RESEARCH COMMUNICATION

A *C* to *T* polymorphism of Urokinase Plasminogen Activator (P141L) is Associated with *Helicobacter pylori* Infection

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Abstract

Urokinase plasminogen activator (uPA) plays an important role in tumor invasion and certain inflammatory diseases. However, few studies have paid attention to how the uPA is associated with *Helicobacter pylori* infection and gastric atrophy. This study investigated associations of a *C*-to-*T* polymorphism of *uPA* (P141L, rs2227564) in exon 6 in 454 Japanese health checkup examinees (126 males and 328 females) aged 35 to 85 without a history of cancer. The *uPA* was genotyped by polymerase chain reaction with two-pair primers. The genotype distribution was in Hardy-Weinberg equilibrium (p=0.52) and the frequency of the T allele was 0.239. The risk of *H. pylori* sero-positivity was significantly reduced with the *T/T* genotype; the odds ratio (OR) relative to the *C/C* genotype was 0.34 (95% confidence interval [CI]: 0.14 to 0.86). Of the sero-negative subjects, 21 with atrophy were infected with *H. pylori* but lost their sero-positivity. After reclassifying them together with the sero-positive subjects, the corresponding OR was 0.40 (95% CI: 0.16 to 1.00), confirming that the *T/T* genotype decreased the risk of *H. pylori* infection. This gene polymorphism was not associated with the risk of gastric atrophy. In conclusion, this study indicated a possibility that the *uPA* minor homozygous genotype was associated with a reduction of *H. pylori* infection risk. Further studies are required to confirm these findings.

Keywords: *Helicobacter pylori* infection - reduced risk - urokinase plasminogen activator - Japanese

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Introduction

Helicobacter pylori (*H. pylori*) infects about half of the population worldwide (Suerbaum et al., 2002; Correa et al., 2008) and long-term infection with H. pylori causes superficial gastritis, gastric atrophy and intestinal metaplasia (Correa et al., 2008; Kawaguchi et al., 1996). However, only some of those infected develop *H. pylori*-related gastric atrophy and gastric cancer, even in Asian countries including Japan with high prevalence of H pylori infection. Moreover, approximately 10 to 20 % of the population will never be infected with *H. pylori* (Fallone, 1999). Their genetic traits, as well as their living environment, affects the outcomes (Correa et al., 2008; Ando et al., 2006; Goto et al., 2005; Goto et al., 2006].

Urokinase plasminogen activator (uPA) is secreted by cells as a 52 kDa single glycoprotein chain. The uPA converts plasminogen to plasmin, which can activate some prometalloproteinases and degrade the extracellular matrix, and is implicated in cancer invasion and metastasis (Huang et al., 2007; Ulisse et al., 2009; Sidenius et al., 2003; Tkachuk et al., 2009). The uPA also plays an important role in the regulation of inflammatory response (Li et al., 2005; Zhou et al., 2009). It has been reported that the antigen level of uPA is higher in those with gastric atrophy than those without atrophy (Farinati et al., 1996), indicating that the uPA could be involved in the inflammatory infiltrate of the mucosa in the presence of *H. pylori* infection.

The *uPA* gene is located on chromosome 10q24. According to the HapMap project, there are several SNPs of the *uPA* identified in the NCBI Database of Single-Nucleotide Polymorphisms. Among them, the *uPA C/T* polymorphism (rs2227564) in the nucleotide sequence encoding the kringle structure may affect the tertiary structure of the whole molecule, resulting in the lower affinity of the uPA for substrates (Yoshimoto et al., 1996). The associations between the uPA gene polymorphism and type 1 diabetes (Majsterek et al., 2005), colonic cancer (Przybyłowska et al., 2002), and invasive phenotype of gastric cancer (Wu et al., 2008) have been reported.

