RESEARCH ARTICLE

No Association between the *CDX2* G543C Polymorphism and Risk of Gastric Atrophy and Cancer

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Abstract

   Ectopic expression of CDX2 in the stomach is closely associated with chronic *Helicobacter pylori* (*H. pylori*) infection and intestinal metaplasia. Whether CDX2 has tumor suppression or tumorigenesis potential remains to be elucidated. In this study, we investigated the association between the *CDX2* G543C polymorphism (silent mutation) and the risk for *H. pylori*-induced gastric atrophy and cancer as well as *H. pylori* infection, using 454 Japanese subjects undergoing a health checkup and 202 gastric cancer patients. The frequency of the minor allele was the same as previously reported in China, but different from that reported in England. *CDX2* G543C was not associated with risk of *H. pylori* infection, gastric atrophy, or gastric cancer, although the point estimate for non-cardiac differentiated gastric cancer as compared to controls with gastric atrophy was 2.22 (95%CI=0.17-29.4). In conclusion, our results indicate that the *CDX2* G543C polymorphism is unlikely to affect the *H. pylori* infection-gastric atrophy-gastric cancer sequence.

Keywords: *CDX2* - polymorphism - *Helicobacter pylori* - gastric atrophy - gastric cancer

Introduction

   Helicobacter pylori (*H. pylori*) induces gastric cancer (Blaser at al., 1995; Asaka et al., 1997), which is divided into two phenotypes, intestinal and diffuse type (Lauren., 1965), corresponding respectively to differentiated and undifferentiated type (Nakamura et al., 1968). Non-cardiac differentiated type develops through the following Correa's cascade; the *H. pylori* infection-gastric atrophy-intestinal metaplasia (IM)-dysplasia-gastric cancer (Correa et al., 1975; 2008). However, the precise molecular mechanisms supporting this progression pathway remain unclear.

   Since a homeobox gene was first discovered in *Drosophila* (Gehring et al., 1990), it has also been identified in other species. Human CDX2 is a member of the caudal-related homeobox gene family and is 94% identical to the mouse counterpart (Mallo et al., 1997). Cdx2 is mainly expressed in the intestine and regulates the differentiation and proliferation of intestinal epithelia in mice (Duluc et al., 1997; Lorentz et al., 1997). Although CDX2 is not expressed in normal gastric epithelium, ectopic expression of CDX2 in the stomach is closely associated with chronic *H. pylori* infection and IM (Bai et al., 2002; Eda et al., 2002; Satoh et al., 2002; Almeida et al., 2003; Kang et al., 2011; Barron et al., 2012). Moreover, previous biological studies showed that CDX2 might play an important role in gastric tumorigenesis (Wang et al., 2012). Nevertheless, another study suggested that CDX2 is a tumor suppressor (Qin et al., 2012).

   Human *CDX2* is localized on 13q12.3-13. Sivagnanasundaram et al. (2001) evaluated somatic and germ line variants in *CDX2* to investigate the association between colorectal cancer and *CDX2* polymorphisms. They determined 6 polymorphisms and 9 haplotypes, but failed to find polymorphisms that relate to the risk for colorectal cancer. However, there has been no evaluation of the association between IM, gastric cancer and *CDX2* polymorphisms in a population-based study.

   The *CDX2* G543C polymorphism in exon 1 at codon 61 (silent mutation) and the *CDX2* T1237C polymorphism in exon 3 at codon 293 (Ser to Pro) have their variant allele more frequently than others and they are in strong linkage disequilibrium (Yagi et al., 1999; Xia et al., 2009). In this study, we investigated how *CDX2* G543C is associated with *H. pylori*-induced gastric atrophy and cancer (non-cardiac differentiated type) as well as *H. pylori* infection.

Materials and Methods

Study subjects
   Detailed information on the healthy controls and gastric cancer patients in this study was reported in our previous paper (Goto et al., 2006). In short, the control group was 454 health checkup examinees (126 males and 328 females) aged 35-85 years with no history of cancer who attended a health checkup program supported by the Nagoya Municipal Government in August and September, 2000. The case group consisted of 202 patients (134 males

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and 68 females) aged 33-94 years with a pathologically confirmed diagnosis of gastric adenocarcinoma, who underwent tumor resection in different hospitals affiliated with Nagoya University. Informed consent was obtained from all subjects. Approval for the study was given by the relevant ethical committees.

