Original Article

Novel association between *Helicobacter pylori* infection and gastrointestinal stromal tumors (GIST) in a multi-ethnic population

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Background: The incidence of gastrointestinal stromal tumors (GIST) is increasing though its epidemiology remains poorly understood. The epidemiological factors involved in GIST were examined in the multi-ethnic population of Hawaii, which has the highest incidence of GIST of all Surveillance, Epidemiology and End Results (SEER) sites in the USA, in order to gain insight into the potential risk factors for GIST.

Methods: Archival tumor tissue from 71 morphologically and immunohistochemically confirmed GIST cases and 65 gastritis-only controls diagnosed between 1998–2017 were evaluated for five *Helicobacter pylori* (*H. pylori*) genes (*HP1177*, *16S rRNA*, *iceA*, *ureB*, *vacA*) by polymerase chain reaction (PCR) and gastritis controls.

Results: Across the ethnically diverse Hawaii population, GIST were significantly more common in Asians compared with whites. The gastric predominance of GIST and higher prevalence of *H. pylori* in Asians than whites in Hawaii led us to examine this infection as a potential causative factor of GIST. Forty-nine (69.0%) GIST cases were gastric in origin. Of 71 GIST cases, 48 (67.6%) were positive for at least one *H. pylori* gene, compared with only 13 (20.0%) of the controls {unadjusted odds ratio (OR): 8.3 [95% confidence interval (CI): 3.8–18.3]; P<0.0001}; 23 (32.4%) of GIST cases were positive for at least two different *H. pylori* genes, compared with only 6 (9.2%) controls [unadjusted OR: 4.7 (95% CI: 1.8–12.5); P=0.002].

Conclusions: *H. pylori* infection is strongly associated with GIST and may play an important role in its tumorigenesis.

Keywords: Gastrointestinal stromal tumors (GIST); *Helicobacter pylori* (*H. pylori*); gastric cancer

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Introduction

Gastrointestinal stromal tumors (GIST) are relatively rare tumors affecting approximately 5,000 newly diagnosed patients annually in the USA, but are the most common mesenchymal tumors of the gastrointestinal tract. GIST were called leiomyomas and leiomyosarcomas in the past, but recent studies have identified interstitial cells of Cajal as the cell of origin of GIST, distinguishing GIST from other mesenchymal GI tumors (1-3). c-KIT (CD117) is expressed in 80–100% and CD34 in 50–80% of GIST, respectively (1,4), and their expression is used to help diagnose GIST. DOG-1 and PKC_{θ} are commonly expressed by GIST as well and are particularly useful in the identification of c-KIT-negative stromal tumors (5-9). Previous reports have identified c-*KIT* and *PDGFRA* mutations in the vast majority of GIST (1,2).

Although there are multiple studies that examine the histology, pathogenesis, and treatment of GIST, existing data regarding the epidemiology of GIST are limited. Few publications to date have addressed potential ethnic differences in the incidence or prevalence of GIST. A population-based study of 292 GIST patients and 292 age- and sex-matched controls from the U.S. National Cancer Institute's Surveillance, Epidemiology and End Results (SEER) registry database, found no statistically significant differences in geographic region, location of residence, or median income, but found significantly more white controls than cases compared with blacks and other non-whites (10). Another retrospective SEER study examined 1,458 malignant GIST cases from 1992 to 2000 in 12 SEER registry regions and found the highest incidence rate in Hawaii (1.06/100,000 person-years compared to the national age-adjusted incidence rate of 0.68/100,000 person-years) (11). This led us to examine the factors associated with Hawaii having the highest incidence of GIST among SEER regions in the USA.

We present the following article in accordance with the MDAR reporting checklist (available at http://dx.doi.org/10.21037/gist-20-2).

Methods

Epidemiology of GIST

The Hawaii SEER registry data were queried and all diagnoses of malignant gastrointestinal stromal sarcoma (ICD-O-3 code 8936) from 1998 to 2009 were identified. The Hawaii Tumor Registry (HTR) has been part of the U.S. SEER program since 1973.

Diagnosis of GIST was based on morphology (spindle, epithelioid, mixed) and immunohistochemical tumor markers (c-KIT, DOG1 or PDGFRA). Prior to 1998, the terms leiomyoma, leiomyosarcoma, neurofibromas, schwannomas were more commonly used to refer to GIST, however because of their location and lack of immunohistochemical staining, were largely indistinguishable from GIST. To avoid historical coding "bias", sampling bias, or classification errors, tumors with these diagnoses (leiomyoma, leiomyosarcoma, neurofibroma, schwannoma) prior to 1998 were excluded from our data set. The HTR database includes patient demographics, tumor histology, primary site topography, tumor behavior, grade, and stage of disease at diagnosis, and immunohistochemistry in more recent cases.

