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Molecular and clinicopathological features of *KIT/PDGFRA* wild-type gastrointestinal stromal tumors

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Abstract

Approximately 10% of gastrointestinal stromal tumors (GISTs) harbor reportedly no *KIT* and *PDGFRA* mutations (wild-type GISTs). The clinicopathological features and oncologic outcomes of wild-type GISTs based on molecular profiles are unknown. We recruited 35 wild-type GIST patients from the two registry studies of high-risk GISTs between 2012 and 2015 and primary GISTs between 2003 and 2014. Molecular profiling of wild-type GISTs was performed by targeted next-generation sequencing (NGS) using formalin-fixed paraffin-embedded tumor samples. Among 35 wild-type GISTs, targeted NGS analysis detected *NF1*, *SDH*, or *BRAF* mutation: 16 NF1-GISTs with various *NF1* mutations, 12 SDH-GISTs (4 with *SDHA* mutations, 4 with *SDHB* mutations, and 4 with SDHB-negative staining), and 5 BRAF-GISTs with the V600E mutation. Two GISTs showed no mutations based on our targeted NGS analysis. Additional gene mutations were infrequent in primary wild-type GISTs and found in *TP53*, *CREBBP*, *CDKN2A*, and *CHEK2*. Most NF1-GISTs were located in the small intestine ($N = 12$;

Abbreviations: AYA, adolescent and young adult; CGP, comprehensive genomic profiling; COSMIC, Catalog of Somatic Mutations in Cancer; FFPE, formalin-fixed paraffin-embedded; HPF, high-powered field; IQR, interquartile range; GIST, gastrointestinal stromal tumor; NF1, neurofibromatosis type 1; NCC, National Cancer Center; NGS, next-generation sequencing; OS, overall survival; PCR, polymerase chain reaction; RFS, recurrence-free survival; VUS, variant of unknown significance.

75%) and showed spindle cell features ($N=15$; 94%) and multiple tumors ($N=6$, 38%) with modest proliferation activities. In contrast, SDH-GISTs were predominantly found in the stomach ($N=11$; 92%), exhibiting epithelioid cell ($N=6$; 50%) and multiple ($N=6$, 50%) features. The overall survival of patients with SDH-GISTs appeared to be better than that of BRAF-GISTs ($p=0.0107$) or NF1-GISTs ($p=0.0754$), respectively. In conclusion, major molecular changes in wild-type GISTs include *NF1*, *SDH*, and *BRAF*. NF1-GISTs involved multifocal spindle cell tumors in the small intestine. SDH-GISTs occurred in young patients and were multifocal in the stomach and clinically indolent.

KEYWORDS
clinicopathological features, gastrointestinal stromal tumor, *NF1*, prognostic outcomes, *SDH*

1 | INTRODUCTION

Gastrointestinal stromal tumor (GIST) is the most frequent mesenchymal tumor of the gastrointestinal tract. The annual incidence of GIST has been estimated to range from 6 to 22 cases per million.[1,2] The median age of GIST patients at diagnosis is 60 years, with no apparent major difference according to sex. Most GISTs arise in the stomach (approximately 60%–65%) or the small bowel (20%–25%) and are rarely found in the rectum or colon (~5%), the esophagus (1%), and in other sites (8%–10%), including outside the gastrointestinal tract. Although positive staining for the KIT protein (>95% GISTs) and/or DOG-1 protein by immunohistochemistry is the hallmark of GIST diagnosis, proliferation of GIST cells is driven by various driver mutations.[3,4] Frequent driver mutations found in GISTs include the *KIT* (60%–70%) and *PDGFRA* (10%–15%) genes.[1–4] Approximately 10% to 15% of GISTs, however, have no mutations in either *KIT* or *PDGFRA*, and are referred to as "wild-type GISTs", which may involve other oncogenic drivers, such as mutations in the *RAS* family genes, *BRAF*, or *NF1* genes; mutations or inactivating alterations of the *SDH* genes; and, very rarely, fusions involving the *TRK* family or *FGFR* family genes.[1,2,5] These mutations or fusions are considered mutually exclusive in primary GIST.[1,2]

Wild-type GIST is distinct from its *KIT*/*PDGFRA*-mutated counterpart, as it is generally unresponsive to tyrosine kinase inhibitors, such as imatinib, the standard therapy for *KIT*/*PDGFRA*-mutated GIST.[1–5] Several case series have indicated that SDH-deficient GISTs represent a major subtype of wild-type GISTs without *KIT* and *PDGFRA* mutation and that they preferentially affect young females, appear as lobulated and/or multifocal tumors in the stomach, and are associated with frequent lymph node metastasis despite an indolent clinical course.[6–12] Some wild-type GISTs may be presented in the context of syndromes with germline mutations involving the SDH complex or of the Carney triad associated with hypermethylation[13,14] and in the context of neurofibromatosis type 1 (NF1).[15–17] Nevertheless, as wild-type GIST is very rare, clinical and pathological features as well as prognostic outcomes are poorly understood.

