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# Impact of the Japanese Gastric Cancer Screening System on Treatment Outcomes in Gastric Gastrointestinal Stromal Tumor (GIST): An Analysis Based on the GIST Registry

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## ABSTRACT

**Background.** Gastrointestinal stromal tumors (GISTs) of the stomach are found incidentally during gastric cancer screening in Japan. This study investigated whether the Japanese gastric cancer screening system helps to improve treatment outcomes in gastric GIST based on an analysis of the GIST registry conducted by the Kinki GIST Study Group.

**Methods.** The registry was designed to collect data on background characteristics, treatment methods, pathologic characteristics, and prognosis of GIST from January 2003 through December 2007 at 40 participating institutions.

**Results.** The study enrolled 672 GIST patients, 482 of whom had gastric GIST. According to the modified National Institutes of Health consensus criteria, 22.6 % of the patients were classified as high risk for recurrence, 18.5 % as intermediate risk, 35.9 % as low risk, and 13.9 % as very low risk. After exclusion of the patients inevaluable for treatment outcome, the study included 137 symptomatic patients (symptomatic group) and 147 asymptomatic patients (asymptomatic group). The diagnosis of the asymptomatic patients was determined through gastric cancer screening. The median tumor size in the asymptomatic group was significantly smaller than in the symptomatic group (3.5 vs. 5.3 cm; $P < 0.0001$). The 5-year recurrence-free survival rate in the asymptomatic high-risk patients (72.4 %) was lower than in their symptomatic counterparts (46.3 %) ($P = 0.017$). More patients in the asymptomatic group underwent laparoscopic surgery (42.2 vs. 27.2 %; $P = 0.0081$).

**Conclusions.** By identifying asymptomatic patients, the Japanese gastric cancer screening system contributes to early detection of gastric GIST and favorable treatment outcomes.

Gastrointestinal stromal tumors (GISTs) are distinctive KIT (CD117)-expressing mesenchymal neoplasms of the gastrointestinal (GI) tract[1] that usually have gain-of-function mutations in either KIT or platelet-derived growth factor receptor alpha.[2–6] They most commonly occur in the stomach.[7]

The National Comprehensive Cancer Network[8] has developed consensus recommendations for the treatment of GIST. In Japan, Clinical Practice Guidelines for GIST have been proposed[9] based on evidence regarding the diagnosis and treatment of GIST established in Western countries. Patterns of care and clinical outcomes of GIST in the Japanese community have not been well documented due to the lack of a nationwide registry.

Therefore, we retrospectively gathered data on prevalence, patient characteristics, methods of diagnosis, disease



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staging, treatment methods, and clinical outcomes of patients with GIST based on the registry organized by the Kinki GIST Study Group.

Because Japan has very high rates of morbidity and mortality associated with gastric cancer, a nationwide gastric cancer screening system using barium upper GI studies or esophagogastroduodenoscopy has been established[10–12] to detect gastric cancer in its early stages and to decrease mortality associated with gastric cancer.[13] Given this screening system, asymptomatic patients with gastric GISTs can be identified incidentally. Because the recurrence rate and prognosis for GIST depend mainly on tumor size, mitotic index, and tumor location,[7] early detection of asymptomatic gastric GISTs may potentially improve treatment outcomes in Japan.

The current study aimed to investigate the clinical impact of the gastric cancer screening system on gastric GIST treatment outcomes using GIST registry data.

## METHODS

### *Study Design and Participants of the GIST Registry*

The GIST registry protocol was designed by the Kinki GIST Study Group. The institutional review board of each participating hospital approved the protocol for patient enrollment. This retrospective observational study was designed to collect data on immunohistologically proven cases of GIST diagnosed and treated between January 2003 and December 2007 at 40 institutions. Enrollment began in April 2011 and ended in March 2012. The risk of recurrence was classified according to the modified National Institutes of Health (NIH) consensus criteria[14] and the Armed Forces Institute of Pathology (AFIP) criteria.[15]

Using data from the GIST registry database, we analyzed the cohort of gastric GIST patients in terms of patient background, methods of diagnosis, treatment methods used, pathologic characteristics, and prognosis. The potential role of the gastric cancer screening system for clinical outcomes in gastric GIST was assessed.

### *Statistical Analysis*

The continuous variables are expressed as medians and ranges. Fisher's exact test was used to compare binary variables, and the Mann–Whitney $U$ test was used to compare continuous variables. Survival curves were estimated using the Kaplan–Meier method and compared using the log-rank test. All $P$ values lower than 0.05 were considered statistically significant. Statistical analysis was performed using JMP software (SAS Institute, Cary, NC, USA).

## RESULTS

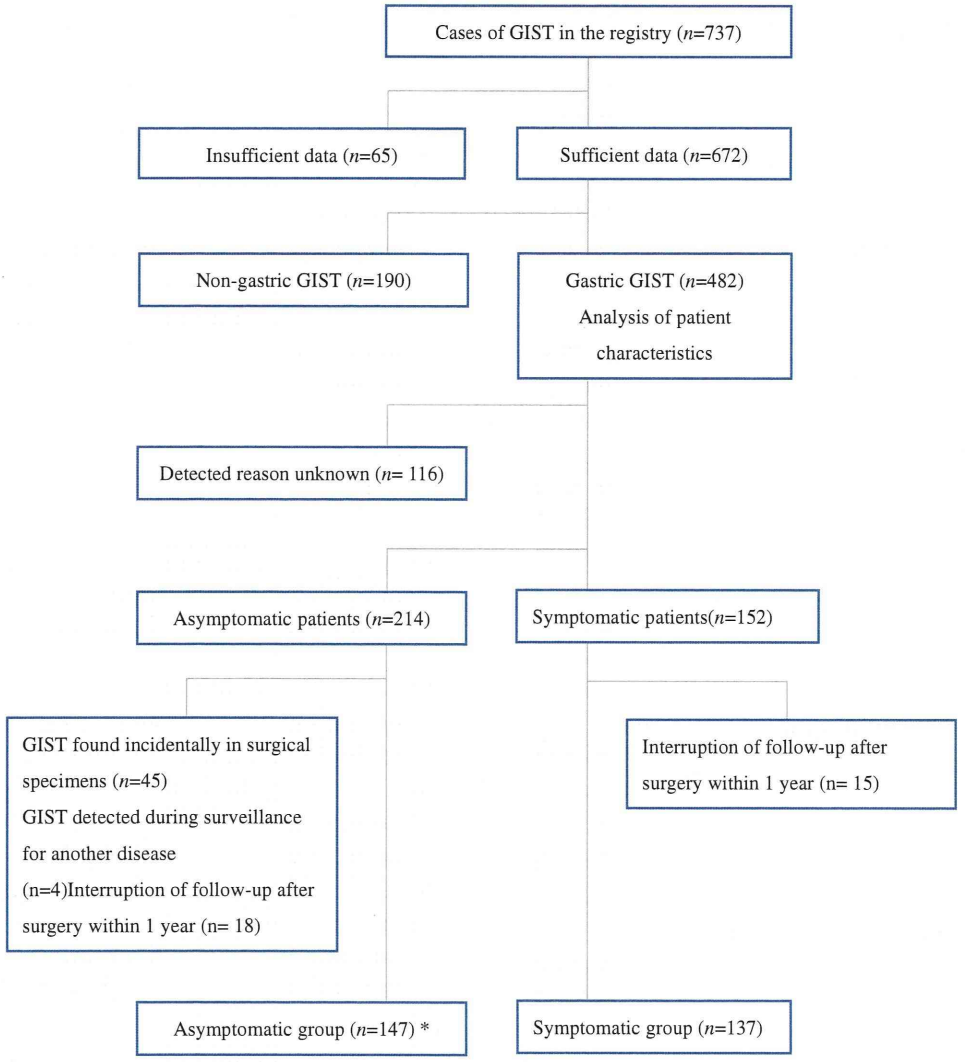
The study enrolled 737 GIST patients from 40 institutions. Figure 1 shows the study profile and how gastric GIST cases were extracted from the registry database. We excluded 65 patients with missing data. Of the 672 GIST patients with sufficient data, 482 (71.7 %) with gastric GIST were included in the analysis of patient characteristics.

Table 1 summarizes the characteristics of the 482 patients (258 males and 224 females) with gastric GIST. They had a median age of 66 years (range 18–92 years) and a median tumor size of 3.6 cm (range 0.1–25.0 cm). The mitotic count was less than 5/50 high-power fields (HPF) in 64.1 % of the patients. According to the modified NIH consensus criteria, 109 patients (22.6 %) were classified as high risk, 89 (18.5 %) as intermediate risk, 173 (35.9 %) as low risk, 67 (13.9 %) as very low risk, and 44 (9.1 %) as unknown risk, whereas according to the AFIP criteria, 59 patients (12.2 %) were classified as high risk, 76 (15.8 %) as moderate risk, 49 (10.2 %) as low risk, 174 (36.1 %) as very low risk, 90 (18.7 %) as no risk, and 34 (7.1 %) as unknown risk. All the patients except for two underwent radical surgery. The percentage of patients who received neoadjuvant or adjuvant imatinib mesylate was respectively 0.8 and 6.6 %.