However, there have been no studies which investigate associations of the *uPA* gene polymorphism with *H. pylori* infection and *H. pylori*-induced gastric atrophy considered as a pre-cancer lesion. Gastric cancer, especially differentiated type, is developed along with the following Correa cascade in the stepwise way; the *H. pylori* infection-gastric atrophy-metaplasia-gastric cancer (Correa et al., 2008, Correa et al., 1975). Whether the uPA influences the Correa cascade has not been investigates. Investigating which steps of Correa cascade the *uPA* polymorphism affects on the way

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to the development of gastric cancer could provide biological researchers with a clue to discovery of the novel mechanism explaining the establishment of *H. pylori*infection and gastric atrophy. Therefore, the aim of this epidemiological study is to evaluate the association with the *uPA* gene poloymorphism and the risk of *H. pylori* infection and gastric atrophy.

Materials and Methods

Subjects

The clinical characteristics of the subjects were described in our previous paper (Katsuda et al., 2003). Briefly, the study subjects were 454 health checkup examinees without a history of cancer (126 males and 328 females) aged 35 to 85, who were enrolled in August and September 2000. The study was approved by the Ethics Committee of the Aichi Cancer Center (Ethical Committee Approval Number 11-12).

Tests for H. pylori antibody and pepsinogens

Anti-*H. pylori* IgG antibody tests, high-molecular-weight campylobacter-associated-protein (HM-CAP) ELISA (Enteric Products Inc., Westbury, NY) was used for identification of *H. pylori*-infected participants. The sensitivity of HM-CAP was reported to be 98.7% with a specificity of 100% in the USA (Evans et al., 1989), though the sensitivity was not so high for Japanese (Matsuo et al., 2000). An ELISA value of 2.3 or over was regarded as indicating *H. pylori* infection-positive status. Pepsinogens 1 and 2 (PG1 and PG2) in serum were measured by radioimmunoassay using a commercially available kit (DINABOT, Tokyo, Japan). We utilized the validated cut off levels employed routinely in Japan, namely PG1 < 70ng/ml and PG1/PG2 < 3.0, to define gastric atrophy (Borch et al., 1989; Kekki et al., 1991; Dinis-Ribeiro et al., 2004).

Determination of the uPA genotype

DNA was extracted from buffy coat fraction by Qiagen QIAamp DNA Blood Mini Kit (QIAGEN Inc.,Valencia,CA). We focused on a nonsynonymous polymorphism of uPA gene (P141L, rs2227564) in the present study, because previous studies suggested that this gene polymorphism could have been functional (Yoshimoto et al., 1996; Przybyłowska et al., 2002). The SNP was genotyped by PCR-CTPP (polymerase chain reaction with confronting two-pair primers) (Kawase et al., 2003). The primers were F1: 5' CCC TCT GGG TTG GAA TGA C, R1: 5' CCA TGC ACT CTT GGA CAA GCG, F2: 5' TGC AGG TGG GCC TAA AGC T, and R2: GGG AGG CAG GTA GGA GAA AG. The PCR was performed with initial denaturation at 95 °C for 10 minutes, followed by 30 cycles of denaturation at 95 °C for 1 minute, annealing at 61.0 °C for 1 minute and extension at 72 °C for 1 minutes. The final extension was at 72 °C for 5 minutes. PCR product was visualized on a 2% agarose gel with ethidium bromide staining (Figure 1).