The U.S. Census data (year 2000) was used to determine the ethnic composition of Hawaii's population as a reference for the ethnicity of GIST cases (12). Pacific Islander is a term that includes Chamorro, Micronesian, Marshallese, Samoan, and Tongan ethnic groups. Ethnicity was self-reported in both the SEER and U.S. Census. The incidence rates for GIST were compared amongst ethnic groups reported to the HTR from 1998–2009. The incidence rates in non-white ethnic groups were compared to the incidence rate in whites.

Histological specimens

The Residual Tissue Repositories of two SEER registries in Hawaii and Iowa were queried for de-identified malignant GIST tissue cases (ICD-O-3 code 8936) cases from 1998–2017. Formalin-fixed and paraffin-embedded (FFPE) GIST tissue sections (cases; n=63) were collected from the HTR and Queen's Medical Center. An additional eight malignant GIST cases from 2000–2015 were provided by the State Health Registry of Iowa due to the smaller number of whites with GIST in Hawaii. All GIST surgical resection specimens contained tumor bordered by non-malignant gastrointestinal tissue.

The controls were chosen from diagnostic esophagogastroduodenoscopy cases performed at the Queen's Medical Center from 2015–2017 found to have gastritis without evidence of tumors. Multiple (4-8) pinch biopsy specimens from the gastric corpus and antrum were obtained from 65 control patients who were referred for evaluation of upper gastrointestinal symptoms or anemia, and were found to have endoscopic and histopathologic

evidence of gastritis without evidence of gastric malignancies or peptic ulcer disease.

DNA extraction and purification

For cases and controls, sequential sections (5 µm thick) were cut from the tissue samples and mounted on glass microscope slides. For cases, one of the slices was stained with hematoxylin and eosin to verify the presence of tumor surrounded by adjacent normal gastrointestinal tissue. For controls, one of the slices was stained with hematoxylin and eosin to verify the presence of gastric tissue. The tissue specimens were scraped from 2–3 sequential unstained slides for each case and control. Both tumor and normal surrounding gastrointestinal tissue was collected for the GIST cases. The DNA was extracted from the paraffin-embedded tissue sections as previously described (13). Briefly, paraffin-embedded sections were placed in 180 µL ATL lysis buffer from the DNAeasy kit (Qiagen, Germany), followed by incubation at 100 ℃ for 30 minutes. The tubes were briefly centrifuged to remove condensate. Twenty µL of proteinase K was added to the content of the microfuge tubes, and the tubes were incubated at 65 ℃ for 16 hours. The lysed emulsion was further purified with the DNAeasy spin-column (Qiagen). DNA was finally recovered in a single elution step with 100 µL AE solution from the DNAeasy kit (Qiagen).

Detection of Helicobacter pylori (H. pylori)

The following polymerase chain reaction (PCR) primer pairs were used to perform PCR amplification of *H. pylori*-specific genes from tumor DNA:

16S rRNA: 109 bp (14)
F: 5'-CTGGAGAGACTAAGCCCTCC-3'
R: 5'-ATTACTGACGCTGATTGTGC-3'
HP1177: 187 bp (14)
F: 5'-ACGAACGCGCAAAAACTTTA-3'
R: 5'-TTGCCATTCTCATCGGTGTA-3'
vacA: 136/163 bp (14)
F: 5'-ATGGAAATACAACAAACACAC-3'
R: 5'-CAACAATGGCTGGAATGAT-3'
ureB: 132 bp (15)
F: 5'-CCCATTTGACTCAATGCGATG-3'
R: 5'-TGGGATTAGCGAGTATGTCGG-3'
iceA1: 218 bp (16)
F: 5'-ATCATAAAGACGGCCGCAAAGAT-3'
R: 5'-AT(A/G)GGGTCATATTGATAACA(A/G)CC-3'
iceA2: 247 bp (16)
F: 5'-CGCTGTTTTTCTAGCGGTGTTTTA-3'
R: 5'-CATTGATCT(A/G)TGTTTGTATGCTTC-3'

(*iceA1* and *iceA2* are allelic variants of *iceA*. Detection of either allele was considered positive for *iceA*).

Human beta-globulin was used as a PCR control for the extraction of human DNA from the tissues:

bgl [beta-globulin (control)]: 110 bp (17)
F: 5'-ACACAACTGTGTTCACTAGC-3'
R: 5'-CAACTTCATCCACGTTCACC-3'

PCR was performed on purified tumor DNA in 20 µL goTaq green PCR buffer (PROMEGA, USA) containing forward and reverse primers. PCR was performed using a GeneAmp PCR System 9700 (Applied Biosystems, USA) with the following conditions: 6 minutes of preincubation at 94 ℃ followed by 40 cycles of 30 seconds each at 94 ℃, 30 seconds at 55 ℃ and 30 seconds at 72 ℃. Final extension was performed for 7 minutes at 72 ℃. The PCR products were resolved on 2% agarose gels, and, the respective bands were cut out of the agarose gels followed by DNA isolation and purification. Sequencing of the PCR products extracted from the gels was performed to verify the PCR products. The purified PCR products were sequenced using the same primers above and the resulting sequences matched using Nucleotide BLAST with matches yielding significance at $P<0.05$. Both negative and positive (patients with positive *H. pylori* infection by Giemsa stain of gastric biopsies) controls were utilized for all PCR assays. The uniformity of DNA quality was determined by detection of the housekeeping gene *bgl* by PCR. The PCR detection of the *H. pylori*-specific genes was performed in duplicate for each sample.