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) and HM-CAP with antigens extracted from clinically isolated Japanese *H. pylori* strains (J-HM-CAP) ELISA (Kyowa Medex, Tokyo, Japan), were used for the identification of *H. pylori*-infection in the participants. An ELISA value of 2.3 or over was regarded as positive for both tests. The infection was confirmed in all gastric cancer cases by culture and bacteriological tests using biopsy specimens before gastric resection. Pepsinogens I and II (PG I and PG II) in serum were measured by radioimmunoassay using a commercially available kit (DINABOT, Tokyo, Japan). Gastric atrophy was defined as PG I< 70 ng/ml and PG I/PG II ratio <3, which are the validated cut off levels employed routinely in Japan (Borch et al., 1989; Kekki et al., 1991; Dinis-Ribeiro et al., 2004)

Genotyping

DNA was extracted from the buffy coat fraction by Qiagen QIAamp DNA Blood Mini Kit (QIAGEN Inc.,Valencia,CA). *CDX2* G543C was genotyped by a TaqMan assay using an ABI PRISM 7300 sequence detection system (Applied Biosystems, Foster City, CA, USA) in accordance with the manufacturer's instructions. Thermal cycling conditions for polymerase chain reaction (PCR) were, first, denaturing at 95°C for 10 min, followed by 40 cycles of 92°C for 15s and 60°C for 60s.

Statistical analysis

The strength of associations of *H. pylori* infection, gastric atrophy and gastric cancer with *CDX2* G543C was measured as odds ratios (ORs). ORs adjusted for sex and age with 95% confidence intervals (CIs) were calculated using logistic regression analysis. Hardy-Weinberg equilibrium was tested for the *CDX2* G543C polymorphism. A p value of p<0.05 was considered statistically significant. All statistical tests were two-sided. These calculations were performed by computer program STATA Version 11 (STATA Corp., College Station, TX).

Results

Characteristics of the study subjects were described previously (Goto et al., 2006). Only two controls could not be genotyped. The distributions of the *CDX2* G543C genotypes were in the Hardy-Weinberg equilibrium with the C allele frequency of 0.11: χ^2=0.01 and p=0.92. As our previous study reported (Goto et al., 2006), 21 seronegative controls with gastric atrophy were expected to have been infected with *H. pylori*. They were combined with a seropositive group and both of them were reclassified as the *H. pylori* infection group. The rate of *H.*

Table 1. The Sex-age-adjusted ORs and 95%CIs of the *CDX2* G543C Genotypes for Gastric Atrophy (GA) among *H. pylori* Infection Controls

CDX2 genotype	n	GA*(%)	OR	95%CI
G/G	213	123(57.8)	1	Reference
G/C	53	33(62.3)	1.22	0.66-2.27
C/C	4	1(25.0)	0.24	0.02-2.40
G/C+C/C	57	34(59.7)	1.09	0.60-1.98

Table 2. The Sex-age-adjusted ORs and 95%CIs of the *CDX2* G543C Genotypes for Gastric Cancer among *H. pylori* Infection Subjects

CDX2 genotype	Cases (n=202)	Controls* (n=270)	OR	95% CI
G/G	147(72.8%)	213(78.9%)	1	Reference
G/C	53(26.2%)	53(19.6%)	1.40	0.88-2.24
C/C	2(0.99%)	4(1.48%)	0.6	20.10-3.75
G/C +C/C	55(27.2%)	57(21.1%)	1.3	40.85-2.12

*One control subject could not be genotyped.

pylori infection in the controls was 59.7%. *CDX2* G543C did not increase the risk for *H. pylori* infection (data not shown) or for gastric atrophy among infection controls (Table 1).