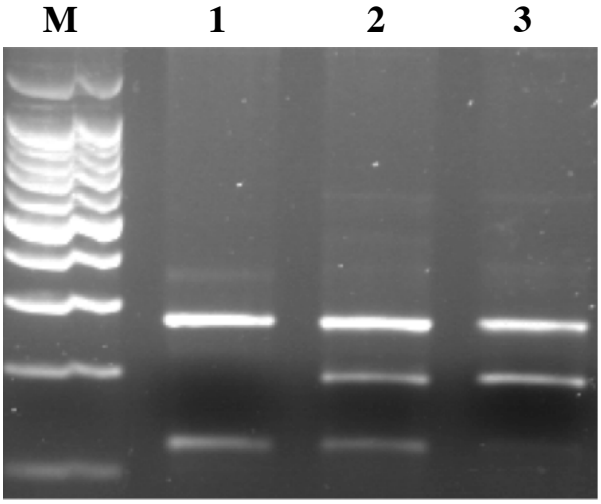

Figure 1. Agarose Gel showing the Different Genotypes of the *uPA* Polymorphism. Lane M, 100-bp marker; lane 1, *C/C* genotype (118 and 266 bp bands); lane 2, *C/T* genotype (118, 187 and 266 bp bands); lane 3, *T/T* genotype (187 and 266 bp bands)

Statistical analysis

Odds ratios (ORs) adjusted for sex and age with 95% confidence intervals (CIs) were calculated using unconditional logistic regression analysis. Hardy-Weinberg equilibrium was tested for the uPA polymorphism by a $\chi 2$ test with 1 df. The data were considered statistically significant at $P<0.05$. These calculations were performed by computer program STATA Version 11 (STATA Corp., College Station, TX).

Results

Study characteristics have been described previously (Katsuda et al., 2003). The number of sero-positivity and gastric atrophy among the subjects was 250 (55.1%) and 158 (34.8%), respectively. The *uPA* genotypes distribution in the subjects was in Hardy-Weinberg

Table 1. Sex-age-adjusted Odds Ratios (ORs) and 95% Confidence Intervals (95% CIs) of Urokinase Plasminogen Activator Genotypes for H. pylori Sero-positivity (HP+)

Genotype	N1	HP+2	OR	95%CI	HP+3	OR	95%CI
C/C	255 (57.3)	146 (57.3)	1	Reference	158 (62.0)	1	Reference
C/T	167 (37.5)	93 (55.7)	0.88	0.58-1.33	99 (59.3)	0.82	0.54-1.26
T/T	23 (5.2)	8 (34.8)	0.34	0.14-0.86	10 (43.5)	0.40	0.16-1.00

[1]Nine subjects could not be genotyped; [2]Three subjects could not be genotyped; [3]Twenty-one sero-negative subjects with atrophy were reclassified as the sero-positive ones, but one could not be genotyped

Table 2. Sex-age-adjusted Odds Ratios (ORs) and 95% Confidence Intervals (95% CIs) of Urokinase Plasminogen Activator Genotypes for Gastric Atrophy (GA) with Sero-positivity and/or GA

Genotype	N[1]	GA[2] (%)	OR	95%CI
C/C	158	94 (59.5)	1	Reference
C/T	99	55 (55.6	0.83	0.50-1.39
T/T	10	6 (60.0)	1.04	0.28-3.87

[1]Four subjects could not be genotyped; [2]Three subjects could not be genotyped

equilibrium (χ^2=0.42, P=0.52). Nine subjects among whom three were sero-positive could not be genotyped. The *T/T* genotype significantly decreased the risk of *H. pylori* sero-positivity (OR=0.34; 95% CI, 0.14 to 0.86) (Table 1). Twenty-one sero-negative subjects with atrophy were considered to have been infected with *H. pylori* because the development of gastric atrophy among those without the bacterial infection is rare (Adamu et al., 2010). After the reclassification of them into sero-positive group, the OR of the genotype was recalculated to evaluate the association of this gene polymorphism with the risk of *H. pylori* infection. The corresponding OR was 0.40 (95% CI, 0.16 to 1.00) (Table 1).

In the next step, it was investigated whether or not the uPA affected the risk of *H. pylori*-induced gastric atrophy. The main comparator groups included not only the sero-positive subjects but also the above 21 seronegative subjects with atrophy. The *uPA* genotype was not found to be associated with the risk of *H. pylori*-induced gastric atrophy (Table 2).