Approval for this research was granted by the Western Institutional Review Board (protocol no. 1168906) and University of Hawaii Human Subjects Committee (2014-302). This study conformed to the provisions of the Declaration of Helsinki (as revised in 2013). Due to the retrospective nature of the data and biospecimen collection, and de-identification of all personal health information, individual consent for this retrospective analysis was waived.

Statistical analysis

In addition to descriptive statistical analyses, bivariate analyses were performed to compare data; chi-square tests were used to determine the statistical significance between the frequency of GIST in various minority ethnic groups. Crude and unconditional logistic regression analyses were performed using SPSS v22 (IBM Corp., Armonk, NY,

Table 1 Characteristics of GIST in the Hawaii SEER database from 1998–2009

Characteristics	Total	Percentage
Age at diagnosis, yr		
<50	26	17.6
50–59	36	24.5
60–69	32	29.2
70–79	33	29.9
>80	20	13.6
Gender		
Male	73	49.7
Female	74	50.3
Location		
Stomach	82	56.0
Small intestine	48	34.0
Colon	5	3.5
Other digestive organ	11	7.5
Stage		
Localized	80	54.4
Regional	26	17.7
Distant	33	22.4
Unstaged	8	5.4

GIST, gastrointestinal stromal tumors; SEER, Surveillance, Epidemiology and End Results.

Table 2 Analysis of GIST cases by ethnicity compared to whites in Hawaii SEER Database from 1998–2009

Ethnicity	P value
Chinese	4.7×10^{-5}
Filipino	0.03
Hawaiian/part-Hawaiian	0.2
Japanese	4.3×10^{-4}
Korean	1.5×10^{-7}
Vietnamese	2.6×10^{-9}
White	1
Pacific Islander	3.3×10^{-7}

GIST, gastrointestinal stromal tumors; SEER, Surveillance, Epidemiology and End Results.

USA) to calculate risk [odds ratio (OR); 95% confidence interval (CI)] of detection of *H. pylori* genes in all 71 GIST cases (from Hawaii and Iowa) versus the 66 controls (from Hawaii). All 95% CI were two-sided. P<0.05 was considered statistically significant.

Results

One hundred and forty-seven patients with a confirmed histologic diagnosis of malignant GIST were reported in the Hawaii SEER database from 1998–2009 (*Table 1*). Fifty-six percent of GIST were in the stomach, 34.0% in the small intestine, and 3.5% in the colon (*Table 1*). There was no significant difference in the prevalence of GIST between men or women of any ethnic group (*Table 1*). Fifty-four point four percent of GIST were staged as localized, 17.7% regional, 22.4% with distant metastases, and 5.4% unstaged at the time of diagnosis (*Table 1*). Ethnicity was reported for all individuals except for 0.7% (no ethnicity reported) of GIST subjects. Compared to whites, malignant GIST incident rates were significantly greater in Japanese ($P=4.3\times10^{-4}$), Chinese ($P=4.7\times10^{-5}$), Korean ($P=1.5\times10^{-7}$), Vietnamese ($P=2.6\times10^{-9}$), Pacific Islander ($P=3.3\times10^{-7}$), and Filipino (P=0.03) ethnic groups in Hawaii (*Table 2*). The gastric and Asian predominance of GIST in the multi-ethnic Hawaii population was reminiscent of a previous study that strongly linked *H. pylori* infection to gastric adenocarcinoma in Hawaii Asians (18). Multiple studies have found a high coincidence of GIST and non-GIST cancers (mostly adenocarcinomas) in the stomachs and gastroesophageal junctions of the same patients, suggesting common risk factors for the two types of tumors (19-23). These data led us to look for an association between *H. pylori* infection and GIST.

Paraffin-embedded GIST tumor specimens were available for 71 GIST and 65 gastritis controls. Of the GIST cases, 11 (15.5%) ranged in age 21 to 49 years, 13 (18.3%) were 50 to 59 years, 14 (19.7%) were 60 to 69 years, 21 (29.6%) were 70 to 79 years, and 12 (16.9%) were >80 years (*Table 3*). Of the controls, 16 (24.6%) ranged in age 21 to 49 years, 11 (16.9%) were 50 to 59 years, 19 (29.2%) were 60 to 69 years, 12 (18.5%) were 70 to 79 years, and 7 (10.8%) were >80 years (*Table 3*). Females comprised 49.3% and 60% of GIST patients and controls, respectively (*Table 3*). Asians made up the majority of both GIST and controls at 42 (59.2%) and 43 (66.2%) (P=0.40),

Table 3 Demographic characteristics of GIST cases and controls for which tissue samples were collected

