HHS Public Access

Author manuscript

J Dig Dis. Author manuscript; available in PMC 2017 January 01.

Published in final edited form as:

J Dig Dis. 2016 January ; 17(1): 20–27. doi:10.1111/1751-2980.12309.

Prostate stem cell antigen gene TT genotype and development of intestinal metaplasia in *Helicobacter pylori* infection

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Abstract

Aim—Gastric cancer is etiologically related to interactions between *Helicobacter pylori* infection, environmental, and host factors. Gastric carcinoma is associated with a cascade of increasing atrophic gastric mucosal damage. Prostate stem cell antigen polymorphisms have been associated with an increased risk of gastric cancer. Here, we examined the interaction between prostate stem cell antigen polymorphisms and *H. pylori* in the progression of *H. pylori* gastritis.

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Disclosure of funding and conflicts of interest

Conflict of Interest

TU has received research grants from Institute for Fermentation, Osaka (IFO), Japan.

DYG is a consultant for RedHill Biopharma and has received research support. He is also a consultant for Otsuka Pharmaceuticals and for BioGaia.

Methods—Prostate stem cell antigen polymorphisms (TT, TC and CC) among *H. pylori* infected and uninfected Bhutanese were compared with the severity of *H. pylori* gastritis (neutrophils, monocytes, atrophy scores, *H. pylori* density, and the presence and extent of intestinal metaplasia) using the updated Sydney system.

Results—Biopsies from 339 patients were included. The proportion of biopsies with intestinal metaplasia was also significantly ($P<0.05$) greater among those with the TT genotype than with either the CT or CC genotype. Despite no significant differences in inflammation or *H. pylori* density scores, the scores for the premalignant condition, intestinal metaplasia in both the gastric corpus and antrum, among *H. pylori* infected with the TT genotype was significantly ($P <0.05$) greater than C allele carriers.

Conclusions—Prostate stem cell antigen TT genotype was associated with more than a 3-fold increase in the prevalence and extent of gastric mucosal intestinal metaplasia compared to C allele carriers among *H. pylori* infected Bhutanese.

Keywords

Helicobacter pylori; Gastric cancer; *Prostate stem cell antigen gene*; Intestinal metaplasia; Single nucleotide polymorphism

Introduction

Helicobactor pylori (*H. pylori*) is an important human pathogen etiologically associated with atrophic gastritis and gastric cancer[1]. *H. pylori* related gastric carcinogenesis is thought to involve an interplay between bacterial-, environment- and host factors[2, 3]. *H. pylori* virulence factors such as CagA, VacA, OipA and DupA associated with an increased host inflammatory response are also associated with an enhanced risk of clinically significant outcomes including peptic ulcer and cancer[4]. Host factors identified as related to gastric cancer have often been recognized on the basis of genetic polymorphisms (e.g. single nucleotide polymorphisms; SNPs) and primarily involve genes related to an enhanced inflammatory response[5]. Genome wide association studies (GWAS) identified that *prostate stem cell antigen* (*PSCA*, rs2294008), a cell surface marker overexpressed in prostate cancer[6], was associated with an increased risk of gastric cancer[7]. This *PSCA* SNP is located in exon 1 of chromosome 8q24.2 and is thought to possibly have tumor suppressor-like role in the stomach.[7] GWAS identified the TT genotype as a possible important genetic risk factor for both diffuse type gastric cancer[7] and for non-cardia gastric cancer[8, 9]. The C allele of this SNP was however associated with an increased risk for duodenal ulcer[10–12] which is interesting in that gastric cancer is associated with atrophic pangastritis whereas duodenal ulcer is associated with corpus sparing antral predominant gastritis. The role of *PSCA* polymorphisms in the pathogenesis of gastric cancer and different patterns of gastritis remains unclear (Figure 1)[12, 13].

Bhutan is a landlocked country surrounded in the north by China and to the south, east and west by India. The prevalence of *H. pylori* infection in Bhutanese is high (eg, >70%)[14]. The *H. pylori* in Bhutanese is typically *cagA* positive with the *cagA* of the more virulent East Asian-type[15]. The age-standardized rate (ASR) of gastric cancer in Bhutan has been

reported to be 17.2/100,000 which is higher than in adjacent India (GLOBOCAN 2012: http://globocan.iarc.fr/).

Here, we compared *PSCA* polymorphisms with the histological grading of gastritis among *H. pylori*-infected Bhutanese to assess the possible role of *PSCA* in the different histologic patterns of gastritis in a non-east Asian population.