The reasons for gastric GIST detection are summarized in Table 1. Symptoms such as bleeding, pain, and palpable mass were present in 152 symptomatic patients. The 214 asymptomatic patients in the study consisted of 165 patients in whom gastric GIST was found at the time of gastric cancer screening (screened asymptomatic patients), 45 cases detected via resected specimens, and 4 cases detected during surveillance for another disease. The reason for gastric GIST was unknown for 116 patients.

To evaluate the clinical impact of the Japanese gastric cancer screening system on treatment outcomes for gastric GIST, we excluded 18 asymptomatic and 15 symptomatic patients with a postsurgery follow-up period shorter than 1 year from 165 screened asymptomatic patients and 152 symptomatic patients, respectively. As a result, we compared 147 asymptomatic patients (asymptomatic group) and 137 symptomatic patients (symptomatic group) in terms of baseline characteristics, risk of recurrence based on modified NIH consensus criteria, treatment received, and 5-year recurrence-free survival (RFS) (Fig. 1).

Table 2 shows the characteristics of the patients in the asymptomatic and symptomatic groups. The asymptomatic group had a lower proportion of women than the symptomatic group (44.9 vs. 57.7 %; $P = 0.031$). The median tumor size in the asymptomatic group was significantly smaller than in the symptomatic group (3.5 vs. 5.3 cm; $P < 0.0001$). The asymptomatic group had significantly




GIST, gastrointestinal stromal tumor

*All GISTs were detected during gastric cancer screening in asymptomatic group

**FIG. 1** Study profile

fewer patients with a mitotic count greater than 5/50 HPF (24.5 vs. 36.5 %; $P = 0.014$). Compared with the symptomatic group, the asymptomatic group had a significantly lower proportion of high-risk patients based on the modified NIH consensus criteria (21.1 vs. 40.9 %; $P = 0.0002$) and the AFIP criteria (9.5 vs. 24.1 %; $P = 0.0004$). The numbers of patients who had received adjuvant imatinib therapy were 7 (4.8 %) in the asymptomatic group and 9 (6.6 %) in the symptomatic group. More patients in the asymptomatic group underwent laparoscopic surgery (42.2 vs. 27.2 %; $P = 0.0081$) and partial gastrectomy (85.7 vs. 61.0 %; $P < 0.0001$) than in the symptomatic group.

After a median follow-up period of 58 months, 40 patients (14.1 %) experienced recurrence. The main sites of recurrence were the liver ($n = 24$; 60 %) and the peritoneal surface ($n = 14$; 35 %). The patients in both groups classified as very low to intermediate risk according to the modified NIH consensus criteria had a favorable treatment outcome in terms of the 5-year RFS rate, which was 97.7 % in the asymptomatic group ($n = 107$) and 98.6 % in the symptomatic group ($n = 71$) (Fig. 2a). In contrast, the high-risk patients in the asymptomatic group ($n = 31$) had a significantly better 5-year RFS rate than the high-risk patients in the symptomatic group ($n = 56$) (72.4 vs. 46.3 %, log-rank test; $P = 0.017$) (Fig. 2b). The median tumor size among the high-risk patients was smaller in the asymptomatic group than in the symptomatic group (5.5 vs. 10.5 cm), but this difference was not statistically significant ($P = 0.065$). The asymptomatic group had fewer patients with a mitotic count greater than 10/50 HPF (38.7 vs. 58.9 %; $P = 0.039$). The

**TABLE 1** Characteristics of the study patients ($n = 482$)

| Factor | *n* (%) |
|---|---|
| Age: years (range) | 66 (18–92) |
| Sex | |
| Males | 258 (53.5) |
| Females | 224 (46.5) |
| Median tumor size: cm (range) | 3.6 (0.1–25.0) |
| ≤2.0 | 90 (18.7) |
| 2.1–5.0 | 244 (50.6) |
| 5.1–10.0 | 98 (20.3) |
| >10.0 | 45 (9.3) |
| Unknown | 5 (1.0) |
| Mitotic count (/50 HPF) | |
| ≤5 | 309 (64.1) |
| >5 | 121 (25.1) |
| Unknown | 52 (10.8) |
| Tumor rupture | |
| Yes | 13 (2.7) |
| No | 453 (94.0) |
| Unknown | 16 (3.3) |
| Risk of recurrence based on the modified NIH criteria | |
| High | 109 (22.6) |
| Intermediate | 89 (18.5) |
| Low | 173 (35.9) |
| Very low | 67 (13.9) |
| Unknown | 44 (9.1) |
| Risk of recurrence based on the AFIP criteria | |
| High | 59 (12.2) |
| Moderate | 76 (15.8) |
| Low | 49 (10.2) |
| Very low | 174 (36.1) |
| None | 90 (18.7) |
| Unknown | 34 (7.1) |
| Neoadjuvant imatinib therapy | |
| Yes | 4 (0.8) |
| No | 478 (99.2) |
| Adjuvant imatinib therapy | |
| Yes | 32 (6.6) |
| No | 450 (93.4) |
| Surgery | |
| Yes | 480 (99.6) |
| No | 2 (0.4) |
| Surgical approach | |
| Open | 308 (64.2) |
| Laparoscopic | 172 (35.8) |
| Procedure | |
| Partial gastrectomy | 325 (67.7) |
| Subtotal gastrectomy | 73 (15.2) |
| Total gastrectomy | 27 (5.6) |
| Extended gastrectomy[a] | 22 (4.6) |
| Other | 4 (0.8) |
| Unknown | 29 (6.0) |
| Reason for GIST detection | |
| Asymptomatic cases | 214 (44.4) |
| Detected during gastric cancer screening | 165 |
| Detected in resected specimen for another disease | 45 |
| Detected during surveillance for another disease | 4 |
| Symptomatic cases | 152 (31.5) |
| Bleeding | 65 |
| Pain | 48 |
| Mass | 15 |
| Other | 24 |
| Unknown | 116 (24.1) |

*HPF* high-power fields, *NIH* National Institutes of Health, *AFIP* Armed Forces Institute of Pathology

[a] Gastrectomy with combined resection of other organs

RFS curves of the patients, excluding the patients in both groups who received adjuvant imatinib, stratified by the modified NIH consensus criteria are shown in Fig. 2c, d, and the difference in RFS between the two groups remained unchanged (72.8 and 42.2 %, respectively; $P = 0.0083$). Figure 2e, f show the RFS curves of both groups stratified by the AFIP criteria. The 5-year RFS rates for the patients in the asymptomatic and symptomatic groups were respectively 95.1 and 88.9 % ($P = 0.077$) for the no-risk to moderate-risk patients and 72.2 and 43.9 % ($P = 0.061$) for the high-risk patients.

The overall survival curves for all the patients and the patients without adjuvant imatinib treatment, classified as high risk of recurrence based on the modified NIH consensus criteria, are shown in Fig. 2g, h. The high-risk patients in the asymptomatic group had a significantly better 5-year overall survival rate than the high-risk patients in the symptomatic group among all the patients (100 vs. 80.3 %; $P = 0.040$) and among the patients without adjuvant imatinib (100 vs. 76.8 %; $P = 0.037$).

The results from the multivariate analysis of RFS in the asymptomatic and symptomatic groups are shown in Table 3. The independent risk factors for recurrence were tumor size (hazard ratio [HR], 3.22; 95 % confidence interval [CI], 1.50–7.54; $P = 0.0022$), mitotic count (HR 5.68; 95 % CI 2.65–13.58; $P < 0.0001$), and presence of symptoms (HR 2.67; 95 % CI 1.22–6.49; $P = 0.013$).

## DISCUSSION

In 2008, clinical practice guidelines for GIST[9] were established on the basis of large cohort studies in Japan and

**TABLE 2** Characteristics of the patients in the asymptomatic and symptomatic groups

| | Asymptomatic group ($n$ = 147) $n$ (%) | Symptomatic group ($n$ = 137) $n$ (%) | $P$ value |
|---|---|---|---|
| Age: years (range) | 66 (25–92) | 66 (18–91) | 0.86 |
| Sex | | | |
| Males | 81 (55.1) | 58 (42.3) | 0.031 |
| Females | 66 (44.9) | 79 (57.7) | |
| Median tumor size: cm (range) | 3.5 (1.1–25) | 5.3 (0.5–25) | <0.0001 |
| ≤2.0 | 15 (10.2) | 10 (7.3) | <0.0001 |
| 2.1–5.0 | 103 (70.1) | 54 (39.4) | |
| 5.1–10.0 | 20 (13.6) | 45 (32.9) | |
| >10.0 | 9 (6.1) | 28 (20.4) | |
| Mitotic count (/50 HPF) | | | |
| ≤5 | 101 (68.7) | 73 (53.3) | 0.014 |
| >5 | 36 (24.5) | 50 (36.5) | |
| Unknown | 10 (6.8) | 14 (10.2) | |
| Risk of recurrence based on the modified NIH criteria | | | |
| High | 31 (21.1) | 56 (40.9) | 0.0002 |
| Very low to intermediate | 107 (72.8) | 71 (51.8) | |
| Unknown | 9 (6.1) | 10 (7.3 %) | |
| Risk of recurrence based on the AFIP criteria | | | |
| High | 14 (9.5) | 33 (24.1) | 0.0004 |
| None to moderate | 124 (84.4) | 90 (65.7) | |
| Unknown | 9 (6.1) | 14 (10.2) | |
| Adjuvant imatinib therapy | | | |
| Yes | 7 (4.8) | 9 (6.8) | 0.51 |
| No | 140 (95.2) | 128 (93.4) | |
| Surgical approach | | | |
| Open | 85 (57.8) | 99 (72.8) | 0.0081 |
| Laparoscopic | 62 (42.2) | 37 (27.2) | |
| Procedure | | | |
| Partial gastrectomy | 126 (85.7) | 83 (61.0) | <0.0001 |
| Subtotal gastrectomy | 8 (5.4) | 26 (19.1) | |
| Total gastrectomy | 5 (3.4) | 8 (5.9) | |
| Extended gastrectomy[a] | 1 (0.7) | 8 (5.9) | |
| Other | 0 | 4 (2.9) | |
| Unknown | 7 (4.8) | 7 (5.2) | |