Characteristics	GIST			Controls (n=65)
	Hawaii only (n=63)	Iowa only (n=8)	Total (n=71)	
Age at diagnosis, yr, n (%)				
<50	10 (15.9)	1 (12.5)	11 (15.5)	16 (24.6)
50–59	13 (20.6)	0 (0.0)	13 (18.3)	11 (16.9)
60–69	14 (22.2)	0 (0.0)	14 (19.7)	19 (29.2)
70–79	17 (27.0)	4 (50.0)	21 (29.6)	12 (18.5)
>80	9 (14.3)	3 (37.5)	12 (16.9)	7 (10.8)
Gender, n (%)				
Male	32 (50.8)	4 (50.0)	36 (50.7)	26 (40.0)
Female	31 (49.2)	4 (50.0)	35 (49.3)	39 (60.0)
Location, n (%)				
Stomach	41 (65.1)	8 (100.0)	49 (69.01)	65 (100.0)
Small intestine	16 (25.4)	0 (0.0)	16 (22.54)	–
Colon	2 (3.2)	0 (0.0)	2 (2.82)	–
Other digestive organ	4 (6.3)	0 (0.0)	4 (5.63)	–

GIST, gastrointestinal stromal tumors.

respectively (*Table S1*). The remainder of GIST and controls were comprised of Hawaiians 9 (12.7%) and 5 (7.7%) (P=0.34), whites 14 (19.7%) and 13 (20.0%) (P=0.97), Pacific Islanders 5 (7.0%) and 4 (6.2%) (P=0.85), and "other" (ethnicity not reported) 1 (1.4%) and 0 (0.0%) (P=0.34), respectively (*Table S1*). Of the primary GIST, 49 (69.0%) were gastric in origin, 16 (22.5%) occurred in the small intestines, 2 (2.8%) in the colon, 2 (2.8%) in the rectum and 2 (2.8%) were detected outside the stomach and intestines (liver, mesentery) (*Table 3*). Among small intestine GIST (n=16), 5 were duodenal (31.3%), 3 ileal (18.8%), 1 overlapped different segments of small intestine (6.3%), and 7 were not otherwise specified (43.8%).

H. pylori infection was detected by positive PCR amplification of five *H. pylori* genes (*HP1177*, *16S rRNA*, *iceA*, *ureB*, *vacA1*). For individual *H. pylori* genes, *HP1177* was detected in 21 GIST and 1 control (P<0.0001), *16S rRNA* in 18 GIST and 2 controls (P=0.0003), *iceA* in 25 GIST and 9 controls (P=0.0041), *ureB* in 15 GIST and 5 controls (P=0.028), and *vacA* in 14 GIST and 4 controls (P=0.021) (*Table S2*). Of the total 71 patients with GIST, 48 (67.6%) were positive for at least one *H. pylori* gene, compared with only 13 (20.0%) of 65 controls [unadjusted OR: 8.3 (95% CI: 3.8–18.3); P<0.0001] (*Table 4*). Furthermore, 23 (32.4%) cases were positive for at least two *H. pylori* genes, compared with only 6 (9.2%) controls [unadjusted OR: 4.7 (95% CI: 1.8–12.5); P=0.002] (*Table 4*). For the eight GIST cases from Iowa, 5 (62.5%) were positive for at least one *H. pylori* gene (*Table 3*). Excluding the eight patients from Iowa did not alter the significance between GIST and *H. pylori* genes compared with controls.

Of the GIST cases that were positive for one or more *H. pylori* genes (n=48), 32 (66.7%) were gastric, 11 (22.9%) were found in the small intestines, 1 (2.1%) in the colon, 2 (4.2%) occurred in the rectum, and 2 (4.2%) were extra-intestinal (liver, mesentery) (*Table S1*). On further analysis of the 11 small intestine GIST cases positive for one or more *H. pylori* genes, 4 (36.4%) were duodenal, 3 ileal (27.3%), 3 (27.3%) overlapping small intestinal segments and 1 (9.1%) not otherwise specified.

Discussion

In our study, we found GIST cases in Hawaii were overrepresented in Asians and Pacific Islanders compared

Table 4 Summary of *H. pylori* gene detection in gastrointestinal tissues of GIST cases and controls

No. of positive *H. pylori* genes	GIST, n (%)			Controls (n=65), n (%)	OR (95% CI)	P value
	Hawaii only (n=63)	Iowa only (n=8)	Total (n=71)			
1	43 (68.3)	5 (6.3)	48 (67.6)*	13 (20.0)*	8.3 (3.8–18.3)*	110^{-4}*
≥2	18 (29.6)	5 (6.3)	23 (32.4)*	6 (9.2)*	4.7 (1.8–12.5)*	210^{-3}*
0	20 (31.7)	3 (37.5)	23 (32.4)	52 (80.0)		
1	25 (39.7)	0 (0.0)	25 (35.2)	7 (10.77)		
2	11 (17.4)	0 (0.0)	11 (15.5)	4 (6.15)		
3	3 (4.8)	4 (50.0)	7 (9.9)	2 (3.08)		
4	3 (4.8)	1 (12.5)	4 (5.6)	0 (0.0)		
5	1 (1.6)	0 (0.0)	1 (1.4)	0 (0.0)		

*, P<0.05 for GIST *vs.* controls. *H. pylori*, *Helicobacter pylori*; GIST, gastrointestinal stromal tumors; OR, odds ratio; CI, confidence interval.

with whites. Recent studies found a higher prevalence of *H. pylori* infection in non-whites than whites in Hawaii (24), which may help explain the higher incidence rates for GIST in Asians and Pacific Islanders than whites in our study.