Methods

This study is part of a series of studies designed to examine the importance of *H. pylori* in Bhutan[14, 15]. Approval for the samples and the protocol including this study was obtained in advance form the Ethics Committee of Jigme Dorji Wangchuk National Referral Hospital, Bhutan and of Oita University Faculty of Medicine, Japan. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the Helsinki declaration.

Subjects

As previously described, we recruited 401 Bhutanese volunteers with no symptom or mild dyspeptic symptoms in December 2010[14]. Exclusion criteria were past treatment for *H. pylori* infection, history of abdominal operation, regular use of nonsteroidal anti-inflammatory drugs, low-doses of aspirin, or potent acid inhibitors. Additionally, we excluded volunteers who took any antibiotics for the previous 4 weeks. Informed consent was obtained from all individual participants included in the study. We excluded 62 samples for lack of insufficient samples or lack of background data resulting in a total of 339 samples (Figure 2).

Gastroscopy and histological examination

At gastroscopy, one biopsy from the gastric corpus and three biopsies from the antrum were obtained. The two samples from the antrum were used for rapid urease test and for *H. pylori* culture. The gastric biopsy specimens from the antrum and corpus were fixed in 10% buffered formalin and serial sections were stained with hematoxylin and eosin and with May-Giemsa stain and scored using the updated Sydney system by a single Japanese pathologist who was blinded to the background[16]. The updated Sydney system scores *H. pylori* density, neutrophils, monocytes, atrophy, intestinal metaplasia in the antrum and corpus into one of 5 grades (0 to 4). Samples with a grade 1 or more were considered positive[17]. Furthermore, *H. pylori* density was also evaluated by immunohistochemistry with polyclonal anti-*H. pylori* antibody as described previously[18].

H. pylori diagnosis and *PSCA* polymorphism genotyping

H. pylori infection was diagnosed using combinations of rapid urease test, culture, histology and serological examination. *H. pylori* was identified on the basis of colony morphology, Gram staining and positive reactions for oxidase, catalase and urease. Serological examination was evaluated with a commercially available ELISA kit (Eiken Co., Ltd., Tokyo, Japan) and rapid urease test was also evaluated with a commercially available kit (CLO or Campylobactor-like organism test; Kimberly-Clark Ballard Medical products,

Roswell, GA). The samples with least two positive results were diagnosed to be *H. pylori*-positive. *H. pylori* negative was defined as negative by all tests.

Genomic DNA was extracted from gastric mucosal biopsy specimens using a commercially available kit (QIAamp DNA Mini kit; QIAGEN, Hilden, Germany). The samples were classified into 3 SNPs of *PSCA* (T/T, T/C and C/C) by PCR-Restriction Fragment Length Polymorphism (PCR-RFLP). The primers were used 5'-AGG TGG AAA GAA GGA CAA AGG G-3' (forward) and 5'-GGC CAA GCC TGC CAT CAA-3'(reverse) and the PCR products were digested with the restriction enzyme NlaIII (New England BioLabs, Beverly, MA)[19].

Statistical Analyses

Hardy-Weinberg equilibrium of allele frequencies at individual assessed by comparing the observed and expected genotype frequencies using the chi squared test. Ages were given as mean and standard deviation, histological score were given as mean and standard error. Chi-square test, Fisher's exact test, Wilcoxon's signed-rank test, Kruskal-Wallis test and Spearman's rank correlation coefficient were used as appropriate for statistical analysis using JMP 10.0 software (SAS, Cary, NC). All $P < 0.05$ were considered to be statistically significant.

Results

Subject Characteristics

Gastric biopsy samples from 339 Bhutanese were included in this study. No significant differences were observed in sex (Table 1). The age of *H. pylori* negative subjects was significantly greater than that of positive subjects (*H. pylori* positives; 37 ± 14 years old, and negatives; 43.4 ± 14 years) (Table 1). The prevalence of *PSCA* genotypes was similar between *H. pylori* positive and negative subjects (Table 1). HapMap project data showed that the prevalence of *PSCA* genotypes and allele frequency of Bhutanese was relatively similar to that of Chinese but dissimilar to that of Japanese and Gujarati Indians living in Texas (Table 2). Clinical presentation included 19 subjects with duodenal ulcer (DU), 22 with gastric ulcer (GU) and 3 with gastric cancer; DU, GU, and gastric cancer were identified by endoscopy. Gastric cancer was confirmed by histopathology. The remaining 295 subjects had endoscopically normal appearing mucosa or endoscopic gastritis without ulcers or cancer. The prevalence of *H. pylori* infection was 94.7% (18/19) in DU, 90.9% (20/22) in GU, 33.3% (1/3) in gastric cancer, and 71.9% (212/295) in subjects without peptic ulcers or gastric cancer.