*HPF* high-power fields, *NIH* National Institutes of Health, *AFIP* Armed Forces Institute of Pathology

[a] Gastrectomy with combined resection of other organs

randomized controlled trials in Western countries.[7,16,17] Because the guidelines have changed the treatment strategy used in clinical practice, improvements in treatment outcome are expected. However, Japan has no prospective registry for documentation of the prevalence, treatment strategies used, and treatment outcomes for GIST. Therefore, a GIST registry was planned by the Kinki GIST Study Group to obtain data on GIST from 2003 to 2007 in Japan. During this period, imatinib became available for the treatment of advanced and recurrent GIST but not for neoadjuvant or adjuvant treatment. As a result, the number of patients receiving neoadjuvant (0.8 %) or adjuvant imatinib (6.6 %) was very low. Currently, this study represents the largest registry of GIST patients in Japan.

In Japan, gastric cancer screening has a long history as a part of the health care system,[18] which may influence the prevalence and outcome of gastric GIST. The current Japanese gastric cancer screening system using barium upper GI or esophagogastroduodenoscopy is mainly organized by local governments. Individuals 40 years of age or older can receive annual screening at mass screening centers or outpatient clinics. The cost of screening is 20–40 U.S. dollars per participant, which is covered by the local government or partially subsidized by respective employers.[18] The current study showed that 71.7 % of the cases in this registry were gastric GIST cases. This percentage is higher than the percentages reported from outside Japan (50–60 %).[7,19] Table 1 shows that our series consisted of smaller gastric GIST tumors, fewer patients with a mitotic count greater than 5/50 HPF, and higher proportion of very-low- to low-risk patients according to the modified NIH consensus criteria.[19] Discussion of this difference involved some limitations because almost all the patients in our series underwent surgery as first-line treatment for gastric GIST. Relatively few patients who received non-surgical treatment such as imatinib as first-line management were enrolled in our registry, partly because most investigators in the Kinki GIST study group were surgeons.

Several studies have demonstrated that small asymptomatic GISTs are grossly detectable in autopsy specimens and stomachs resected from patients with gastric cancer[20,21] and incidentally have found that small GISTs are likely to have benign behavior and a good prognosis.[22,23] In the current study, the operative indications for gastric GIST consisted of histologically proven GISTs larger than 5 cm in diameter and those smaller than 5 cm with malignant findings such as ulceration, irregular margins, or larger size.[9] However, in daily practice, patients with small gastric GISTs often undergo surgery at diagnosis.

As shown in Table 2, there were clear differences between the asymptomatic and symptomatic groups. Compared with the symptomatic group, the asymptomatic group had a higher proportion of males, a smaller median tumor size, a lower mitotic count, fewer high-risk cases, and more frequent treatment with laparoscopic surgery and partial gastrectomy. Because the subjects screened for gastric cancer consisted mainly of workers, the male-to-female

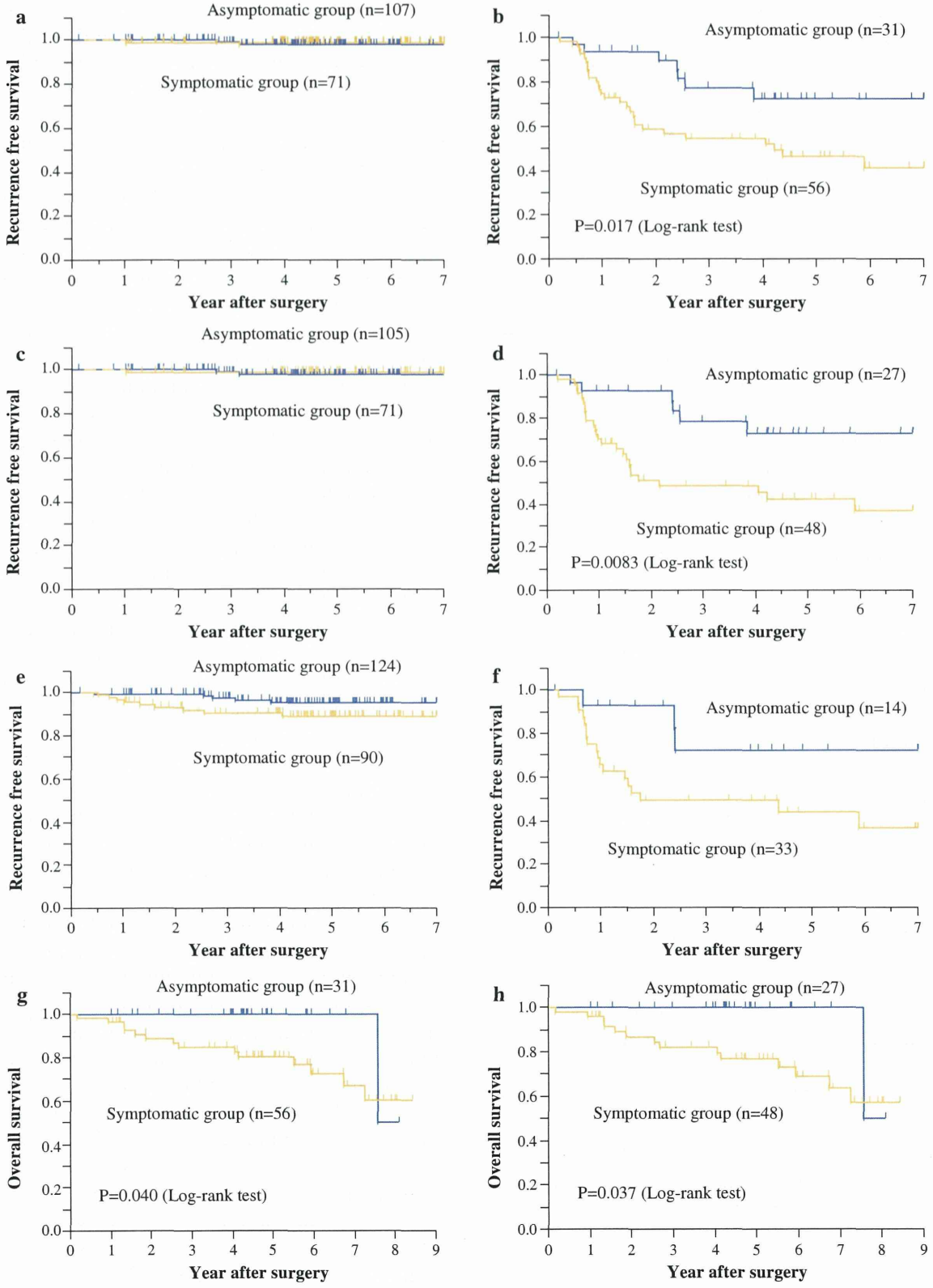



**FIG. 2** Recurrence-free survival curves of patients in asymptomatic (*blue line*) and symptomatic (*yellow line*) group having very low to intermediate risk (**a**) and high risk (**b**) stratified by the modified NIH consensus criteria and excluding patients who received adjuvant imatinib (**c**) and (**d**), having non to moderate risk (**e**) and high risk (**f**) stratified by the AFIP criteria. Overall survival curves of patients in asymptomatic and symptomatic group having high risk stratified by the modified NIH consensus criteria (**g**) and excluding patients who received adjuvant imatinib (**h**)

**TABLE 3** Multivariate analysis of recurrence-free survival in the asymptomatic and symptomatic groups ($n = 284$)

| Variable | | $n$ | Univariate | | | Multivariate | | |
|---|---|---|---|---|---|---|---|---|
| | | | HR | 95 % CI | $P$ value | HR | 95 % CI | $P$ value |
| Age (years) | >70 | 95 | 1.05 | 0.53–2.00 | 0.88 | 1.01 | 0.48–2.01 | 0.98 |
| | ≤70 | 189 | | | | | | |
| Gender | Male | 139 | 1.58 | 0.85–3.04 | 0.15 | 1.58 | 0.81–3.18 | 0.18 |
| | Female | 145 | | | | | | |
| Size (cm) | >5 | 102 | 6.00 | 3.03–12.92 | <0.0001 | 3.22 | 1.50–7.54 | 0.0022 |
| | ≤5 | 182 | | | | | | |
| Mitotic count (HPF) | >5/50 | 90 | 7.97 | 3.79–18.81 | <0.0001 | 5.68 | 2.65–13.58 | <0.0001 |
| | ≤5/50 | 170 | | | | | | |
| Group | Symptomatic | 137 | 3.51 | 1.78–7.57 | 0.0002 | 2.67 | 1.22–6.49 | 0.013 |
| | Asymptomatic | 147 | | | | | | |

*HR* hazard ratio, *CI* confidence interval, *HPF* high-power fields

ratio was inevitably high. The remaining differences were mainly due to the effects from early detection of gastric GIST. Complete (R0) resection without tumor rupture remains the only curative treatment for primary localized gastric GIST.[24,25] The feasibility and effectiveness of less invasive surgery for gastric GIST remain unknown.[26] No prospective randomized controlled trials have directly compared less invasive approaches with conventional therapy. Laparoscopic resection is considered a good surgical procedure for gastric GIST because GIST usually does not invade the adjacent organs, and nodal involvement is rare. Several studies have described the clinical utility, safety, and oncologic equivalence of less invasive surgery for relatively small gastric GISTs relative to conventional open surgery.[27–29] Patients with asymptomatic gastric GIST are more likely to undergo less invasive procedures.