Previous studies demonstrated strong associations between *H. pylori* infection and two other types of gastric cancers: adenocarcinoma (18) and MALT lymphoma (25). The association between *H. pylori* infection and gastric adenocarcinoma led the International Agency for Research on Cancers to declare *H. pylori* a carcinogen (26). One study, published only in abstract form, did report an association between GIST and *H. pylori* infection (27). In this study, of 71 malignant GIST cases and 65 non-GIST controls, gastrointestinal specimens were positive for *H. pylori* infection by Giemsa stain in 62.9% and 30%, respectively (P=0.013). Our study utilized PCR detection of *H. pylori* infection in gastrointestinal tissue specimens since this has been shown to be more sensitive than histochemical methods for *H. pylori* infection (28,29) and allows unequivocal distinction between various *Helicobacter* species, such as *H. helmanii*, that can also reside in the stomach (30). In the regression analyses performed on our subjects, GIST cases showed large ORs for *H. pylori* infection detected by PCR compared with controls. Due to the much lower number of GIST cases in whites than Asians in Hawaii, we obtained 8 GIST specimens from eight white patients from the University of Iowa to determine if this could be due to sampling error. The majority of the GIST cases from Iowa were positive for *H. pylori* infection as well. Even when the subset of 63 GIST cases and 66 matched (age, gender, ethnicity) controls from Hawaii were analyzed separately, the ORs for the association between *H. pylori* and GIST remained large and significant. Thus, our results strongly suggest an association between gastrointestinal *H. pylori* infection and GIST.

With regards to the controls, we selected patients referred for evaluation of upper gastrointestinal symptoms or anemia, who were found to have endoscopic and histopathologic evidence of gastritis without evidence of gastric malignancies or peptic ulcer disease after careful consideration. We did consider patients with known peptic ulcer disease as controls but previous studies have shown an inverse correlation between duodenal ulcers and gastric cancer, and a direct correlation between gastric ulcers and gastric cancer (31-34). Thus, including duodenal ulcer patients as controls would have potentially skewed the ORs for *H. pylori* infection in favor of GIST cases. Furthermore, using patients with gastric ulcers as controls would possibly have diminished the likelihood ratios for *H. pylori* infection and GIST, but may have included patients at risk for both gastric ulcers and GIST.

In our study, *H. pylori* genes were detected not just in gastric GIST but in small intestinal, colonic, and extra-intestinal GIST as well. This is not unexpected since *H. pylori* has been detected in the small and large intestines of patients with gastrointestinal diseases and even in the liver of patients with liver cancers (35-37). Nevertheless, the majority of primary GIST tumors in Hawaii occur in the stomach, where *H. pylori* infection is most prevalent. Gastric adenocarcinoma is much more frequent than gastric MALT lymphoma and GIST, likely due to the fact that the latter malignancies originate in the submucosal layers of the

stomach which underlie the epithelium and therefore are more distant from *H. pylori* and carcinogens.

In the USA, there is no recommendation for gastric cancer screening in the general population due to the relatively low overall rate of gastric cancer. However, clinicians should be aware of the increased frequency of GIST, in addition to gastric adenocarcinoma and MALT lymphoma, in patients at increased risk for or with *H. pylori* infection. As GIST arise within the gastrointestinal submucosa, they first appear as raised lesions covered by normal appearing mucosa and frequently require endoscopic ultrasound with fine needle aspiration for further characterization (38). Future studies should determine if eradication of *H. pylori* infection decreases the rate of GIST.

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Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at http://dx. doi. org/10. 21037/gist–20–2

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx. doi. org/10. 21037/gist–20–2). Dr. CFL reports grants from National Cancer Institute, during the conduct of the study. The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Approval for this research was granted by the Western Institutional Review Board (protocol No. 1168906) and University of Hawaii Human Subjects Committee (2014-302). This study conformed to the provisions of the Declaration of Helsinki (as revised in 2013). Due to the retrospective nature of the data and biospecimen collection, and de-identification of all personal health information, individual consent for this retrospective analysis was waived.