The updated Sydney System score between *H. pylori* positives and negatives

We compared histological scores between *H. pylori* infected vs. uninfected at each gastric site. As expected, neutrophils, monocytes and atrophy scores in *H. pylori* positives were significantly higher than these in negatives in both the antrum and corpus (all $P < 0.01$) (Table 3). Intestinal metaplasia was overall uncommon and the differences in severity and proportion with intestinal metaplasia were not different irrespective of *H. pylori* infection (Table 3). The scores of neutrophils and monocytes had significant correlations with *H.*

pylori density of *H. pylori* positives (Table 4). There was no correlation between inflammatory cell scores and the score of intestinal metaplasia (Table 4).

The updated Sydney System score separated by *PSCA* genotypes

Because the gastric mucosa of *H. pylori*-negative subjects was typically normal (Table 3), we focused on possible relationships between *H. pylori* infection and *PSCA* genotypes. Separating each histological score by *PSCA* genotypes, we found that the intestinal metaplasia score among those with TT genotype was significantly greater than that of C allele carriers in both the gastric corpus and antrum (corpus; TT vs CT; P = 0.02 and TT vs CC; P <0.01, antrum; TT vs CT; P = 0.01 and TT vs CC; <0.01) (Table 5). There was no relationship between *PSCA* genotype and gastric mucosal damage as expressed by scores for neutrophils, monocytes or atrophy (Table 5). Although, the numbers with clinical disease were low, we found no relationship between *PSCA* genotype and clinical presentation; CC:CT:TT (number [%]) = 8 (44.4%): 6 (33.3%): 4 (22.2%) for DU, 11 (55%): 8 (40%): 1 (5%) for GU, 1 (100%):0:0 for gastric cancer and 104 (52.3%): 71 (37.2%): 24 (12.0%) for subjects without ulcers or cancer).

Figure 3 shows the comparison of intestinal metaplasia among each *PSCA* genotype. In the corpus, the proportion of intestinal metaplasia in TT genotype was significantly greater than in either the CT or CC genotype (TT genotype: 10%, CT genotype: 1.1%, CC genotype: 0%, *P* <0.01 and <0.05) (Figure 3). In the antrum, the pattern was similar (TT genotype: 30%, CT genotype: 10%, CC genotype: 8.4%, *P* <0.01 and = 0.02) (Figure 3). The proportion with intestinal metaplasia in the antrum was significantly greater than that in the corpus consistent with intestinal metaplasia starting in the antrum and then progressing into the corpus (CC; *P* <0.01, CT; *P* = 0.02, TT; *P* = 0.04) (Figure 3).

As shown in Table 5 and Figure 3, the histological scores for intestinal metaplasia and the proportion with intestinal metaplasia with the TT genotype of *PCSA* with *H. pylori* positive was significantly greater than these among C allele carriers without other mucosal changes in each genotype.

Discussion

H. pylori infection results in progressive gastric mucosal damage that may advance to atrophic gastritis, increasing intestinal metaplasia and gastric carcinoma[20]. In this study, we aimed to investigate whether the *PSCA* genotype was linked to the progression of *H. pylori*-associated gastric mucosal damage as evidenced by the presence of atrophy and intestinal metaplasia. Intestinal metaplasia is considered a premalignant condition. For example, in a cohort study of 4,655 healthy asymptomatic subjects over 7 years, the risk of gastric cancer in severe chronic atrophic gastritis with extensive intestinal metaplasia was higher than that in chronic atrophic gastritis without intestinal metaplasia[21]. Overall, gastric cancer risk correlates with atrophy/intestinal metaplasia, rather than with neutrophil/monocyte infiltration[22].