In this study, we pooled wild-type GIST cases from two prospective registry studies, the primary GIST registry, and the STAR ReGISTry study[18] and examined clinicopathological features and oncologic outcomes of wild-type GIST patients based on molecular features obtained from targeted next-generation sequencing (NGS) analysis.

2 | PATIENTS AND METHODS

Wild-type GISTs, as defined by the lack of *KIT* (exons 9, 11, 13, 17) and *PDGFRA* (exons 12, 14, 18) mutations determined by conventional polymerase chain reaction (PCR) and Sanger sequencing or by targeted NGS analysis, were collected from our two prospective registry cohorts: the primary GIST cohort and the high-risk GIST cohort. The primary GIST cohort consisted of consecutive 253 patients with primary GIST who underwent surgery at Osaka University Hospital and Osaka Police Hospital between 2003 and 2014 and were prospectively registered (Figure S1). For various reasons, *KIT* and *PDGFRA* mutations were not assessable for 31 GISTs. After informed consent, genotyping of the *KIT* and *PDGFRA* genes was performed for 222 GISTs. *KIT* mutations were detected in 183 GISTs (82.4%) and *PDGFRA* mutations in 22 (9.9%); 17 GISTs (7.7%) were diagnosed as wild-type.

Regarding the high-risk GIST cohort, between December 2012 and December 2015, a total of 541 patients diagnosed with primary high-risk GISTs by local pathology were recruited to the prospective observational registry study, the STAR ReGISTry study, after complete resection (R0 or R1).[18] The modified NIH consensus criteria[19] were used for the risk stratification. Among the 541 patients, 4 tumors did not meet the inclusion criteria, 3 patients were excluded from the study because no pathological specimens were available, and 19 tumors diagnosed as non-GISTs in central pathology were excluded; 515 patients were eligible for further evaluation (Figure S1).[18] Among these 515 high-risk GISTs, *KIT* and *PDGFRA* mutations were not assessable for 22 GISTs for various reasons, including lack of informed consent. Of the remaining 493 high-risk GISTs, *KIT* mutations were detected in 457 GISTs (92.6%) and *PDGFRA* mutations in 18 (3.7%); 18 GISTs (3.7%) were diagnosed as wild-type. Collectively, 35 wild-type GISTs, involving 17 GIST patients from the primary

GIST cohort and 18 from the high-risk GIST cohort, were analyzed in this study. Both registry studies prospectively enrolled primary GIST patients who underwent surgery irrespective of whether they were sporadic, syndromic, or familial. The data cutoff date was 31 December 2019.

2.1 | Definitions of wild-type GIST subgroups in this study

Among the 35 wild-type GISTs, surgical specimens were not obtained from one patient in the primary GIST cohort and one in the high-risk GIST cohort. Both patients, whose GISTs were negative for SDHB immunostaining, were considered to have SDH alterations and were included in the SDH-GIST group.[13,14] The other 33 GISTs were subjected to targeted NGS analysis, as mentioned below. Twenty-nine GISTs showed mutation in *NF1* ($N=16$), *SDHA* or *SDHB* ($N=4$ or $N=4$, respectively), or *BRAF* ($N=5$), as indicated in Table 1. No mutations were found in specimens from four GIST patients, two of which, one from the high-risk cohort and the other from the primary GIST cohort, were negative for SDHB immunostaining and were considered SDH-GIST. Thus, NF1-GIST consisted of 16 GISTs with various mutations in *NF1*. SDH-GISTs included eight GISTs with mutations in *SDHA* or *SDHB* and four GISTs with negative immunostaining for SDHB staining. Five BRAF-GISTs harbored the typical V600E mutations in *BRAF*.[20,21] In two wild-type GISTs, we could not detect potential driver mutations and/or alterations in our study.

