Associations of TNF-A-1031TT and -857TT genotypes with Helicobacter pylori seropositivity and gastric atrophy among Japanese Brazilians

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Abstract

**Background.** Our previous study in a Japanese population showed elevated Helicobacter pylori seropositivity in those with tumor necrosis factor (TNF) A-1031TT and -857TT genotypes. This study examined the associations of this seropositivity and serum pepsinogen (PG) levels with these genotypes in Japanese Brazilians.

**Methods.** The subjects were 963 individuals (399 males and 564 females), aged 33 to 69 years, from four regions (Sao Paulo, Curitiba, Mogi das Cruzes, and Mirandopolis) in Brazil. Gastric atrophy was evaluated with serum pepsinogen (PGI < 70 ng/dl and PGI/II < 3), and TNF-T-1031C and C-857T were genotyped by polymerase chain reaction with confronting two-pair primers (PCR-CTPP).

**Results.** The frequency of TNF-A T-1031C was 68.4% TT, 28.4% TC, and 3.3% CC, and that of C-857T was 64.5% CC, 31.7% CT, and 3.8% TT, whose distributions were in Hardy-Weinberg equilibrium. No significant associations of the genotypes with H. pylori seropositivity or gastric atrophy were found. However, male participants with TNF-A -1031CC and -857CC showed the lowest seropositivity (43.8% out of 16), and males with TNF-A -1031TT and -857TT showed the highest (61.5% out of 13).

**Conclusion.** This study demonstrated that the associations between H. pylori seropositivity and TNF-A genotypes were not marked for Japanese Brazilians. The genotypes were not associated with gastric atrophy among the seropositive individuals.

Key words Helicobacter pylori · Gastric atrophy · TNF-A · Polymorphism

Introduction

The lifetime (age, 0 to 84 years) cumulative rate of gastric cancer was estimated to be 14.7% for Japanese males and 4.8% for Japanese females. Based on these estimates, the following two assumptions: (1) the relative risk of Helicobacter pylori infection for gastric cancer is 5, and (2) half of the population is infected with H. pylori, indicate that the cumulative rate of gastric cancer is 21.2% for infected males and 8.0% for infected females, while the rate is 4.2% for uninfected males and 1.6% for uninfected females. These assumptions are applicable for Japanese in their 40s or 50s. Although H. pylori infection has such a large impact on gastric cancer, the steps from the infection, to gastric atrophy, to carcinoma are not fully understood in terms of gene-environment interactions.

Tumor necrosis factor (TNF) α, a cytokine induced by H. pylori, inhibits gastric acid secretion. The TNF-A gene on chromosome 6p21.3 encoding TNF-α is known to have five biallelic single-nucleotide polymorphisms in the promoter region; T-1031C, C-863A, C-857T, G-308A, and G-238A. Among Japanese, the -308A and -238A alleles are rare (1.7% and 2.0% among 575 controls, respectively), and C-863A is tightly linked with T-1031C. The -1031C allele is also linked with the -857C allele, and no haplotypes for -1031C linked with -857C were found in our previous study in 1361 Japanese.

Significant associations of the TNF-A -308A allele with H. pylori infection have been reported in Italy, and such associations with CagA-positive H. pylori infection have been reported in Korea. A small study in Korea showed that the -308A allele was significantly more frequent in patients infected with CagA- H. pylori (9 out of 46) than in healthy controls with unknown H. pylori infection status (7 out of 113). In Germany, the -308A allele was not found among 14 H. pylori-positive female patients with duodenal...
ulcer, while 26.8% of 98 *H. pylori*-positive female patients without duodenal ulcer had at least one -308A allele. In that study, no difference in G-308A genotype distribution was observed between the *H. pylori*-positive and -negative subjects.\(^7\) Those with the -308A allele were reported to have higher TNF-α production in whole blood cell culture 24h after stimulation by lipopolysaccharide.\(^1.13\) Concerning TNF-A T-1031C and C-857T, the relation to TNF-α expression is inconsistent.\(^6,12,13\) However, in an Italian study, TNF-A -857TT was more frequent in CagA+ subjects than in all subjects, including *H. pylori*-negative subjects, indicating that TNF-A -857TT was less frequent in *H. pylori*-negative subjects.\(^8\) Our previous study showed that those harboring -1031TT and -857TT had the highest *H. pylori* seropositive rate.\(^7\)