PSCA is a member of the lymphocyte antigen 6 (LY-6) family of glycosylphosphatidylnositol (GPI) –anchored cell surface proteins and one of the most

abundant transcripts in differentiated tumors (ex. urothelial tumor)[23]. Although the gene was first found as a prostate-specific antigen overexpressed in prostate cancer, it was subsequently shown that other normal organs such as stomach, colon, and trophoblast[6, 24]. PSCA expression in cancer depends on each organ (e.g. prostate, pancreas, kidney and ovary are up-regulated, whereas, stomach and esophagus are down-regulated)[23, 25–27]. In the stomach PSCA protein is mainly expressed in neuroendocrine cells [24]. PSCA is thought to have an inhibitory role in proliferation of differentiating gastric epithelial cells and leads the down-regulation in gastric cancer tissues as a tumor suppresser gene[7, 23]. There are several hypotheses about the differences of pathogenicity among each genotype. In one of the reasonable hypotheses, T allele carrier's express a PSCA protein with an additional fragment of nine amino acid at the N-terminal protein (long PSCA, 123 amino acids), whereas C allele carrier's express shorter a PSCA protein (114 amino acids)[11]. Although long PSCA has N- terminal protein localizing to the plasma membrane, short PSCA resides in the cytoplasm[28]. This genetic variation is thought to result in the differences in biological function of PSCA.

A number of studies have confirmed that the *PSCA* TT genotype is associated with an increased risk of atrophic gastritis and gastric cancer and a reduced risk of duodenal ulcer[78–12]. Conversely, the CC genotype is related to an increased risk of duodenal ulcer and a reduced risk of gastric cancer. To date the focus on gastric carcinogenesis has largely been on inflammation[2, 3]. The presence of atrophy and intestinal metaplasia are currently thought to be end products of the inflammatory response. However, the details of the mechanisms remain unclear and include both the aggressive feature of inflammation and those factors acting to limit the inflammatory response. The fact that the presence of severity and location of gastric inflammation did not differ in relation to *PSCA* genotype suggests the role of *PSCA* in gastric cancer involves factors other than those directly related to the type and severity of inflammation. However, the presence and location of intestinal metaplasia did relate to *PSCA* genotype suggesting factors more directly related to progression of intestinal metaplasia are involved. The available data suggest that the T genotypes predispose to corpus atrophy whereas the C genotype is either not conducive to the development of corpus atrophy or is actually related to mechanisms protecting the corpus from *H. pylori*-induced inflammation and its consequences. Gastric cancer is associated with low to absent gastric acid secretion. In contrast, duodenal ulcer disease is associated with the combination of minimal corpus inflammation with marked antral inflammation which allows robust acid secretion and the high duodenal acid load required for development of duodenal ulcer disease[29]. .The potential mechanisms that differ in relation to *PSCA* genotype include those responsible for blunting the effects of *H. pylori* associated inflammation and/or its ability to damage genes which result in a reduction of acid secretion or those associated with mucosal repair [2]. For example, duodenal ulcer disease is also associated with a high parietal cell mass and a high acid output. The gastric pits in the corpus transmit 140–160 mmol hydrochloric acid from the parietal cells to the lumen. This high acidity prevents deep colonization of the corpus mucosa by *H. pylori* and *H. pylori*-related mucosal inflammatory cell-derived IL-1β from inhibiting parietal cell secretion[1]. However, a reduction in acid secretion such as associated with parietal cell vagotomy or the use of a proton pump inhibitor rapidly can rapidly result in with extension of *H. pylori* into the corpus pits and development of corpus

gastritis [30, 31]. It is possible that the CC genotype may be related in some why to parietal cell mass or to the regulation of gastric acid secretion. However, this study was not designed to comprehensively evaluate either the structure or function of the corpus mucosa. Subsequent studies should consider a more comprehensive evaluation of gastric acid secretion, mapping of the gastric corpus as well as studies of genes involved in repair of mucosal and DNA damage.

Our study confirms and extends prior studies relating *PSCA* genotypes and *H. pylori*-related diseases[8, 32]. We evaluated a non-east Asian population with a relatively uniform population, diet, and environment. However, our study has the several limitations. First, the sample size was relatively small and the range of mucosal damage among the participants was limited. Second, the virulence of the *H. pylori* infecting Bhutanese is uniform which did not allow any analysis of the potential effects of different *H. pylori* virulence factors. Finally, the protocol allowed only two biopsy specimens for histology so that the full histologic evaluation by the by updated Sydney system could not he obtained. However, the results suggest that the possibilities for the differences in outcome with the different genotypes need to be expanded to include both protective factors and those related to the control of gastric acid secretion.