2.2 | Targeted NGS analysis of cancer-associated genes

For targeted NGS analysis, we modified an NCC Oncopanel test (ver. 4), which is a comprehensive genomic profiling (CGP) test to examine mutations and copy number alterations of 114 genes together with rearrangements of 12 oncogenes.[22] The modified version (ver. 5) used in the study was supplemented with seven genes including four genes encoding SDH complex components (Figure S2). Tumor DNA was prepared from sections of formalin-fixed paraffin-embedded (FFPE) tumor tissue samples using a QIAamp DNA FFPE Tissue Kit (Qiagen). The tumor cell content of tissue samples was estimated by counting the nuclei of tumor and nontumor cells in the adjacent section and was confirmed to be ≥30%. A mixture of unrelated blood samples was used as normal control DNA. NGS library preparation, NGS runs, and bioinformatics analysis were performed as previously described,[22] with some modifications. The average read depths were 115 to 1137 (median 483). Nonsynonymous variants and splicing site variants with ≥10% variant allele frequencies (VAFs) were defined as mutation candidates. Variants that were registered at >1% frequency in single-nucleotide pleomorphism (SNP) databases were excluded as germline polymorphisms. The SNP databases used were 1000 Genomes, ESP6500, Human Genetic Variation Database, ToMMo, and in-house Japanese germline SNP data. Because the remaining 120 variants (Table S1) were considered to still contain many rare SNPs and passenger mutations, those with >5 registrations as confirmed somatic variants in the Catalog of Somatic Mutations in Cancer (COSMIC) database, those registered as pathogenic or likely pathogenic in the ClinVar database, those found in splicing sites, and those resulting in stop-gain or frameshift were selected as potentially pathogenic mutations. For genes known as drivers of wild-type GISTs (*NF1*, *SDHA*, *SDHAB*, *SDHC*, *SDHD*, and *BRAF*), other nonsynonymous variants without any registration in the above SNP databases were also selected to prevent exclusion of novel pathogenic mutations. Based on these criteria, two *SDHA* variants (R188W and H198R) and one *SDHB* variant (R94T) were found. In addition, genes with <0.5-fold copy number decreases were considered homozygous deletion candidates and were judged by manual inspection.

The study was conducted in accordance with the World Medical Association Declaration of Helsinki, Ethical Principles for Medical Research Involving Human Subjects (Amended in Seoul in October 2008), and the Ethics Guidelines for Clinical Research (Ministry of Health, Labor and Welfare Notice No. 415, 2008). Ethical approval was initially obtained from the institutional review board (IRB) of the National Cancer Center. Written informed consent was obtained from all participants.

All data generated or analyzed during this study are available within the article and its supplementary information files. Raw sequence data are not publicly available in online repository due to informed consent.

2.3 | Statistical analysis

Statistical analyses were performed using the chi-squared test, Fisher's exact test, Student's *t*-test, and the Mann–Whitney U test. Recurrence-free survival (RFS) was calculated from the date of initial surgery to the date of first tumor recurrence or to the date of death, excluding living patients without recurrence at the time of data collection. Overall survival (OS) was calculated from the date of initial surgery to the date of any death, excluding living patients. RFS and OS were compared between three groups using the Kaplan–Meier life table method with the log-rank test with a post hoc test of the Mantel–Cox method. The *p*-values were two sided, and *p*-values less than 0.10 were considered significant. The data were analyzed using the Statistical Package of IBM SPSS Statistics 25, version 28.0 (IBM Corp.).

3 | RESULTS

3.1 | Mutations in wild-type GISTs

In total, 16 NF1-GIST patients with various *NF1* mutations, 12 SDH-GISTs (4 with *SDHA* mutations, 4 with *SDHB* mutations, and 4 with SDHB-negative staining), and 5 BRAF-GISTs were included (Table 1).

TABLE 1 Results of targeted NGS analysis and clinical features of case series.