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Supplementary

Table S1 GIST cases used for *H. pylori* detection

Sample	Gender	Ethnicity	Age	Place of birth	Tumor site	Tumor class	Tumor grade	Stage
GIST 1	Male	Filipino	60–69	Asia NOS	Gastric antrum	Malignant primary	2	Localized
GIST 2	Female	Japanese	70–79	Hawaii	Lesser curvature of stomach, NOS	Malignant primary	1	Regional, extensive
GIST 3	Male	White	60–69	USA (not Hawaii)	Gastric antrum	Malignant primary	2	Localized
GIST 4	Male	Pac Island excl Hawaii	60–69	Pacific Islands	Fundus of stomach	Malignant primary	1	Localized
GIST 5	Female	Other	21–49	Hawaii	Small intestine, NOS	Malignant primary	1	Localized
GIST 6	Male	Hawaiian	21–49	Hawaii	Overlapping lesion of stomach	Malignant primary	3	Regional, extensive
GIST 7	Male	Japanese	60–69	Hawaii	Stomach, NOS	Malignant primary	Unknown, not stated, or N/A	Localized
GIST 8	Female	Filipino	60–69	Asia NOS	Duodenum	Malignant primary	1	Localized
GIST 9	Female	Japanese	70–79	Hawaii	Colon, NOS	Malignant primary	Unknown, not stated, or N/A	Regional, extensive
GIST 10	Male	Japanese	21–49	USA (not Hawaii)	Duodenum	Malignant primary	1	Regional, extensive
GIST 11	Female	Filipino	50–59	Asia NOS	Gastric antrum	Malignant primary	Unknown, not stated, or N/A	Localized
GIST 12	Female	Chinese	21–49	Unknown, not stated, or N/A	Ileum	Malignant primary	1	Distant
GIST 13	Female	Chinese	>80	Hawaii	Greater curvature of stomach, NOS	Malignant primary	Unknown, not stated, or N/A	Distant
GIST 14	Male	Chinese	70–79	Hawaii	Duodenum	Malignant primary	Unknown, not stated, or N/A	Localized
GIST 15	Female	Japanese	60–69	Hawaii	Overlapping lesion of small intestine	Malignant primary	1	Localized
GIST 16	Female	Hawaiian	50–59	Hawaii	Small intestine, NOS	Malignant primary	3	Distant
GIST 17	Male	Hawaiian	21–49	Hawaii	Small intestine, NOS	Malignant primary	Unknown, not stated, or N/A	Distant
GIST 18	Male	Chinese	70–79	Hawaii	Overlapping lesion of stomach	Malignant primary	Unknown, not stated, or N/A	Regional, extensive, lymph nodes
GIST 19	Male	Filipino	50–59	Asia NOS	Greater curvature of stomach, NOS	Malignant primary	Unknown, not stated, or N/A	Localized
GIST 20	Female	Korean	70–79	Asia NOS	Duodenum	Malignant primary	4	Regional, extensive
GIST 21	Female	Japanese	70–79	Hawaii	Overlapping lesion of stomach	Malignant primary	4	Localized
GIST 22	Female	Korean	50–59	Unknown, not stated, or N/A	Overlapping lesion of stomach	Malignant primary	Unknown, not stated, or N/A	Localized
GIST 23	Male	Filipino	>80	Asia NOS	Ileum	Malignant primary	Unknown, not stated, or N/A	Distant
GIST 24	Female	Chinese	70–79	Hawaii	Ileum	Malignant primary	1	Localized
GIST 25	Female	Japanese	>80	Hawaii	Duodenum	Malignant primary	4	Localized
GIST 26	Male	Japanese	70–79	Unknown, not stated, or N/A	Stomach, NOS	Malignant primary	Unknown, not stated, or N/A	Unknown, not stated, or N/A
GIST 27	Male	White	50–59	Unknown, not stated, or N/A	Stomach, NOS	Malignant primary	Unknown, not stated, or N/A	Unknown, not stated, or N/A
GIST 28	Male	Filipino	60–69	Unknown, not stated, or N/A	Rectosigmoid junction	Malignant primary	Unknown, not stated, or N/A	Unknown, not stated, or N/A
GIST 29	Female	Japanese	>80	Unknown, not stated, or N/A	Stomach, NOS	Malignant primary	Unknown, not stated, or N/A	Unknown, not stated, or N/A
GIST 30	Male	Chinese	21–49	Unknown, not stated, or N/A	Stomach, NOS	Malignant primary	Unknown, not stated, or N/A	Unknown, not stated, or N/A
GIST 31	Female	Hawaiian	60–69	Unknown, not stated, or N/A	Stomach, NOS	Malignant primary	Unknown, not stated, or N/A	Unknown, not stated, or N/A
GIST 32	Female	Japanese	70–79	Unknown, not stated, or N/A	Stomach, NOS	Malignant primary	Unknown, not stated, or N/A	Unknown, not stated, or N/A
GIST 33	Female	Japanese	>80	Unknown, not stated, or N/A	Stomach, NOS	Malignant primary	Unknown, not stated, or N/A	Unknown, not stated, or N/A
GIST 34	Male	Japanese	60–69	Unknown, not stated, or N/A	Small intestine, NOS	Malignant primary	1	Unknown, not stated, or N/A
GIST 35	Female	Chinese	60–69	Unknown, not stated, or N/A	Small intestine, NOS	Malignant primary	1	Unknown, not stated, or N/A
GIST 36	Female	Japanese	50–59	Unknown, not stated, or N/A	Small intestine, NOS	Malignant primary	1	Unknown, not stated, or N/A