In summary, *PSCA* polymorphism TT was associated with an increased prevalence of intestinal metaplasia in *H. pylori*-infected Bhutanese. The patients with TT genotype of *PSCA* increased the risk of development of intestinal metaplasia more than 3-fold compared with C allele carrier. Gastric atrophy/intestinal metaplasia is final stage of mucosal change of the gastric inflammatory response resulting in gastric cancer and the data suggest that *PSCA* polymorphisms may be related to the development of intestinal metaplasia in the *H. pylori* infected stomach. We failed to find a relation between *PSCA* polymorphisms and intensity of acute and chronic cellular inflammation suggesting the additional mechanisms need to also be reconsidered such as mechanisms related to control of acid secretion. Gastric cancer is related to gastric atrophy and genetic instability. It is possible that *PSCA* genotype influences *H. pylori* induced genetic instability by interacting with repair mechanism such as those involved in DNA methylation, microRNA expression, or *H. pylori* induced DNA breakage. This study was not designed to evaluate gastric acid secretion or the expression of genes involved in repair of mucosal or DNA damage. Such studies might be useful to clarify the links between increased risk of gastric inflammation intestinal metaplasia and genetic polymorphisms of *PSCA*.

Acknowledgments

We thank Lotay Tshering (Department of Surgery, Jigme Dorji Wangchuck National Referral Hospital, Thimphu, Bhutan) providing us the gastric biopsy samples.

Funding

This report is based on work supported in part by grants from the National Institutes of Health (DK62813), and Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan (24406015, 24659200, 25293104, 26640114 and 15H02657). This work was also supported by the Japan Society for the Promotion of Science (JSPS) Institutional Program for Young Researcher Overseas Visits and the Strategic Funds for the Promotion of Science and Technology from Japan Science and Technology Agency (JST).

Abbreviations

PSCA	*Prostate stem cell antigen*
H. pylori	*Helicobacter pylori*
cagA	Cytotoxin-associated gene A
vacA	Vacuolating cytotoxin A
oipA	Outer inflammatory protein
DupA	duodenal ulcer promoting gene A product
SNP	Interleukin Single nucleotide polymorphism
GWAS	Genome wide association studies

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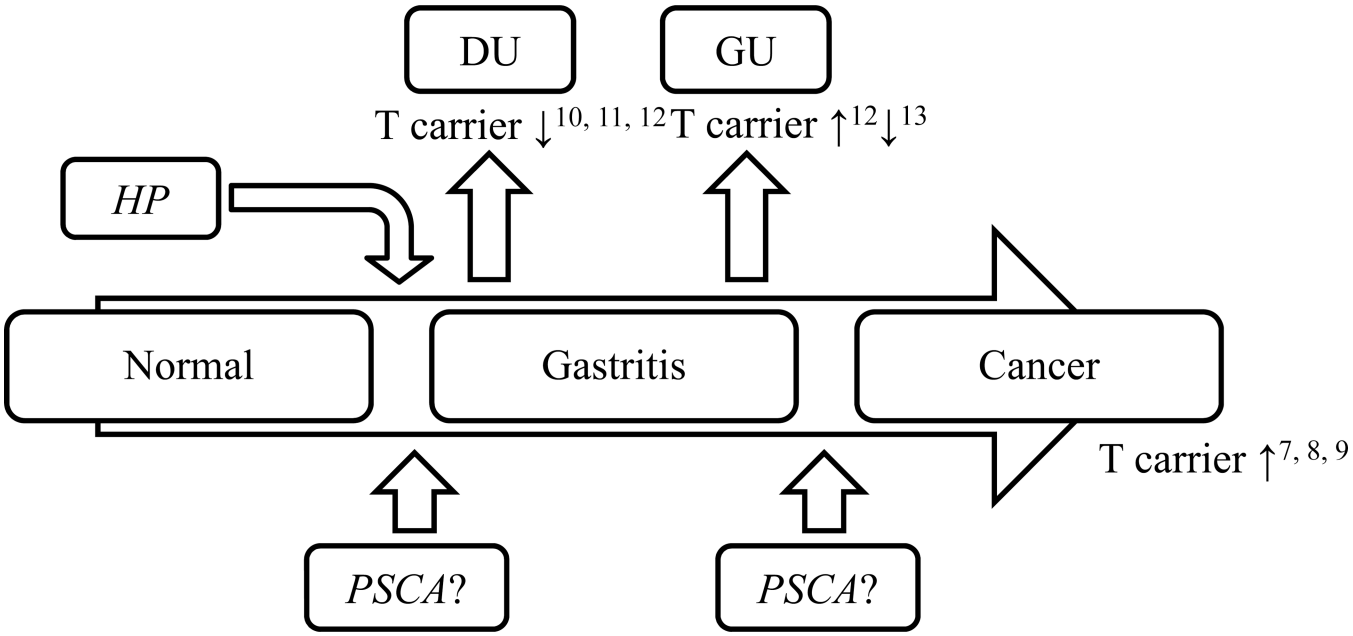

Figure 1. Relationship between *PCSA* genotypes and gastroduodenal disease

Although the relation between gastric cancer, gastroduodenal ulcer and *PSCA* genotypes have been shown, the relationship between gastric histological changes and *PSCA* genetic polymorphism in *H. pylori* infection remains unclear. Superscript on figure means reference number.