Patient No.	Targeted NGS analysis	Cohort	Tumor cell content	Read depth	Detected mutation (VAF)/CNA	Wild-type GIST group	Multiple GISTs	Café au lait spots	Subcutaneous neurofibromas	Family history	SDHB-IM
1	Yes	High-risk GIST	100%	115.0	NF1 p.R1276* (73.5%) CREBBP p.L708fs*5 (20.7%)	NF1	No	Yes	Yes	-	Positive
2	Yes	High-risk GIST	100%	483.0	NF1 p.R1534* (52.8%) NF1 p.S340fs*12 (36.3%)	NF1	No	No	Yes	No	Positive
3	Yes	High-risk GIST	100%	549.2	NF1 p.Q209* (51.4%)	NF1	Yes	Yes	Yes	Yes	Positive
4	Yes	High-risk GIST	100%	951.9	NF1 p.E1123* (39.6%)	NF1	Yes	Yes	Yes	-	Positive
5	Yes	High-risk GIST	100%	1137.0	NF1 p.Q1101fs*4 (45.0%) NF1 c.G7869 + 1A (splicing site) (44.2%) CDKN2A homozygous deletion	NF1	No	No	No	No	Positive
6	Yes	High-risk GIST	80%	338.0	NF1 p.Y489C (70.6%)	NF1	No	-	-	-	Positive
7	Yes	High-risk GIST	60%	546.4	NF1 p.Q1447fs*22 (55.7%)	NF1	No	No	No	No	Positive
8	Yes	High-risk GIST	100%	301.1	NF1 p.W777R (74.4%)	NF1	No	Yes	Yes	Yes	Positive
9	Yes	High-risk GIST	100%	570.6	NF1 p.R1870Q (splicing site) (35.6%)	NF1	No			No	Positive
10	Yes	Primary GIST	80%	226.2	NF1 p.M645V (51.1%)	NF1	No	No	No	No	-
11	Yes	Primary GIST	70%	349.1	NF1 c.T3496 + 2G (splicing site) (63.2%)	NF1	No	No	No	No	Positive
12	Yes	Primary GIST	100%	416.9	NF1 p.Q756* (46.2%) TP53 p.E11Q (91.4%)	NF1	Yes	Yes	Yes	Yes	-
13	Yes	Primary GIST	100%	479.6	NF1 p.I679fs*21 (51.6%)	NF1	Yes	Yes	Yes	No	Positive
14	Yes	Primary GIST	100%	639.1	NF1 p.C1682* (48.8%) NF1 p.Y235fs*45 (22.5%)	NF1	Yes	Yes	Yes	No	Positive
15	Yes	Primary GIST	95%	620.7	NF1 p.Q1255* (46.4%) NF1 p.A1916fs*4 (33.2%)	NF1	Yes	Yes	Yes	No	Positive
16	Yes	Primary GIST	95%	667.7	NF1 p.R461* (88.9%)	NF1	No	Yes	Yes	No	Positive
17	Yes	High-risk GIST	50%	589.9	SDHB p.L157* (79.5%)	SDH	No	-	-	-	Negative
18	Yes	High-risk GIST	60%	365.1	SDHA p.N40fs*18 (41.3%) CHEK2 p.R519* (46.2%)	SDH	Yes	-	-	-	Negative
19	Yes	High-risk GIST	100%	443.9	SDHB p.R94T (86.6%)	SDH	No	-	-	-	Positive*
20	Yes	Primary GIST	95%	399.0	SDHA p.Y259fs*21 (80.6%)	SDH	Yes	-	-	No	-
21	Yes	Primary GIST	80%	832.0	SDHB p.Q214H (splicing site) (92.7%)	SDH	No	-	-	No	-
22	Yes	Primary GIST	75%	394.9	SDHA p.H198R (81.8%)	SDH	Yes	-	-	No	-
23	Yes	Primary GIST	60%	390.7	SDHB p.R46Q (71.7%)	SDH	No	-	-	No	-

(Continues)

TABLE 1 (Continued)

Patient No.	Targeted NGS analysis	Cohort	Tumor cell content	Read depth	Detected mutation (VAF)/CNA	Wild-type GIST group	Multiple GISTs	Café au lait spots	Subcutaneous neurofibromas	Family history	SDHB-IM
24	Yes	Primary GIST	80%	346.9	SDHA p.R188W (34.1%)	SDH	Yes	-	-	No	-
25	Yes	High-risk GIST	95%	786.1	None	SDH: due to SDHB-negative	No	-	-	-	Negative
26	Yes	Primary GIST	30%	243.3	None	SDH: due to SDHB-negative	Yes	-	-	No	Negative
27	No	High-risk GIST				SDH: due to SDHB-negative	No	-	-	-	Negative
28	No	Primary GIST				SDH: due to SDHB-negative	Yes	-	-	No	Negative
29	Yes	High-risk GIST	100%	527.4	BRAF p.V600E (62.4%)	BRAF	No	-	-	-	Positive
30	Yes	High-risk GIST	90%	503.8	BRAF p.V600E (46.5%)	BRAF	No	-	-	-	Positive
31	Yes	High-risk GIST	90%	1078.4	BRAF p.V600E (41.2%) TP53 p.R202H (47.2%)	BRAF	No	-	-	-	Positive
32	Yes	Primary GIST	90%	645.9	BRAF p.V600E (46.0%)	BRAF	No	-	-	-	-
33	Yes	Primary GIST	99%	481.6	BRAF p.V600E (32.2%)	BRAF	No	-	-	-	Positive
34	Yes	High-risk GIST	100%	993.4	None	Not detected	No	-	-	-	Positive
35	Yes	Primary GIST	50%	226.2	None	Not detected	No	-	-	-	Positive

Note: "-": Data not available or no description in hospital medical records. Positive*: Most GIST tumor cells are negative for SDHB immunohistochemistry, and a part of tumor cells are positive; hence, a tumor was positive.