Discussion

This study found that the *uPA* polymorphism was significantly associated with a reduction of *H. pylori* infection risk. No significant association between the uPA gene polymorphism and gastric atrophy was found. To our knowledge, the roles of the uPA/uPAR system as important inflammatory mediators (Li et al., 2005; Zhou et al., 2009) have not yet been well investigated in *H. pylori* infection. However, the biological mechanism explaining the association should be discussed. The factors related to gastric acid secretion have been considered important as the increase of gastric acid is a bactericidal condition (McColl et al., 1998; El-Omar et al., 2001). The previous study reported that NF-κB is highly expressed and activated in the endocrine cells, which stimulate release of acid into the stomach by gastrin (Doger et al, 2006). Therefore, there was a possibility that NF-κB can raise a powerful and fulminant reaction to combat the bacterial infection of the stomach through stimulation of acid secretion into the stomach. Hence, we could not deny a possibility that the uPA could be involved in the immune response accompanied by acid secretion into the stomach, because the uPA/uPAR system is regulated by NF-κB (Novak et al., 1991) and plays an important role in inflammation the same as NF-κB does (Li et al., 2005; Zhou et al., 2009; Doger et al, 2006). In addition, the uPA *C/T* polymorphism (rs2227564) leading to Pro141Leu could be functional. A higher level of the uPA antigen in colorectal cancer samples with the C/C genotype was observed (Przybyłowska et al., 2002). Consequently, based on our hypothesis that those with the *T/T* genotype had a low level of uPA and were less likely to cause inflammation leading to the abolition of gastric acid, they could probably keep bactericidal acidification of the stomach. Further studies to elucidate the functional relevance of the uPA polymorphism are required.

Regarding the minor allele frequency of the uPA (rs2227564), there was no difference between this study's data and the HapMap data base of Japanese subjects; the former was 0.239 and the latter was 0.233. The minor allelic frequencies in other populations were reported in the HapMap database as 0.356 in Han Chinese in Beijing, 0.208 in CEPH samples (Utah residents with ancestry from northern and western Europe) and 0.000 in Yoruba subjects from Ibadan, Nigeria, referring to ss69083728. It would be important to investigate the association between this SNP and *H. pylori* infection in other Asians and Caucasians.

It was necessary to discuss how 21 sero-negative subjects with atrophy should be understood. The spontaneous eradication of *H. pylori* through severe gastric atrophy has been reported to result in the loss of *H. pylori* serological marker (Asaka et al., 2000; Lambert et al., 1995). Of the 21 sero-negative subjects, 10 satisfied the severe gastric atrophy criteria of PG1<30 ng/ml and PG1/PG2<2, which were adopted in the previous studies (Hishida et al., 2009; Tahara et al., 2009; Yanaoka et al., 2008). They lost their sero-positivity due to severe atrophy. The remaining 11 had mild atrophy because they met the condition of atrophy but not the condition of severe atrophy. Judging from the previous reports that gastric atrophy is strongly associated with *H. pylori* infection (Adamu et al., 2010) and that spontaneous regression or clearance of *H. pylori* is rare under normal circumstances excluding severe atrophy (Freeman, 1997; Bair et al., 2009; Xia et al., 1997), they must have had eradication therapy. Therefore, it was certain that the above 21 sero-negative subjects did not develop atrophy without *H. pylori* infection but had been infected with *H. pylori* in the past. To determine whether this gene polymorphism was associated with *H. pylori* infection, we reclassified them together with sero-positive subjects, because unless those with the *T/T* genotype had a significantly decreased the risk of sero-positivity after the reclassification, we could not conclude that this gene polymorphism was associated with H. pylori infection. Even under this condition, we could confirm that the *T/T* genotype reduced the risk of *H. pylori* infection significantly; the OR=0.40 (95%CI: 0.16 to 1.00) (Table1).

In conclusion, the present results suggest the novel association of the *uPA* gene polymorphism with the risk of *H. pylori* infection. This genetic trait may provide a clue to finding a new mechanism associated with the establishment of infection with *H. pylori*. Our study needs replication studies in other independent populations to confirm these results and biological studies to investigate the underlying mechanisms.

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