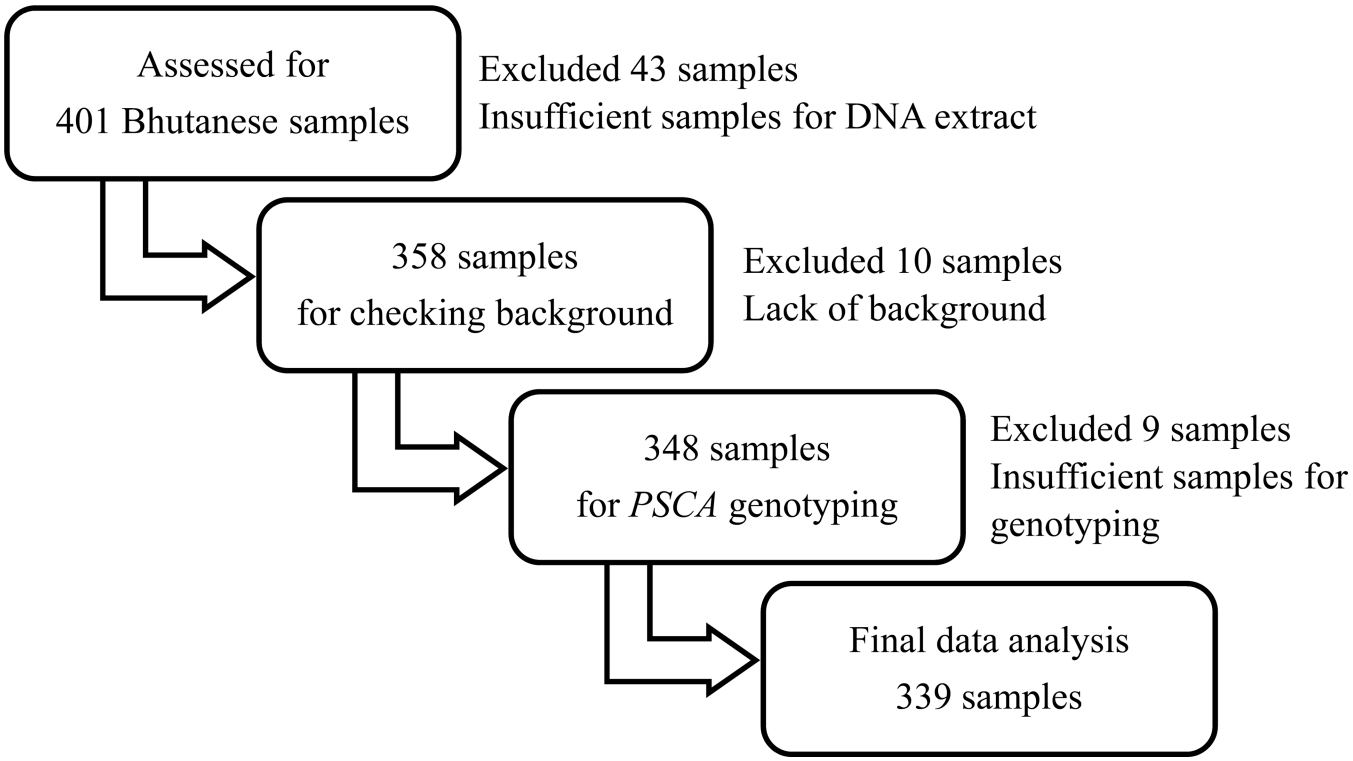

Figure 2. Design of study

We recruited 401 Bhutanese volunteers. After excluding samples where background data was unknown or the samples were insufficient for DNA extraction or genotyping, a total of 339 samples remained.