CDX2 G543C was not associated with the risk for gastric cancer among infection subjects, which included differentiated and undifferentiated phenotypes (Table 2). In order to elucidate whether *CDX2* G543C could affect Correa's cascade, we restricted the case to a subgroup with non-cardiac differentiated gastric cancer, although information on their phenotypes was not available for 16 cases. All in the subgroup had gastric atrophy. The comparison between the subgroup and controls with gastric atrophy showed that there is no association between *CDX2* G543C and the risk for non-cardiac differentiated gastric cancer (the OR for the *C/C* genotype=2.22:95%CI=0.17-29.4).

Discussion

It has been reported that *H. pylori* infection provokes aberrant CDX2 expression in the stomach (Kang et al., 2011). CDX2 initiates and develops IM through stimulation of intestinal proliferation and differentiation, leading to the development of gastric cancer, especially differentiated (intestinal) type (Barron et al., 2012). This may reflect the fact that *H. pylori* is associated with the development of non-cardiac differentiated type cancer (Correa et al., 1975; Blaser at al., 1995; Asaka et al., 1997; Correa et al., 2008). However, the role of CDX2 in carcinogenesis remains controversial. Some studies indicated Cdx2 has tumorigenic potential (Almeida et al., 2003; Mutoh et al., 2004; Kang et al., 2011; Wang et al., 2012). Among them, Wang et al. (2012) showed the human gastric cancer cell lines (MGC-803) are suppressed by using Cdx2 small interference (si) RNA. Other studied suggested that Cdx2 is a tumor suppressor (Bai et al., 2002; Bonhomme et al., 2003; Qin et al., 2012). Qin et al. (2012) showed Cdx2 expression is decreased through the progression from IM to gastric cancer in a stepwise

way, suggesting that Cdx2 may be a tumor suppressor. That is, some factors may limit Cdx2 expression from reaching threshold above which Cdx2 can work as a tumor suppressor, potentially inducing gastric cancer. Xie et al. (2010) also previously reported that overexpression of Cdx2 inhibits MGC-803 cells in the same way as Cdx2 siRNA. These suggest that the role of Cdx2 in the development of gastric cancer remains elusive and/or Cdx2 could have the dual roles of both tumorgenesis and tumor suppression. Our results show *CDX2* G543C is not associated with risk of gastric cancer. Even after the stratification of the *CDX2* G543C genotypes among cases by phenotype and location of gastric cancer in order to evaluate their effect on Correa's cascade, *CDX2* G543C was not found to be related to the risk for non-cardiac differentiated gastric cancer.

According to *CDX2* cDNA sequence (accession number Y13709), *CDX2* G543C and *CDX2* T1237C are the same as *CDX2* G545C and *CDX2* T1239C, respectively, which Sivagnanasundaram et al. (2001) reported. They have the variant allele more frequently than other polymorphisms that ever reported and they are in strong linkage disequilibrium. Based on these findings, first *CDX2* G543C was examined, finding no associations of *CDX2* G543C with *H. pylori*-induced gastric atrophy and cancer. Therefore, we did not proceed to evaluate the association with *CDX2* T1237C. The frequency of the C allele in our study was 0.11 with little difference from 0.12 in China (Xia et al., 2009), although that Yagi et al. (1999) reported in Japan was 0.07. They were higher than those reported by Sivannanasundaram et al. (2001); 0.04 from CEPH (Centre d'Etude du Polymorphism Humain) and 0.06 from UK population.

There were limitations in this study. We did not have the histological information on whether the subjects had IM. It is well known that *H. pylori* is spontaneously eliminated through IM replacement, resulting in the loss of *H. pylori* serological markers (Lambert et al., 1995; Asaka et al., 2001). Twenty one seronegative subjects with atrophy were thus likely to have metaplasia, though this was not enough to evaluate the effect of *CDX2* G543C on the progression from IM to intestinal gastric cancer. In this study, we used gastric atrophy defined by a serological test as a surrogate for IM, resulting in insufficient evaluation of the effect of *CDX2* G543C on Correa's cascade in a stepwise way.

In conclusion, our study indicates that the *CDX2* G543C polymorphism is not associated with *H. pylori*-induced gastric atrophy and cancer. Further studies are needed to confirm whether CDX2 polymorphism could affect the gastric carcinogenesis pathway.

Acknowledgements

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