GIST 37	Female	Japanese	>80	Unknown, not stated, or N/A	Stomach, NOS	Malignant primary	1	Unknown, not stated, or N/A
GIST 38	Female*	Hawaiian	50–59	Unknown, not stated, or N/A	Cardia, esophagogastric junction	Malignant primary	1	Unknown, not stated, or N/A
GIST 39	Female	Japanese	50–59	Unknown, not stated, or N/A	Stomach, NOS	Malignant primary	1	Unknown, not stated, or N/A
GIST 40	Male	Japanese	70–79	Unknown, not stated, or N/A	Stomach, NOS	Malignant primary	1	Unknown, not stated, or N/A
GIST 41	Male	Hawaiian	21–49	Unknown, not stated, or N/A	Stomach, NOS	Malignant primary	1	Unknown, not stated, or N/A
GIST 42	Male	Filipino	>80	Unknown, not stated, or N/A	Stomach, NOS	Malignant primary	1	Unknown, not stated, or N/A
GIST 43	Female*	Hawaiian	70–79	Unknown, not stated, or N/A	Stomach, NOS	Malignant primary	1	Unknown, not stated, or N/A
GIST 44	Male	White	60–69	Unknown, not stated, or N/A	Stomach, NOS	Malignant primary	1	Unknown, not stated, or N/A
GIST 45	Male	Pac Island excl Hawaii	21–49	Unknown, not stated, or N/A	Stomach, NOS	Malignant primary	1	Unknown, not stated, or N/A
GIST 46	Female	Pac Island excl Hawaii	21–49	Unknown, not stated, or N/A	Stomach, NOS	Malignant primary	1	Unknown, not stated, or N/A
GIST 47	Female	White	50–59	Unknown, not stated, or N/A	Stomach, NOS	Malignant primary	1	Unknown, not stated, or N/A
GIST 48	Male	White	70–79	Unknown, not stated, or N/A	Stomach, NOS	Malignant primary	1	Unknown, not stated, or N/A
GIST 49	Male**	Japanese	70–70	Unknown, not stated, or N/A	Stomach, NOS	Malignant primary	1	Unknown, not stated, or N/A
GIST 50	Female	Japanese	>80	Unknown, not stated, or N/A	Stomach, NOS	Malignant primary	2	Unknown, not stated, or N/A
GIST 51	Female	Japanese	>80	Unknown, not stated, or N/A	Stomach, NOS	Malignant primary	2	Unknown, not stated, or N/A
GIST 52	Male	Hawaiian	70–79	Unknown, not stated, or N/A	Stomach, NOS	Malignant primary	3	Unknown, not stated, or N/A
GIST 53	Male	Hawaiian	50–59	Unknown, not stated, or N/A	Small intestine, NOS	Malignant primary	3	Unknown, not stated, or N/A
GIST 54	Male	Pac Island excl Hawaii	60–69	Unknown, not stated, or N/A	Stomach, NOS	Malignant primary	3	Unknown, not stated, or N/A
GIST 55	Male	White	50–59	Unknown, not stated, or N/A	Specified parts of peritoneum (including omentum and mesentery)	Malignant primary	3	Unknown, not stated, or N/A
GIST 56	Male	Japanese	50–59	Unknown, not stated, or N/A	Stomach, NOS	Malignant primary	3	Unknown, not stated, or N/A
GIST 57	Male	Pac Island excl Hawaii	21–49	Unknown, not stated, or N/A	Stomach, NOS	Malignant primary	4	Unknown, not stated, or N/A
GIST 58	Female	Chinese	60–69	Unknown, not stated, or N/A	Stomach, NOS	Malignant primary	4***	Unknown, not stated, or N/A
GIST 59	Female	Korean	70–79	Unknown, not stated, or N/A	Liver	Malignant primary	4***	Unknown, not stated, or N/A
GIST 60	Male	Chinese	50–59	Unknown, not stated, or N/A	Rectum, NOS	Malignant primary	Unknown, not stated, or N/A	Unknown, not stated, or N/A
GIST 61	Male	Japanese	60–69	Unknown, not stated, or N/A	Anus, NOS	Malignant primary	Unknown, not stated, or N/A	Unknown, not stated, or N/A
GIST 62	Male	Filipino	70–79	Unknown, not stated, or N/A	Stomach, NOS	Malignant primary	Unknown, not stated, or N/A	Unknown, not stated, or N/A
GIST 63	Female	Chinese	70–79	Unknown, not stated, or N/A	Stomach, NOS	Malignant primary	1	Unknown, not stated, or N/A
GIST 64	Female	White	82	Iowa	Overlapping lesion of stomach	Malignant primary	2	Localized
GIST 65	Male	White	48	Iowa	Lesser curvature of stomach, NOS	Malignant primary	1	Localized
GIST 66	Female	White	72	Iowa	Overlapping lesion of stomach	Malignant primary	Unknown, not stated, or N/A	Localized
GIST 67	Female	White	81	Iowa	Overlapping lesion of stomach	Malignant primary	Unknown, not stated, or N/A	Regional, extensive
GIST 68	Male	White	70	Iowa	Stomach, NOS	Malignant primary	4	Localized
GIST 69	Female	White	81	Iowa	Cardia, esophagogastric junction	Malignant primary	Unknown, not stated, or N/A	Regional, extensive
GIST 70	Male	White	72	Iowa	Cardia, esophagogastric junction	Malignant primary	Unknown, not stated, or N/A	Localized
GIST 71	Male	White	72	Iowa	Cardia, esophagogastric junction	Malignant primary	Unknown, not stated, or N/A	Localized