Abbreviations: NGS, next-generation sequencing; GIST, gastrointestinal stromal tumor.

In two GIST patients, we could not detect any mutations and any deficiency from our analyses. Most of the mutations found in *NF1*, *SDHA*, and *SDHB* were truncating (frameshift and splicing site) mutations, while some were missense mutations, including variants of unknown significance (VUSs). All mutations found in *BRAF* were the typical missense mutation of V600E.

Few other gene mutations were detected by our targeted NGS analysis in the 33 wild-type GISTs (Table 1). Three NF1-GISTs carried a *TP53* amino acid substitution mutation, a *CREBBP* frameshift mutation, or *CDKN2A* homozygous deletion. One SDH-GIST had a frameshift mutation in *CHEK2*, and one BRAF-GIST had a *TP53* mutation. For the other 28 GISTs, we could not detect additional mutations in other genes. Four NF1-GISTs harbored two different mutations in *NF1*.

3.2 | Clinicopathological features of GISTs with NF1, SDH, or BRAF mutations

The 35 patients with wild-type GISTs included 16 (46%) men and 19 (54%) women, with a median age of 54 years. The wild-type GISTs were located in the stomach ($N=16$; 46%) or in the small intestine (17; 49%), with one each located in the esophagus or extragastrointestinal tissues (Table 2). The median tumor size was 6.0 cm, and the median mitotic count was 4.0/50 high-powered fields (HPFs). Tumor rupture was observed in five (14%) patients. Histologically, 28 GISTs consisted of spindle cell types or four epithelioid types, with three of mixed cell type. Thirty-four wild-type GISTs (97%) were strongly positive for KIT immunostaining; one (3%) was weakly positive for KIT. Twenty-eight wild-type GISTs were evaluated by DOG1 immunostaining: 27 were positive (96%), and 1 (4%) was weakly positive.

Most NF1-GISTs were in the small intestine ($N=12$; 75%), and 38% (6 out of 16 patients) had multiple tumors, as reported previously.[15–17] All NF1-GISTs but one showed the spindle cell type, and the proliferation activities appeared to be modest (mitosis count: 5.2 ± 5.4/50 HPF and Ki67: 4.7 ± 5.7%) compared with the other wild-type GISTs (Table 3). Nine NF1-GIST patients of 16 had multiple café au lait spots, and 10 patients had subcutaneous multiple neurofibromas (Table 1). Three patients had a family history of NF1, whereas no family history was reported for 10 patients.

The age of onset was younger in patients with SDH-GISTs (37 ± 14 years old) than in those with other wild-type GISTs and *KIT*- or *PDGFRA*-mutated GISTs (Table 3).[6–12] SDH-GISTs were mainly found in the stomach (11 out of 12; the other one in esophagogastric junction), and six patients with SDH-GISTs (50%) showed multiple tumors with nodular growth. Histologically, six cases exhibited epithelioid components, which were significantly more frequent than NF1-GISTs, BRAF-GISTs, and *KIT*-mutated GISTs.[6–12] No patients were found with a family history or paraganglioma and/or pulmonary chondroma in this study.

The number of BRAF-GISTs is limited, and the age of onset appears to be young. BRAF-GISTs are mainly located in the small intestine with spindle cell features.[20,21,23]

TABLE 2 Patient characteristics.

