronary events.

Several studies have shown the role of cytokine polymorphisms in modulating the levels of systemic inflammation that can be mediated by chronic low-grade infections such as periodontal infections, Chlamydia pneumoniae, and *H. pylori* infection, leading to an increased risk of atherosclerosis [21]. Since *IL-1* polymorphisms are correlated with *H. pylori*-related gastric diseases, including gastric cancer, *IL-1* polymorphisms could thus be important determinants of the inflammatory response in patients with *H. pylori* infection. *IL-1* genetic polymorphisms are reported to influence *H. pylori*-related gastric mucosal *IL-1β* production [8], and cytokines such as *IL-1β* and tumor necrosis factor alpha are important in the regulation of gastric acid secretion, and they inhibit parietal cell acid secretion through multiple pathways [22]. Therefore, *IL-1β* plays an important role for the continuous chronic infection of *H. pylori* because the inhibition of gastric acid enables larger colonization of *H. pylori* bacteria [23]. Moreover, *in vitro* experiments have shown that the cag pathogenicity island (cag PAI) genes upregulate a superset of host genes, including immune response genes, which leads to a heightened inflammatory response [24]. Animal model studies revealed that, in contrast to infection with CagA positive *H. pylori*, CagA negative strains do not induce severe gastric inflammation, atrophy, or carcinogenesis; this suggests that there is an essential role of the cag PAI genes in

Fig. 1. Kaplan-Meier analysis of cardiovascular event rates, (A) according to the presence of *Helicobacter (H.) pylori.* A Kaplan-Meier analysis demonstrated a significantly higher probability of adverse outcomes in *H. pylori* positive patients than in *H. pylori* negative patients (log-rank test, P = 0.011). (B,C) according to the presence of *H. pylori* in the conditions of (B) absence or (C) presence of *interleukin (IL)-1* polymorphisms. There were no significant differences in cardiovascular events rates between *H. pylori* positive and *H. pylori* negative in absence of *IL-1* polymorphism (log-rank test, p = 0.63), while there were significant differences in presence of *IL-1* polymorphism (log-rank test, p = 0.01). (D) in cytotoxin-associated gene-A (CagA) positive (red line), *H. pylori* positive and CagA negative (orange line) and *H. pylori* negative and CagA negative (blue line) patients. We divided subjects into 3 groups of CagA positive (n = 91), CagA negative/ *H. pylori* positive (n = 46) and CagA negative/ *H. pylori* negative (n = 193) according to CagA and *H. pylori* seropositivity. There were significant differences in cardiovascular events rates among the 3 groups (log-rank test, p = 0.045). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

H. pylori-related diseases [25]. Our study results showed that patients with CagA are at a higher risk of MI, and the multivariate logistic regression analysis suggested that this risk of MI might be inflated by the host genetic factor of *IL-1* polymorphisms. Therefore, there is a possibility that the bacterial virulence factor CagA and *IL-1* polymorphisms might cause a synergistic effect of inflammatory response and the development of MI.

Moreover, in the present study, we found that CagA-positive patients with *IL-1* polymorphisms had worse prognoses after ACS compared to the patients who did not have these polymorphisms. Thus, combined bacterial and host genetic factors may play an important role for subsequent cardiovascular events, and the assessment of these factors might improve the risk stratification of clinical outcomes. The present study suggested that there would be a treatment strategy for the eradication of *H. pylori* in patients with acute coronary syndrome. However, further large scale and prospective studies are needed to clarify our findings.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijcha.2020.100498.

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