One interesting finding of this study was that the asymptomatic high-risk patients had a significantly better prognosis than the symptomatic high-risk patients in terms of RFS. Two possible explanations can be considered. First, the presence of symptoms is itself a risk factor for recurrence. Second, the asymptomatic group had a smaller median tumor size and a lower mitotic count. The multivariate analysis shown in Table 3 indicated that tumor size, mitotic count, and the presence of symptoms were independent risk factors for recurrence. Therefore, the presence of symptoms may be prognostic. The asymptomatic and symptomatic patients at high risk clearly differed in terms of tumor size and mitotic count according to the modified NIH consensus criteria. We calculated the estimated risk of recurrence for the two groups according to the Joensuu contour maps [9] now available for estimating the risk of GIST recurrence within the first 10 years after surgery. The estimated risk of recurrence within 10 years was 20–40 % for the asymptomatic group compared with 60–80 % for the symptomatic group. The actual 5-year recurrence rates for the asymptomatic and symptomatic high-risk patients in our study were 27.6 and 53.7 %, respectively. The Joensuu contour maps can be a very important tool for predicting survival among high-risk patients.

Because adjuvant treatment with imatinib is the most important determinant of RFS, we compared the RFS and the overall survival of patients without adjuvant treatment between the two groups to exclude the effect from adjuvant treatment. As shown in Fig. 2c, d, and h, the RFS and the overall survival of the high-risk patients in the asymptomatic group still were significantly better. Another question focused on which criteria were better for selecting high-risk patients who may need adjuvant treatment. The RFS of the patients classified by the AFIP criteria is shown in Fig. 2e, f. We observed a certain number of recurrences in the no-risk to moderate-risk patients, and the RFS differed between the asymptomatic and symptomatic groups, although the difference was not significant. Likewise, the RFS in the high-risk patients differed between the groups, but the difference was not significant. The characteristics of the two criteria differed, and it was more convenient to select high-risk gastric GIST patients for recurrence using the modified NIH criteria.[7]

In conclusion, thorough identification of asymptomatic patients and early detection of gastric GIST through gastric cancer screening in Japan increased the opportunity for less invasive surgery and contributed to better treatment outcomes.

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Hospital, Osaka Rosai Hospital, Osaka Medical College Hospital, Matsushita Memorial Hospital, Kyoto City Hospital, Shiga Medical Center for Adults, Hyogo Cancer Center, Hoshigaoka Koseinenkin Hospital, Kinki University Hospital, Kansai Electric Power Hospital, National Hospital Organization Osaka Minami Medical Center, Tokyo Metropolitan Cancer and Infectious diseases Center Komagome Hospital, Osaka General Medical Center, Yodogawa Christian Hospital, Osaka Medical Center for Cancer and Cardiovascular Diseases, Kansai Medical University Takii Hospital, Sumitomo Hospital, Suita Municipal Hospital, The Hospital of Hyogo College of Medicine, Osaka City Sumiyoshi Hospital, Kansai Medical University Hirakata Hospital, Nissei Hospital, Otemae Hospital, Bellland General Hospital, Minoh City Hospital, Osaka Ekisaikai Hospital, Higashiosaka City General Hospital, and Rinku General Medical Center.

**CONFLICT OF INTEREST** There are no conflict of interest.

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# Subgroups of Patients With Very Large Gastrointestinal Stromal Tumors With Distinct Prognoses: A Multicenter Study

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**Background and Objectives:** Any gastrointestinal stromal tumors (GISTs) larger than 10 cm are classified as "high risk" according to the modified National Institutes of Health consensus criteria. We conducted a multicenter study to identify a subgroup with moderate prognosis even within the "high-risk" group.

**Methods:** We retrospectively collected data on 107 patients with tumors ≥10 cm from a multicenter database of GIST patients. Patients with macroscopic residual lesions or tumor rupture were excluded. The relationship between recurrence-free survival (RFS) and clinicopathological factors was analyzed.

**Results:** The median tumor size and mitotic count were 12.5 cm and 8/50 HPF. The RFS rate was 58.5% at 3 years, 52.1% at 5 years. Only mitotic count was an independent prognostic factor of RFS in the multivariate analysis ($P = 0.001$). The hazard ratio for recurrence in the subgroup with mitotic count >5/50 HPF was 2.91 (95% confidence interval, 1.53 to 5.56). The subgroup with mitotic count ≤5/50 HPF showed significantly better RFS than the mitotic count >5/50 HPF subgroup ($P < 0.001$).

**Conclusions:** Mitotic count is closely associated with outcome in patients with large GISTs. This suggests that the subset of large GISTs with low mitotic counts may be considered as "intermediate-risk" lesions.

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KEY WORDS: large GISTs; high risk; mitosis; mitotic count

## INTRODUCTION

Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors in the gastrointestinal tract. GISTs are thought to share a common progenitor cell with the interstitial cells of Cajal, and usually have activating mutations in either c-kit or platelet-derived growth factor receptor alpha (PDGFRA) [1–4]. Tyrosine kinase inhibitors such as imatinib and sunitinib are highly effective against GISTs with c-kit or PDGFRA mutations [5–8]. Since large-scale, randomized controlled trials have recently demonstrated a survival advantage with adjuvant imatinib after GIST resection [9,10], accurate evaluation of the risk of recurrence is needed. Fletcher et al. [11] proposed a simple prognostic classification system using only the size and mitotic count of the resected tumor. Joensuu [12] modified the criteria to include not only size and mitotic count, but also tumor location and the presence of tumor rupture; this modified National Institutes of Health (NIH) consensus criteria is now widely used in clinical practice [13]. With this modified criteria, GISTs with size greater than 10 cm, mitotic count more than 10/50 high power fields (HPF), or rupture are all classified as "high risk." However, the 10-year recurrence-free survival (RFS) of GISTs larger than 10 cm was reported to be over 30%, while that of GISTs with a mitotic count of more than 10/50 HPF was reported as under 20% in a population-based cohort study [14]. Furthermore, almost all GISTs with tumor rupture recur after surgery [14]. Since the "high risk" group may be heterogeneous in terms of prognosis, we evaluated the long-term outcomes of over 100 patients with GISTs that were 10 cm or larger in order to identify a subgroup with moderate prognosis even within the "high-risk" group.



## METHODS

### Patients

From the registries conducted by the Kinki GIST Study Group and the department of Gastroenterological Surgery of Osaka University Hospital, we retrospectively collected data of 2,002 patients with GISTs who underwent resection. We identified 107 eligible GIST patients who underwent resection between October 25, 1986 and May 28, 2010. All tumors were immunohistologically diagnosed as GISTs, and were 10 cm or larger. Histological evaluations were performed by the pathologists at each institution. Patients with macroscopic residual lesions (R2) or tumor rupture were excluded from this study. None of patients had peritoneal dissemination or distant metastasis at the time of surgery. Tumors with invasion into adjacent organs underwent combined resection. No patients underwent neoadjuvant or adjuvant treatment until recurrence. Data on patients' age at surgery, sex, tumor location, type of surgery, tumor size, mitotic count, and survival outcome were

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collected. Genetic mutations were not analyzed in this study. This study was approved by the Steering Committee of the Kinki GIST Study Group.

### Statistics

The primary endpoint of this study was RFS. RFS was defined as the time from surgery to either the first recurrence or death from any cause. Overall survival (OS) was defined as the time from surgery to death from any cause. Data for patients who had not had an event were censored as of the date of the final observation. Survival curves were estimated using the Kaplan–Meier method and compared using the log-rank test. The impact of clinicopathological factors (age, sex, tumor location, tumor size, and mitotic count) on survival was analyzed with univariate and multivariate analyses using the Cox proportional hazards model. *P* values less than 0.05 were considered statistically significant. All statistical analyses were performed using SPSS Statistics software, version 20 (IBM Corp., Armonk, NY).