The present study examined whether the associations of TNF-A T-1031C and C-857T genotypes with *H. pylori* seropositivity were reproduced among Japanese Brazilians, who maintain Japanese lifestyles, and have a high gastric cancer mortality rate, similar to that of Japanese in Japan.\(^14\) The study was publicized through associations of Japanese, such as Kenjinkai (associations named after each prefecture of Japan), Japanese Brazilian country clubs, and nonprofit societies of Japanese Brazilians. After written informed consent had been obtained from the volunteers, lifestyle data and blood samples were collected at the rooms of the various associations on the occasions of festivals and sport competitions (undokai) from March to May 2001. Those volunteers with a history of disease such as ulcer and gastric cancer were not excluded. The participants numbered 662 in Sao Paulo, 90 in Curitiba, 110 in Mogi das Cruzes city, and 107 in Mirandopolis city. Nine participants aged 33 to 34 years were included, and 6 participants aged less than 30 or more than 75 years were excluded. The latter were too young or too old, according to the eligibility criteria, to be included. Their seropositivity and lifestyle data were regarded as being different from those of the other ages. The age eligibility for participation was determined in an arbitrary way, so was considered that the inclusion of participants aged 33 to 35 years would make no difference to the result. The remaining 963 subjects (399 males and 564 females) of full Japanese ancestry were the subjects of the present analysis. This group consisted of 97 immigrants (Issei), 763 second generation (Nisei), and 103 third generation (Sansei).\(^15,17\)

Anti-*H. pylori* antibody was tested with high-molecular-weight campylobacter-associated-protein (HM-CAP) enzyme-linked immunosorbent assay (ELISA; Enteric Products, Westbury, NY, USA) by SRL (Tokyo, Japan). According to the commonly used definition, 2.3 EV (ELISA value) or higher was regarded as *H. pylori*-seropositive. Gastric atrophy was evaluated with serum pepsinogens (PG1 < 70ng/dl and PG1/II < 3). The pepsinogens were measured by E-plate Eiken Pepsinogen I and II (Eiken, Tokyo, Japan) at Mitsubishi Kagaku, BCL (Tokyo, Japan). Genotyping was conducted by polymerase chain reaction with confronting two-pair primers (PCR-CTPP),\(^18\) using 25μl of PCR mixture, as described in our previous study.\(^7\)

Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated by an unconditional logistic model. Age was adjusted using dummy variables for 10-year age groups. A trend test for genotypes on ORs was performed by allocating sequence numbers such as 1, 2, or 3. STATA Version 7 (STATA, College Station, TX, USA) was used for these calculations. Command “genhwi” of STATA was used for examining Hardy-Weinberg equilibrium.

### Results

#### Genotyping

Due to the possible degradation of DNA, clear bands on PCR-CTPP were not observed in 15 participants for TNF-A C-1031T and 13 participants for TNF-A C-857T, resulting in 948 participants and 950 participants, respectively, being successfully genotyped. The genotype distributions were in Hardy-Weinberg equilibrium: \(\chi^2 = 0.226; P = 0.635\) for TNF-A T-1031C with 0.175 of C allele, and \(\chi^2 = 0.016; P = 0.899\) for TNF-A C-857T with 0.196 of T allele.

#### *H. pylori* seropositivity

Table 1 shows the *H. pylori* seropositivity according to sex. There was no difference in the seropositivity rate between the sexes. For those aged 33 to 49 years, the seropositivity rate in males was higher than in females, and for those aged 50 years or over, the rate was slightly lower than in females. The seropositivity rate was the highest in genotype TNF-A -1031TT for each sex, and in genotypes TNF-A -857TT and in TNF-A -1031TT & -857TT for males.

Age-adjusted OR and 95% CI values for each sex, and age-sex adjusted OR and 95% CI for all participants are listed in Table 2. No significant ORs were observed. The trend test was not statistically significant for any genotypes, although a trend in the OR was observed for the combinations of T-1031C and C-857T in males (Table 2). Among those aged 50 years or over or ever smokers, a similar trend was observed, though it was not significant (Table 3).

#### Gastric atrophy

Table 4 shows the percentages of those with gastric atrophy among the seropositive individuals. Only 14 male parti-