*, Specimen with concurrent gastric adenocarcinoma; **, concurrent diagnosis of pancreatic well-differentiated neuroendocrine tumor; ***, liver metastasis. *H. pylori*, *Helicobacter pylori*; GIST, gastrointestinal stromal tumors.

Table S2 Summary of *H. pylori* results for GIST cases and controls

GIST cases							Controls						
H. pylori gene	*bgl control*	*HP1177*	*16S rRNA*	*iceA*	*ureB*	*vacA*	*H. pylori* gene	*bgl control*	*HP1177*	*16S rRNA*	*iceA*	*ureB*	*vacA*
Blank control	–	–	–	–	–	–	Blank control	–	–	–	–	–	–
Negative control	–	–	–	–	–	–	Negative control	–	–	–	–	–	–
Positive control	+	+	+	+	+	+	Positive control	+	+	+	+	+	+
GIST 1	+		+				Control 1	+			+		
GIST 2	+						Control 2	+					
GIST 3	+						Control 3	+					
GIST 4	+			+			Control 4	+					
GIST 5	+				+		Control 5	+			+		
GIST 6	+		+	+	+	+	Control 6	+				+	+
GIST 7	+						Control 7	+					
GIST 8	+						Control 8	+					
GIST 9	+			+			Control 9	+					
GIST 10	+						Control 10	+					
GIST 11	+						Control 11	+					
GIST 12	+		+	+			Control 12	+					
GIST 13	+			+			Control 13	+	+				+
GIST 14	+						Control 14	+					
GIST 15	+		+	+			Control 15	+					
GIST 16	+		+	+			Control 16	+					
GIST 17	+		+				Control 17	+			+		
GIST 18	+		+	+			Control 18	+					
GIST 19	+	+	+	+		+	Control 19	+					
GIST 20	+		+				Control 20	+					
GIST 21	+						Control 21	+					
GIST 22	+						Control 22	+					
GIST 23	+		+	+		+	Control 23	+					
GIST 24	+				+		Control 24	+			+		
GIST 25	+		+	+	+	+	Control 25	+					
GIST 26	+						Control 26	+			+	+	
GIST 27	+			+			Control 27	+					
GIST 28	+						Control 28	+					
GIST 29	+		+		+		Control 29	+					
GIST 30	+		+		+	+	Control 30	+					
GIST 31	+		+			+	Control 31	+			+		
GIST 32	+	+	+		+		Control 32	+					
GIST 33	+				+	+	Control 33	+			+		
GIST 34	+	+					Control 34	+			+		
GIST 35	+						Control 35	+					
GIST 36	+						Control 36	+			+	+	
GIST 37	+			+			Control 37	+					
GIST 38	+						Control 38	+					
GIST 39	+						Control 39	+					
GIST 40	+						Control 40	+					
GIST 41	+					+	Control 41	+		+		+	+
GIST 42	+				+		Control 42	+					
GIST 43	+						Control 43	+					
GIST 44	+	+			+		Control 44	+					
GIST 45	+	+					Control 45	+					
GIST 46	+	+	+	+	+	+	Control 46	+		+		+	+
GIST 47	+	+	+				Control 47	+					
GIST 48	+	+					Control 48	+					
GIST 49	+	+					Control 49	+					
GIST 50	+						Control 50	+					
GIST 51	+						Control 51	+					
GIST 52	+	+		+			Control 52	+					
GIST 53	+						Control 53	+					
GIST 54	+						Control 54	+					
GIST 55	+	+					Control 55	+					
GIST 56	+	+					Control 56	+					
GIST 57	+	+					Control 57	+					
GIST 58	+	+					Control 58	+					
GIST 59	+		+			+	Control 59	+					
GIST 60	+	+					Control 60	+					
GIST 61	+					+	Control 61	+					
GIST 62	+	+					Control 62	+					
GIST 63	+	+					Control 63	+					
GIST 64	+	+		+	+	+	Control 64	+					
GIST 65	+	+		+		+	Control 65	+					
GIST 66	+		+	+	+		Total	65	1	2	5	9	4
GIST 67	+	+		+	+								
GIST 68	+												
GIST 69	+												
GIST 70	+												
GIST 71	+	+		+		+							
Total	71	21	18	25	15	14							

H. pylori, *Helicobacter pylori*; GIST, gastrointestinal stromal tumors.