## RESULTS

The baseline characteristics of the 107 patients are shown in Table I. The median age was 63 years, and the patients were evenly divided by sex. The most common tumor location was the stomach (68.2%), followed by the small intestine (20.6%). More than half of the patients underwent partial resection of the stomach or small intestine, and a quarter of the patients needed combined resection of other organs. The median tumor size was 12.5 cm. The proportion of patients with mitotic count >5/50 HPF was 55.1%, and the median value of mitotic count was 8/50 HPF.

The median follow-up time in this study was 49 months. The RFS rate of all patients was 58.5% at 3 years, 52.1% at 5 years, and 43.9% at 10 years (Fig. 1A), while the OS rate was 88.1% at 3 years, and 79.4% at 5 years, and 63.5% at 10 years (Fig. 1B). The subgroups of which tumor location was stomach or small intestine did not show the significant difference on RFS ($P = 0.40$) (Fig. 2A) and OS ($P = 0.76$) (Fig. 2B), while the subgroup with mitotic count ≤5/50 HPF had significantly better RFS than the group with mitotic count >5/50 HPF ($P < 0.001$) (Fig. 3A). These two subgroups based on mitotic count also had significantly different OS ($P = 0.020$) (Fig. 3B). In the univariate and multivariate analyses, only mitotic count emerged as a significant prognostic factor of RFS, and the hazard ratios of recurrence in the subgroup with mitotic count >5/50 HPF were 2.91 (95% confidence interval, 1.53 to 5.56; $P = 0.001$) (Table II). Neither tumor location nor tumor size affected survival. If the cutoff of mitosis was set on 10/50 HPF, the hazard ratio of recurrence in the multivariate analysis was 2.41 (95% confidence interval, 1.34 to 4.35; $P = 0.003$).

**TABLE I. Clinicopathological Characteristics**

| | n = 107 |
|---|---|
| Age (years) | |
| Median | 63 |
| Range | 33–91 |
| Sex | |
| Male | 54 (50.5%) |
| Female | 53 (49.5%) |
| Tumor location | |
| Stomach | 73 (68.2%) |
| Small intestine | 22 (20.6%) |
| Duodenum | 6 (5.6%) |
| Large intestine | 3 (2.8%) |
| Omentum | 2 (1.9%) |
| Esophagus | 1 (0.9%) |
| Type of surgery | |
| Partial resection of stomach | 39 (36.4%) |
| Partial resection of small intestine | 23 (21.5%) |
| Distal or proximal gastrectomy | 17 (15.9%) |
| Total gastrectomy | 17 (15.9%) |
| Other procedure | 11 (10.3%) |
| Combined resection of other organs | |
| Yes | 26 (24.3%) |
| No | 81 (75.7%) |
| Tumor size (cm) | |
| Median | 12.5 |
| Range | 10.0–30.0 |
| Mitotic count | |
| ≤5/50 HPF | 48 (44.9%) |
| 6—10/50 HPF | 14 (13.1%) |
| >10/50 HPF | 45 (42.1%) |

HPF, high power fields.

## DISCUSSION

There are several different staging systems to predict the prognosis of GISTs. According to the modified NIH consensus criteria, which is frequently used in clinical practice, any GISTs larger than 10 cm in size is classified into a "high risk" group regardless of the mitotic count or tumor location [12,13]. In other words, the significance of tumor size, mitotic count, and tumor location are given the same weight in terms of the risk of recurrence, but the survival of these three categories are clearly distinct [14]. Our study demonstrated that prognosis of GISTs that are 10 cm or larger without tumor rupture was significantly affected by mitotic count, but not tumor size or tumor location. If the cutoff of mitosis was changed from 5/50 HPF to 10/50 HPF, the result was similar to the original one. The prognosis of patients with very large tumors and low mitotic count was relatively good, even though they are in the "high risk" group in the modified NIH consensus criteria, if there was no tumor rupture at the time of surgery.

Herein, we report the 5-year RFS rate of GIST patients with tumors 10 cm or larger to be 52.1%. Some previous studies have reported the prognosis of very large GISTs based on subgroup analyses. DeMatteo et al. [15] reported that the 5-year RFS rate in 39 patients with tumors 10 cm or larger was approximately 45% in their retrospective study. In another large-scale retrospective study by Joensuu et al. [14], the 5-year RFS rates in 176 patients with tumors between 10.1 and 15.0 cm and 115 patients with tumors >15.0 cm were approximately 50% and 35%, respectively. These studies included also cases involving tumor rupture, whereas our study excluded cases with tumor rupture. Since it is well known that almost all patients with tumor rupture at surgery experience recurrence [14,16,17], we examined the survival impact of only tumor location, tumor size, and mitotic count in our study. Taking into account the differences in the patient between these studies and our study, the survival results of patients with very large GISTs seem to be similar. However, our study is the first to evaluate RFS and OS only in patients with very large GISTs without tumor rupture.

Although tumor location is commonly considered to be a prognostic factor, there was no significant difference of survival between tumor location of stomach and small intestine in this study. Indeed, our results were not different from those in the previous studies. For instance, if we presume a GIST patient with tumor of 12.5 cm and 8 mitosis/50 HPF (these variables are median values in our study), the 5-year RFS is predicted as <10% regardless of tumor location (stomach or small intestine) by Memorial Sloan-Kettering Cancer Center nomogram [18]. Furthermore, Miettinen staging system predicts the recurrence rate of that case as 86% in either location [19]. Thus, it is considered that tumor location did not affect survival for large GIST with high mitotic count.

This study has some limitations. This is a retrospective study and included old cases that were resected in 1980s and 1990s. Although all patients were definitely diagnosed with GISTs by immunohistochemical examination, tyrosine kinase inhibitors such as imatinib or sunitinib were not available after recurrence for most of the older cases. This may

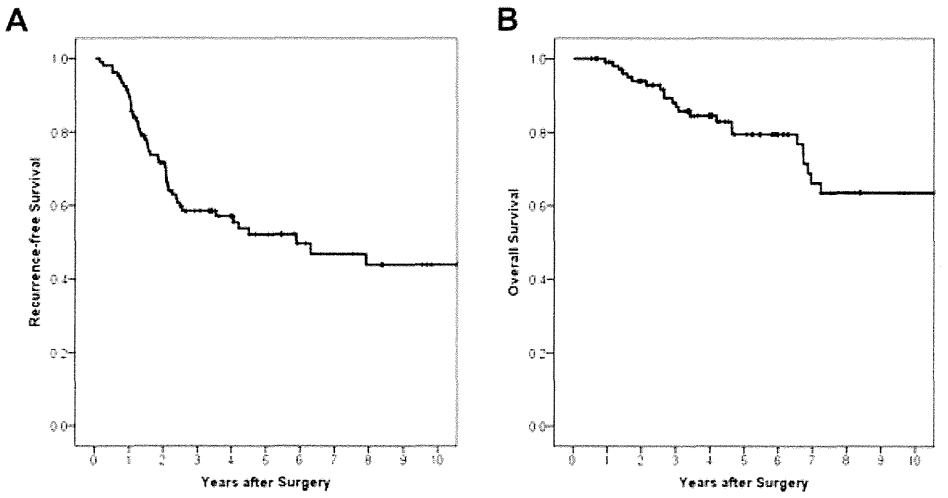


Fig. 1. Recurrence-free survival (**A**) and overall survival (**B**) in all patients with tumors ≥10 cm in size.

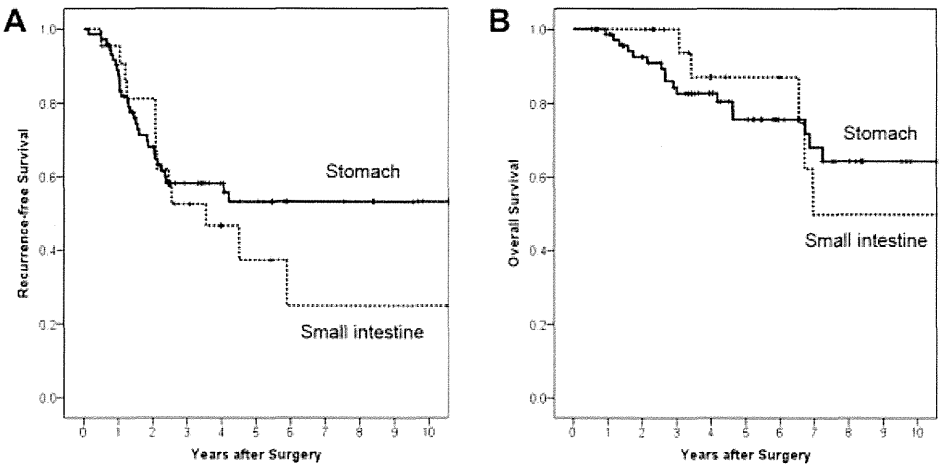


Fig. 2. Recurrence-free survival (**A**) and overall survival (**B**) stratified by location (stomach versus small intestine).