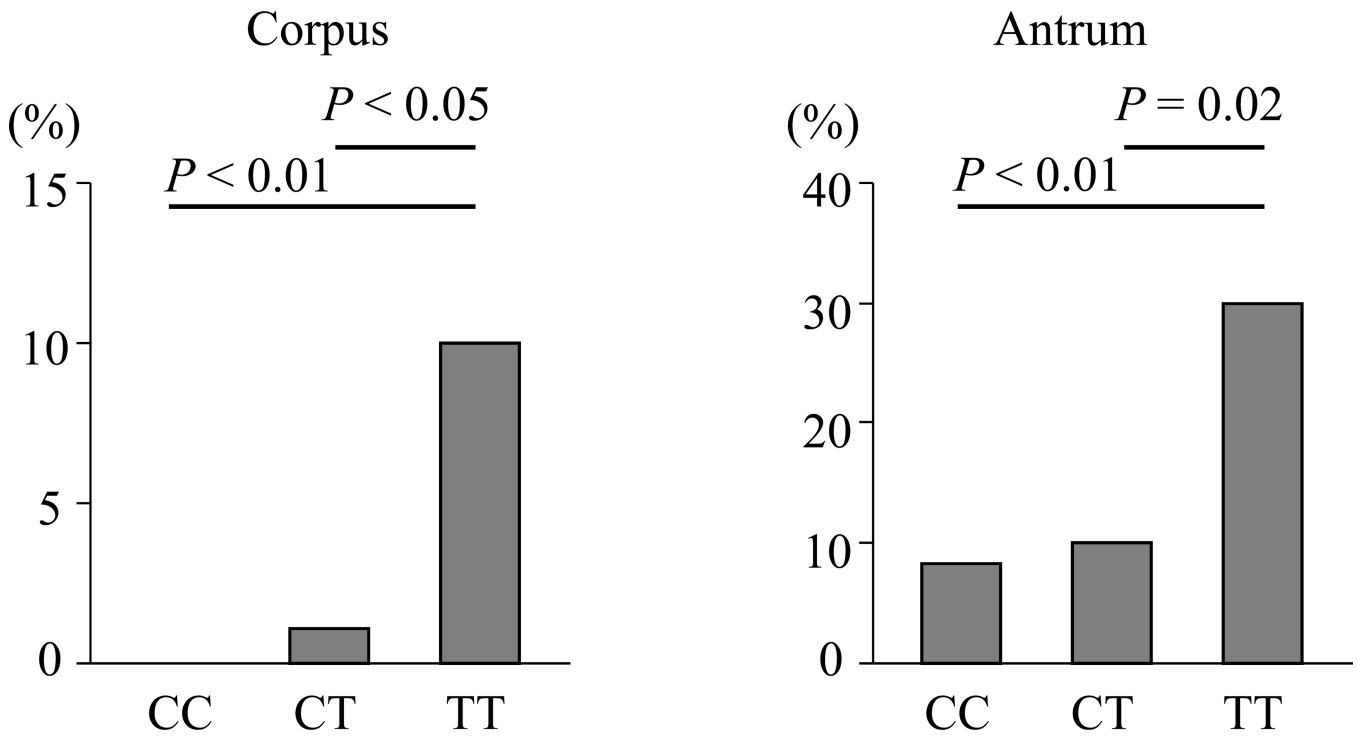

Figure 3. The proportion of intestinal metaplasia with *H. pylori* infection in each gastric site

The proportion with intestinal metaplasia was significantly greater with the TT genotype than with the CT or CC genotypes in both the corpus and antral mucosa (P <0.05). In all genotypes, the proportion of intestinal metaplasia in the antrum was significantly higher than that in the corpus (P < 0.05).

Table 1

Patients characteristics

	Total N = 339 (%)	***H. pylori* positive N = 251 (%)**	***H. pylori* negative N = 88 (%)**	***P* value**
Man Female	150 : 189 (44.2 : 55.8)	118 : 133 (47.0 : 53.0)	32 : 56 (36.4 : 63.6)	$P = 0.10$
Age	38.7 ± 14.5	37.0 ± 13.9	43.4 ± 15.0	$P < 0.01$
PSCA CC CT TT	176 : 127 : 36 (52.0 : 37.5 : 10.6)	131 : 90 : 30 (52.2 : 35.8 : 12.0)	45 : 37 : 6 (51.1 : 42.0 : 6.8)	$P = 0.32$

Prostate stem cell antigen; *PSCA*

No significantly differences in sex and prevalence of *PSCA* genotypes between *H. pylori* positive and negative subjects. Age of *H. pylori* negative subjects was significantly greater than that of positive subjects.