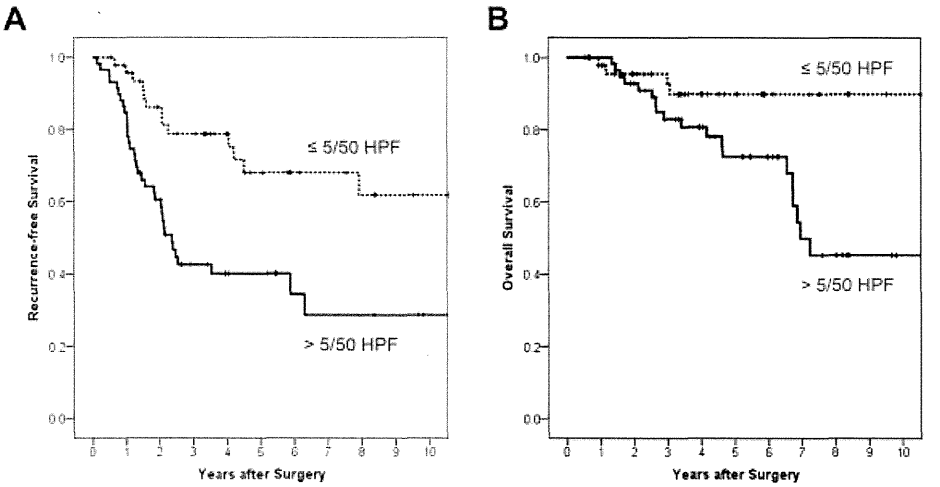


Fig. 3. Recurrence-free survival (**A**) and overall survival (**B**) stratified by mitotic count. HPF, high power fields.

**TABLE II. Association of Clinicopathological Factors with Recurrence-Free Survival**

| | Univariate analysis | | Multivariate analysis | |
|---|---|---|---|---|
| | Hazard ratio (95% CI) | *P* value | Hazard ratio (95% CI) | *P* value |
| Age (≤63 years) | 1.42 (0.80–2.52) | 0.24 | 1.32 (0.73–2.38) | 0.36 |
| Sex (male) | 1.81 (1.01–3.24) | 0.045 | 1.65 (0.89–3.04) | 0.11 |
| Tumor location (not stomach) | 1.21 (0.68–2.14) | 0.53 | 1.35 (0.76–2.41) | 0.31 |
| Tumor size (>12.5 cm) | 1.10 (0.63–1.94) | 0.73 | 1.33 (0.75–2.37) | 0.33 |
| Mitotic count (>5/50 HPF) | 2.99 (1.57–5.68) | 0.001 | 2.91 (1.53–5.56) | 0.001 |

CI, confidence interval; HPF, high power fields.
Age and tumor size were dichotomized by the median value.

have some effect on OS, so we analyzed RFS as the primary endpoint of this study. Another limitation of our study is the lack of available data on genetic mutations, which have been reported to have prognostic importance [20–22]. Further study of the clinical significance of genetic mutations in very large GISTs is needed. An international phase II trial of neoadjuvant imatinib for gastric GISTs 10 cm or larger is currently underway in eastern Asia (UMIN-CTR, UMIN000003114). Since this trial prospectively collects data on genetic mutations, the impact of genetic mutations on the survival of patients with very large GISTs may be clarified in the future.

## CONCLUSION

Although GISTs larger than 10 cm are classified into a "high risk" group in the modified NIH consensus criteria, it may be reasonable to consider tumors with low mitotic count as "intermediate-risk" lesions. In this retrospective series of large GISTs, mitotic count ≤5/50 HPF was associated with improved outcomes as compared to those with mitotic count >5/50 HPF.

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*Original Article*

# Gastrointestinal stromal tumors with exon 8 c-*kit* gene mutation might occur at extragastric sites and have metastasis-prone nature

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**Abstract:** Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors of the human gut. Most sporadic GISTs have somatic gain-of-function mutations of the c-*kit* gene. The mutations are frequently found at exon 11, sometimes at exon 9 and rarely at exon 13 or 17. Recently, exon 8 c-*kit* gene mutations were reported in very minor proportion of sporadic GISTs. We also found 3 GISTs with exon 8 c-*kit* gene mutations in approximately 1,000 sporadic GISTs examined. In the present report, we showed the clinicopathological data of those GISTs. One case had a deletion of codon 419 of aspartate, and 2 cases had a substitution of 3 amino acids of codon 417 to codon 419 to tyrosine. The former was the same mutation recently reported in 2 GIST cases, but the latter has not been reported in any GISTs. All three cases occurred at extragastric sites and two of three showed distant metastasis. Since the remaining case was regarded as high risk for recurrence, imatinib adjuvant treatment has been done without evidence of metastasis. Our results confirmed the idea that exon 8 mutations are minor but actually existing abnormalities in sporadic GISTs, and suggested that such GISTs have a feature of extragastric development and a metastasis-prone nature. Since the exon 8 mutations appeared to be really sensitive to imatinib as shown in the present case study, accurate genotyping including exon 8 of the c-*kit* gene is necessary in GISTs to predict response to imatinib in both the unresectable/metastatic and adjuvant settings.

**Keywords:** c-*kit* gene, exon 8, gain-of-function mutation, GIST, imatinib, sunitinib

## Introduction

Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors of the human gut. Most GISTs express a receptor tyrosine kinase (TK), KIT [1, 2], encoded by proto-oncogene c-*kit* [3-6]. Interstitial cells of Cajal (ICCs), which are present in the gastrointestinal (GI) wall and regulate the GI motility through their spontaneous impulse generation [7], are also positive for KIT. Since ICCs are the only proper cells in GI tract that express KIT, GISTs are now considered to originate from ICCs or precursor of ICCs.

KIT consists of an extracellular (EC) domain with five immunoglobulin-like repeats, a transmembrane domain, a juxtamembrane (JM) domain, and TK I and II domains split by the kinase insert [3-6]. A ligand for KIT is stem cell factor (SCF) [8-10]. The binding of SCF and KIT induces autophosphorylation of KIT, and its downstream pathways such as Ras-MAPK, PI3K-Akt and Jak-Stat systems are activated to induce proliferation and differentiation of ICCs, mast cells, melanocytes, germ cells and erythroblasts. On the other hand, gain-of-function mutations of the c-*kit* gene cause constitutive autophosphorylation of KIT without SCF stimulation, and subsequently activate the downstream signaling molecules. Thus, the gain-of-function mutations could be a cause of GISTs, mast cell neoplasms, seminomas, melanomas

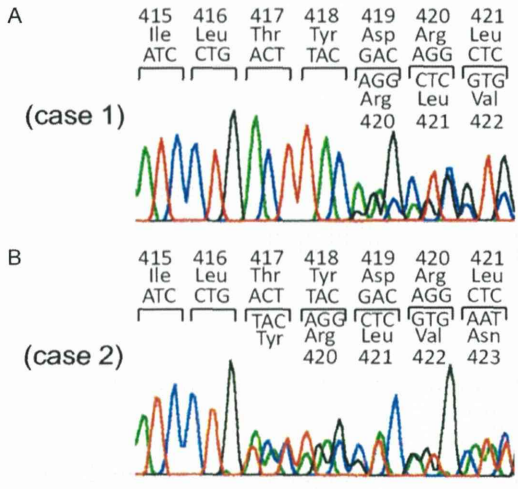


**Figure 1.** Detection of exon 8 c-*kit* gene mutations. A. Heterozygous mutation of Del-Asp419 is shown in case 1. B. Heterozygous mutation of ThrTyrAsp (417-419) Tyr is shown in case 2. Case 3 has the same heterozygous mutation of ThrTyrAsp (417-419) Tyr (data not shown).

and acute leukemias [1, 11-15]. In sporadic GISTs, most of them have somatic gain-of-function mutations of the c-*kit* gene [1]. The mutations are frequently found at exon 11 encoding JM domain (70-80%), at exon 9 encoding EC domain (approximately 10%) and rarely at exon 13 encoding TK I domain and exon 17 encoding TK II domain (less than 2% each) [16, 17]. In approximately a half of c-*kit* gene mutation-negative GISTs, the mutations of platelet-derived growth factor receptor alpha (PDGFRA) gene which shows a quite similar structure to KIT are observed at exon 18 encoding TK II domain (approximately 10%), rarely at exon 12 encoding JM domain (less than 2%), and more rarely at exon 14 encoding TK I domain (less than 1%) [18, 19]. On the other hand, several types of germline gain-of-function mutations of the c-*kit* gene have been detected in approximately 20 families with multiple GISTs [20-23]. Although the mutations in the familial GISTs are also most frequently detected at exon 11, one family with the mutation at exon 8 encoding EC domain, 3 families with the mutation at exon 13 and 4 families with the mutation at exon 17 have been reported [20-23]. Development of multiple GISTs with ICC hyperplasia is commonly observed in patients with the familial GISTs, but some families have mast cell neoplasms and/or hyperpigmentation of the digital, perioral and perineal regions.

Imatinib is a selective TK inhibitor for KIT and PDGFRA [24], which is clinically used for treatment of metastatic or unresectable GISTs [25]. It generally shows a remarkable effect on most GIST cases, but its effectiveness is well-known to be dependent on the types of the c-*kit* and PDGFRA gene mutations [26, 27]. Most GISTs with exon 11 c-*kit* gene mutations show best response to imatinib treatment whereas the particular type of the PDGFRA gene mutation at exon 18, a substitution of aspartate to valine at codon 842 (Asp842Val), is resistant to imatinib [19]. Imatinib is now being used for GISTs after complete resection when they are regarded as high risk tumors for recurrence [28]. On the other hand, sunitinib and regorafenib are multi-targeted TK inhibitors. After the failure of imatinib treatment, sunitinib is administered as a second-line drug [29] and regorafenib as a third-line one in GISTs [30].

In contrast with sporadic GISTs, most of the c-*kit* gene mutations are present at exon 17 in sporadic mast cell neoplasms. However, some sporadic mast cell neoplasms have mutations at exon 9, at exon 11 and even at exon 8 [31]. Recently, moreover, exon 8 c-*kit* gene mutations were also reported in very minor proportion of sporadic GIST cases [32]. Types of exon 8 c-*kit* gene mutations reported in mast cell neoplasms are various [31], but deletion of aspartate at codon 419 (Del-Asp419) is the only type of exon 8 c-*kit* gene mutation reported in GISTs [32]. In the present study, we examined whether GISTs have exon 8 c-*kit* gene mutations in approximately 1,000 sporadic cases, and found 3 such cases. One case had Del-Asp419 and two had substitution of 3 amino acids of codon 417 to codon 419 to tyrosine, hereafter designated as ThrTyrAsp (417-419) Tyr. Here, we showed clinicopathological features of these GISTs.

## Materials and methods

### *Analyses of c-kit and PDGFRA gene mutations*

We have analyzed c-*kit* and PDGFRA gene mutations in approximately 1,000 authentic GIST cases. When fresh-frozen samples were available, total RNA was extracted using RNeasy Mini Kit (QIAGEN, Valencia, CA). Almost all coding regions of the c-*kit* and PDGFRA genes were amplified by polymerase chain

**Table 1.** Clinicopathological characteristics of GISTs with exon 8 c-*kit* gene mutations

| Case No. | Age | Sex | Size (cm) | Site | KIT | CD34 | Mitosis/50HPFs | Metastasis | Survival period (months)[a] |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 53 | M | 8.0 | SI | + | + | 51 | + (B) | 159 (dead) |
| 2 | 56 | M | 3.5 | D | + | _ | 13 | + (L, P) | 102 (dead) |
| 3 | 41 | M | 8.5 | D | + | + | 32 | _[b] | 29 (alive) |

HPF, high power field; M, male; SI, small intestine; D, duodenum; B, bone; L, liver; P, peritoneum. [a]Survival period after the first GIST resection; [b]This case is during adjuvant imatinib therapy.

reaction (PCR) after reverse transcription of the extracted RNA as described previously [1, 19]. When fresh-frozen samples were not available, genomic DNA was extracted from paraffin sections using QIAamp DNA Mini Kit (QIAGEN). Exons 8, 9, 11, 13 and 17 of the c-*kit* gene and exons 12, 14 and 18 of the PDGFRA gene were amplified by PCR. Primers used for PCR were as described previously [17, 19], and a forward primer of 5'-CCATTTCTGTTTTCCTGTAG-3' and a reverse primer of 5'-CTCTGCATTATAAGCAGTGC-3' were used for genomic DNA analysis at exon 8 of the c-*kit* gene. Direct sequencing of the amplified products was carried out with ABI BigDye terminator ver.3.1 (Applied Biosystems, Foster City, CA) and ABI Prism 3100-Avant Genetic Analyzer (Applied Biosystems). The informed consent for the present study was obtained, and the study was approved by the institutional review boards.

*Histology and immunohistochemistry of GISTs*

Resected GIST tissues were fixed with 10% formalin and embedded in paraffin. Sections (3 micrometer thick) were cut and used for hematoxylin and eosin staining and immunohistochemistry. Immunohistochemistry was performed using ENVISION+ KIT HRP (DAB) system (DAKO, Glostrup, Denmark). Rabbit polyclonal antibody against human KIT (A4502; DAKO) and mouse monoclonal antibody against human CD34 (Novocastra Laboratories, Newcastle upon Tyne, UK) were used as the primary antibodies.

## Results

*Detection of exon 8 c-kit gene mutations*

We examined whether exon 8 c-*kit* gene mutations were detected in approximately 1,000 sporadic GISTs. Among them, one GIST had Del-Asp419 and 2 GISTs had ThrTyrAsp (417-419) Tyr (**Figure 1A** and **1B**).

*Clinicopathologicl features of GISTs with exon 8 c-kit gene mutations*

Brief clinicopathological features of 3 GIST cases with exon 8 c-*kit* gene mutations were shown in **Table 1**. Characteristically, all GISTs with the mutation occurred outside the stomach (2 at the duodenum and 1 at the small intestine). All three tumors consisted of spindle shaped cells (**Figure 2A**, **2D** and **2G**). Immunohistochemistry revealed that the tumor cells of all cases were diffusely and strongly positive for KIT (**Figure 2B**, **2E** and **2H**). CD34 was positive in cases 1 and 3 but negative in case 2 (**Figure 2C**, **2F** and **2I**). All three GISTs had high mitotic figures (**Table 1**), suggesting that they were highly aggressive. In fact, two of three GISTs showed metastasis. The patient of case 3 has been receiving imatinib adjuvant therapy until now, and no recurrence is evident.

*Clinical history of patients with exon 8 c-kit gene mutations*

*Case 1:* A 53-year-old Japanese man received partial resection of the small bowel for small intestinal GIST (8.0 cm in diameter) which had Del-Asp419 at exon 8 of the c-*kit* gene. A focus of GIST metastasis developed at the 5th thoracic vertebra approximately 7 years after the complete resection of the primary tumor. He underwent partial resection of the metastatic lesion, but the remaining tumor at the metastatic site enlarged 9 months after the partial metastatectomy. Imatinib therapy (400 mg/day) was started, and size of the metastatic lesion decreased. However, the metastatic tumor regrew 22 months after the initial imatinib administration, and imatinib was changed to sunitinib. The imatinib-resistant lesion had been controlled during 16 months under sunitinib administration. The metastatic tumor grew again, and it was resected as far as possible. In spite of multidisciplinary therapy including rein-

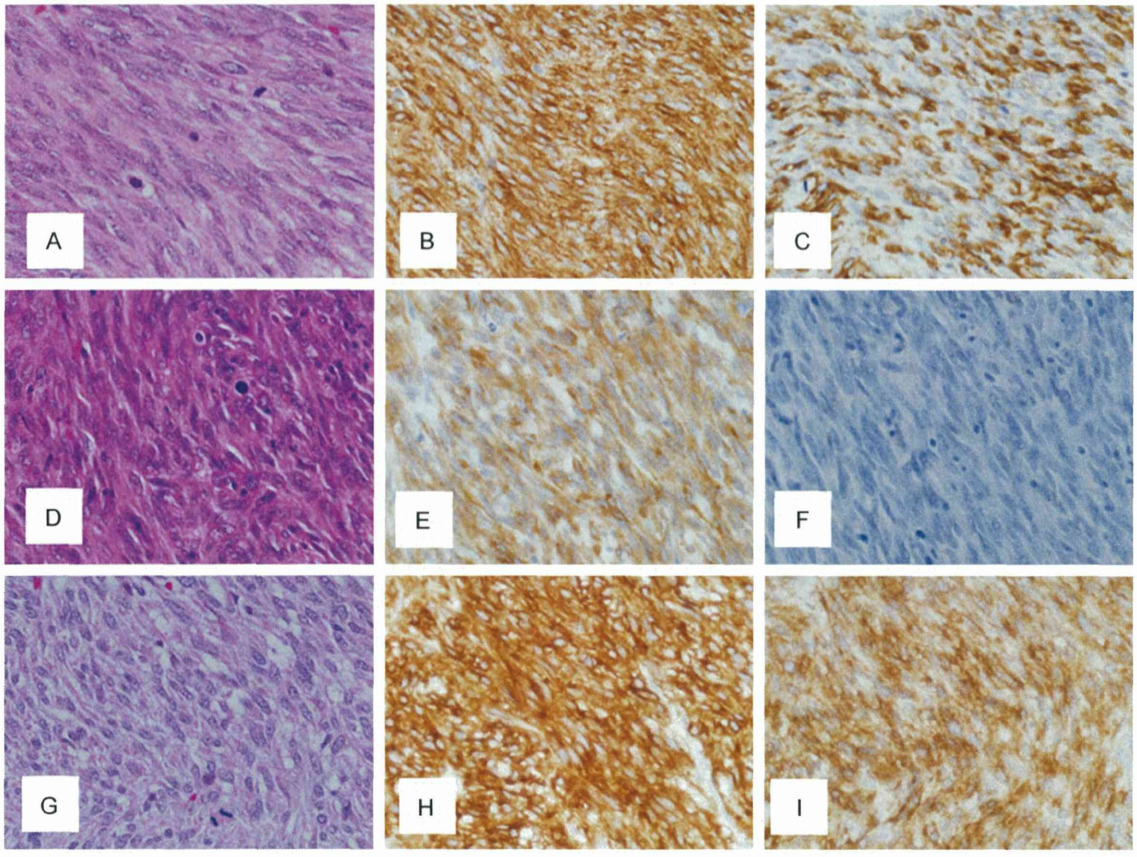


**Figure 2.** Histopathology and immunohistochemistry of 3 GISTs with exon 8 c-*kit* gene mutations. A. Hematoxylin and eosin staining reveals that the tumor cells in case 1 are spindle-shaped. B. Tumor cells in case 1 are immunohistochemically KIT-positive. C. Tumor cells in case 1 are also immunohistochemically CD34-positive. D. Tumor cells in case 2 are spindle-shaped by hematoxylin and eosin staining. E. Immunohistochemistry reveals that tumor cells in case 2 are KIT-positive. F. Immunohistochemistry reveals that tumor cells in case 2 are CD34-negative. G. Hematoxylin and eosin staining shows that the tumor cells in case 3 are spindle-shaped. H. Tumor cells in case 3 are immunohistochemically KIT-positive. I. Tumor cells in case 3 are immunohistochemically CD34-positive.

troduction of imatinib or sunitinib, irradiation, and metastatectomy, the drug-resistant remaining lesion could not be controlled. He died 13 years and 3 months after the first operation for small intestinal GIST. Sequencing of c-*kit* cDNA derived from the resected samples revealed that the sunitinib-resistant lesion had second Asp822Lys at exon 17 in addition to Del-Asp419. Imatinib- and sunitinib-resistant characteristics are considered to be resulted from this second mutation.