Table 2

The prevalence of *PSCA* genotypes in Asia

	Genotypes (%)			Allele (%)	
	CC	**CT**	**TT**	**C**	**T**
Bhutanese	52.0	37.5	10.6	70.7	29.3
HapMap-HCB	53.5	41.9	4.7	74.4	25.6
HapMap-JPT	12.8	50	37.2	37.8	62.2
HapMap-CHD	48.2	44.6	7.2	70.5	29.5
HapMap-GIH	25.0	52.3	22.7	51.1	48.9

Hap-Map is quoted from International HapMap Project site http://hapmap.ncbi.nlm.nih.gov/.

HCB; Han Chinese in Beijing, China, JPT; Japanese in Tokyo, Japan, CHD; Chinese in Metropolitan Denver, Colorado, GIH; Gujarati Indians in Houston, Texas.

HapMap project data shows that the prevalence of genotypes and allele frequency of Bhutanese was relatively similar to that of Chinese.

Table 3

The updated Sydney System score between *H. pylori* positive and negative in each gastric site

Region	Histology	*H. pylori positive*	*H. pylori negative*	*P* value
Corpus	Neutrophils	0.86 ± 0.04 (1)	0.08 ± 0.06 (0)	< 0.01
	Monocytes	1.10 ± 0.04 (1)	0.35 ± 0.06 (0)	< 0.01
	Atrophy	0.51 ± 0.04 (0)	0.23 ± 0.02 (0)	< 0.01
	IM	0.02 ± 0.01 (0)	0.03 ± 0.02 (0)	= 0.69
Antrum	Neutrophils	1.36 ± 0.04 (1)	0.18 ± 0.07 (0)	< 0.01
	Monocytes	1.63 ± 0.04 (2)	0.59 ± 0.07 (1)	< 0.01
	Atrophy	1.36 ± 0.04 (1)	0.97 ± 0.07 (1)	< 0.01
	IM	0.16 ± 0.03 (0)	0.11 ± 0.05 (0)	= 0.47

Intestinal metaplasia; IM

Values are shown as mean±SE (median).

Comparing histological scores between *H. pylori* infected vs. uninfected, neutrophils, monocytes and atrophy scores in *H. pylori* positives were significantly higher than these in negatives in both the antrum and corpus. The differences in severity and proportion with intestinal metaplasia were not different irrespective of *H. pylori* infection.

Table 4

The correlation between the updated Sydney System score and *H. pylori* density in each gastric site.

Histology	Corpus		Antrum	
	***P* value**	**R**	***P* value**	**R**
Neutrophils	< 0.01	0.43	< 0.01	0.45
Monocytes	< 0.01	0.28	< 0.01	0.37
Atrophy	< 0.01	0.22	< 0.01	0.16
IM	0.77	0.02	0.52	−0.04

Intestinal metaplasia; IM, Correlation coefficient; R

However the scores of neutrophils and monocytes had significant correlations with *H. pylori* density of *H. pylori* positives, there was no correlation between inflammatory cell scores and the score of intestinal metaplasia.

Table 5

The updated Sydney System score separated by *PSCA* genotypes with *H. pylori* positive in each gastric site

	Histology	CC	CT	TT	*P* value (TT vs CT)	*P* value (TT vs CC)
Corpus	Neutrophils	0.79 ± 0.06 (1)	0.99 ± 0.07 (1)	0.77 ± 0.12 (1)	0.16	0.70
	Monocytes	1.06 ± 0.05 (1)	1.19 ± 0.06 (1)	0.97 ± 0.11 (1)	0.11	0.40
	Atrophy	0.44 ± 0.06 (0)	0.61 ± 0.07 (1)	0.47 ± 0.12 (0)	0.37	0.71
	IM	0.00 ± 0.02 (0)	0.02 ± 0.02 (0)	0.13 ± 0.04 (0)	= 0.02	< 0.01
Antrum	Neutrophils	1.34 ± 0.07 (1)	1.38 ± 0.08 (1)	1.40 ± 0.14 (1)	0.65	0.61
	Monocytes	1.61 ± 0.06 (2)	1.69 ± 0.07 (2)	1.53 ± 0.13 (1)	0.26	0.42
	Atrophy	1.36 ± 0.05 (1)	1.34 ± 0.07 (1)	1.43 ± 0.11 (1)	0.49	0.56
	IM	0.12 ± 0.04 (0)	0.14 ± 0.05 (0)	0.33 ± 0.09 (0)	= 0.01	< 0.01

Intestinal metaplasia; IM, Values are shown as mean ± SE (median).

Separating each histological score by *PSCA* genotypes, the intestinal metaplasia score among those with TT genotype was significantly greater than that of C allele carriers in both the gastric corpus and antrum.