*Case 2:* A 56-year-old Japanese man underwent partial resection of the duodenum for duodenal GIST (3.5 cm in diameter) which showed ThrTyrAsp (417-419) Tyr at exon 8 of the c-*kit* gene. Two years after the complete resection of duodenal GIST, multiple liver metastases were found by abdominal CT. Imatinib administration (400 mg/day) was started, and the metastatic foci had been controlled for 18 months. Because of regrowth of one of the metastatic foci in the liver, partial hepatectomy was performed. In spite of continuation of imatinib administration after the metastatectomy, peritoneal masses developed. Although the peritoneal lesions were resected, multiple peritoneal masses developed again. He received sunitinib therapy with 4 month control period. After the failure of sunitinib, regorafenib was administered on clinical trial, but it showed no apparent effect. He died 8 years and 4 months after the first operation for duodenal GIST. Sequencing of c-*kit* cDNA derived from the imatinib-resistant hepatic tumor and peritoneal tumor revealed that both lesions had Asp910Tyr at

exon 18 in addition to ThrTyrAsp (417-419) Tyr at exon 8. This second mutation is considered to be a cause of imatinib-resistant character.

*Case 3:* A 41-year-old Japanese man received pancreaticoduodenectomy for duodenal GIST (8.5 cm in diameter) which had ThrTyrAsp (417-419) Tyr at exon 8 of the c-*kit* gene. Since it was judged as a high risk tumor for recurrence, adjuvant imatinib treatment (400 mg/day) has been done for 29 months after the operation. At present, he is under imatinib treatment and there is no evidence of recurrence.

## Discussion

We reported here 3 cases of sporadic GISTs with exon 8 c-*kit* gene mutations. Frequency of the mutations is low (approximately 0.3%), but at least 3 GISTs really had two types of the mutations such as Del-Asp419 and ThrTyrAsp (417-419) Tyr.

Exon 8 c-*kit* gene mutations was first reported in acute myeloid leukemia in 1999 [33]. In acute myeloid leukemia [33, 34], various types of exon 8 c-*kit* gene mutations are known including ThrTyr (417&418) His, ThrTyrAsp (417-419) Asn, ThrTyrAsp (417-419) Ile, ThrTyrAsp (417-419) Phe, ThrTyrAsp (417-419) Tyr, ThrTyrAsp (417-419) Val, ThrTyrAsp (417-419) ArgAla, ThrTyrAsp (417-419) ArgGly, TyrAsp (418&419) Gly, TyrAsp (418&419) Ser, Asp-419Phe, Del-Asp419 and AspArg (419&420) PhePheAspGly. In pediatric mastocytosis, on the other hand, exon 8 c-*kit* gene mutations were reported in 2010 [31]. The types of the exon 8 c-*kit* gene mutations reported in pediatric mastocytosis are Del-Asp419, Ins-PhePhe between codon418 and codon419, ThrTyrAsp (417-419) Tyr and Cys443Tyr [31]. Many types of exon 8 c-*kit* gene mutations are common in acute myeloid leukemia and pediatric mastocytosis. Although acute myeloid leukemia does not appear to have any highly frequent mutation types, Del-Asp419 appears to be the most frequent mutation type in pediatric mastocytosis.

Exon 8 c-*kit* gene mutations in GISTs were first reported in familial GIST cases with germline c-*kit* gene mutation in 2005 [23], and the type of the mutation was Del-Asp419. The patients in the family members had not only multiple GISTs but also mastocytosis. Recently, 2 sporadic GIST cases with Del-Asp419 were found [32]. Therefore, Del-Asp419 was the only mutation type reported in GISTs with exon 8 c-*kit* gene mutation. In the present study, we reported one GIST case with Del-Asp419 and 2 GISTs cases with ThrTyrAsp (417-419) Tyr. Described above, ThrTyrAsp (417-419) Tyr has been reported in acute myeloid leukemia and sporadic pediatric mast cell neoplasms [31, 33, 34]. Thus, these are the first reported GIST cases with ThrTyrAsp (417-419) Tyr among sporadic and familial GIST cases.

In the previous report, 2 sporadic GISTs with exon 8 c-*kit* gene mutations occurred at the small bowel [32]. In the present study, 3 GISTs with exon 8 c-*kit* gene mutations were present at the duodenum in 2 cases and at the small intestine in 1 case. Since all of the reported GIST cases with exon 8 c-*kit* gene mutations developed at the small intestine or duodenum, those GISTs appear to arise from extragastric sites. However, the number of GIST cases with exon 8 c-*kit* gene mutations is too small to draw conclusions, and further effort to collect those GIST cases is needed.

In the previous report of sporadic GISTs with exon 8 c-*kit* gene mutations, one GIST developed multiple metastatic foci in the peritoneum [32]. The other case did not show metastasis. However, the patient had been receiving adjuvant imatinib therapy because the tumor was regarded as intermediate to high risk for recurrence [32]. In the present study, 2 cases of GISTs with exon 8 c-*kit* gene mutations showed distant metastasis; one was in bone and the other was in liver and peritoneum. The other case in this study does not show metastasis, but the patient also has been receiving adjuvant imatinib therapy because the tumor was classified as high risk for recurrence. These results suggested that GISTs with exon 8 c-*kit* gene mutations might be prone to be metastatic. In fact, all 3 cases in the present study had high mitotic figures, suggesting GISTs with exon 8 c-*kit* gene mutations might be aggressive. More cases with exon 8 c-*kit* gene mutations have to be collected to clarify the possibility.

In the previous report, exon 8 c-*kit* gene mutations such as Del-Asp419, ThrTyrAsp (417-419) Tyr, Cys443Tyr, and ThrTyrAsp (417-419) Ile were demonstrated to be ligand-independently autophosphorylated [25, 31, 34]. In the previous report, moreover, exon 8 c-*kit* gene mutations of Del-Asp419 and ThrTyrAsp (417-419) Ile

were demonstrated to be sensitive to imatinib in vitro [25, 34]. As described above, imatinib was reported to be administered to one sporadic GIST case with Del-Asp419 because of being regarded as intermediate to high risk for recurrence [32]. The patient did not show recurrence for 24 months under adjuvant imatinib treatment, but the case cannot clearly demonstrate whether the mutation is sensitive to imatinib in vivo. On the other hand, our 2 cases with distant metastasis showed apparent clinical effect of imatinib. Recently, imatinib tends to be selectively used only for GISTs with imatinib-sensitive mutations. In many GIST cases, the mutations are usually examined only at exons 9, 11, 13 and 17 of the c-*kit* gene and exon 12, 14, 18 of the PDGFRA gene. Therefore, GISTs with exon 8 c-*kit* gene mutation could be erroneously regarded as so-called wild-type GISTs which are usually resistant to imatinib, and those cases might not be considered to be candidates for imatinib treatment. We have to examine whether the exon 8 c-*kit* gene mutations are present in so-called wild-type GISTs to prevent loss of opportunity for imatinib treatment in those patients.

Asp816Val c-*kit* gene mutation at exon 17 is often observed in sporadic mast cell neoplasms [11, 12]. However, any GISTs have not been reported to possess this mutation. Namely, there is tumor-type specificity in Asp816Val. However, this is an exceptional correlation between tumor-types and particular mutations. Many types of the c-*kit* gene mutations are common in mast cell neoplasms and GISTs. Indeed, both of mast cell neoplasms and GISTs have the same exon 8 c-*kit* gene mutations, at least Del-Asp419 and ThrTyrAsp (417-419) Tyr. The cause of tumor-type specificity in Asp816Val remains to be clarified.

Finally, we showed that GISTs with exon 8 c-*kit* gene mutations might have features of extragastric development and metastasis-prone nature. Since the exon 8 c-*kit* gene mutations appeared to be sensitive to imatinib, accurate genotyping including not only exons 9, 11, 13 and 17 but also exon 8 of the c-*kit* gene is necessary to predict response to imatinib in both unresectable/metastatic and adjuvant settings.

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## Disclosure of conflict of interest

Seiichi Hirota received research fund from Novartis Pharma K.K. Japan. The other authors have no conflict of interest.

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