Total	(N=35)
Age (median, IQR; years)	54 (35–66)
Gender	
Male	16 (46%)
Female	19 (54%)
PS	
0	33 (94%)
1	2 (6%)
Location	
Esophagus	1 (2.5%)
Stomach	16 (46%)
Intestine	17 (49%)
Others	1 (2.5%)
Neoadjuvant therapy	
(–)	33 (94%)
(+)	2 (6%)
Surgery	
Open	19 (54%)
Laparoscopic	16 (46%)
Curability of surgery	
R0	28 (80%)
R1	2 (6%)
R2	5 (14%)
Adjuvant therapy	
(–)	21 (60%)
(+)	14 (40%)
Tumor size (median, IQR; cm)	6.0 (3.2–8.0)
Mitosis (median, IQR; /50HPF)	4.0 (1.0–13.0)
Tumor rupture	
No	30 (86%)
Yes	5 (14%)
Risk classification	
Very low	2 (6%)
Low	6 (17%)
Intermediate	4 (11%)
High	23 (66%)
Histological types	
Spindle	28 (80%)
Epithelioid	4 (11%)
Mixed	3 (9%)
Genotyping	
NF1	16 (46%)
SDH	8 (23%)
BRAF	5 (14%)
Unavailable	6 (17%)
SDHB-IM-negative	4 (11%)
No mutations	2 (6%)

Abbreviations: HPF, high-power field; IQR, interquartile range; PS, performance status; SDHB-IM-negative, SDHB was negative in SDHB immunostaining.

TABLE 3 Clinicopathological features of wild-type GISTs according to genotyping.

Mutation		NF1 (*N* = 16)	SDH (*N* = 12)	BRAF (*N* = 5)
Age (mean ± SD; years)		60 ± 15	37 ± 14	54 ± 14
Gender	Male	9	5	1
	Female	7	7	4
Location	Esophagus	0	1[a]	0
	Stomach	3	11	1
	Intestine	12	0	4
	Others	1	0	0
Multiplicity	No	10	6	5
	Yes	6	6	0
Risk classification	Very low	1	0	1
	Low	4	0	1
	Intermediate	0	4	0
	High	11	8	3
Local curability	R0 or R1	13	9	5
	R2	3	3	0
Tumor size (mean ± SD; cm)		8.0 ± 7.9	6.9 ± 2.9	4.8 ± 2.8
Mitosis (mean ± SD; /50HPF)		5.2 ± 5.4	14.3 ± 24.7	5.7 ± 8.7
Ki67 (mean ± SD; %)		4.7 ± 5.7	9.2 ± 9.6	3.1 ± 2.7
Tumor rupture	No	15	8	5
	Yes	1	4	0
Histological type	Spindle	15	6	5
	Epithelioid	0	4	0
	Mixed	1	2	0
Recurrence	No recurrence	10	8	4
	Recurrence	6	4	1
Disease-specific OS	Alive	14	12	4
	Death	2	0	1
OS	Alive	12	12	3
	Death	4	0	2

Abbreviations: GIST, gastrointestinal stromal tumor; HPF, high-powered field; OS, overall survival.
[a]Located in the esophago-gastric junction.

3.3 | Prognostic outcomes of patients with wild-type GISTs

During a median follow-up of 5.4 years (interquartile range [IQR]: 4.2–9.1 years), there were 11 recurrences and 6 deaths. The 5-year RFS of patients with wild-type GISTs was 73.1% (95% CI: 58.0–88.2), and the 5-year OS was 90.9% (95% CI: 0.81–100.7). The estimated median RFS was 7.5 years (95% CI: 6.2–8.9), with an estimated median OS of 10.1 years (95% CI: 8.9–11.4). Although the rates of curative surgery and risk classification were not balanced among the NF1-GISTs, SDH-GISTs, and BRAF-GISTs, the RFS was very similar among the three groups (Figure 1). In contrast, the OS of patients with SDH-GISTs appears to be significantly better than that of patients with BRAF-GISTs ($p = 0.0107$) or NF1-GISTs ($p = 0.0754$), respectively (Figure 1), though noncurative surgery (R0 = 8, R1 = 1 and R2 = 3) and proliferation activity (mean mitotic count = 14.3/50 HPF; mean Ki67 value = 9.2%) rates were relatively high in SDH-GISTs (Table 3). Four patients (33%) with SDH-GISTs had recurrences, but none died during the follow-up period. For the NF1-GIST group, six patients (38%) had relapses, two patients died from the disease, and the other two died due to other cancers.

4 | DISCUSSION

Wild-type GISTs are reported to account for 10%–15% of total GISTs, but values vary depending on cohorts and settings.[1,2] Most reports are series of wild-type GISTs but are not population based.[6–12,15–17,20,21] In our study, wild-type GISTs accounted for 7.7% (17/222) of the primary GIST cohort subjected to *KIT* and *PDFGRA* mutational research and for 3.7% (18/493) of the high-risk GIST cohort, indicating that the proportion of wild-type GISTs in the

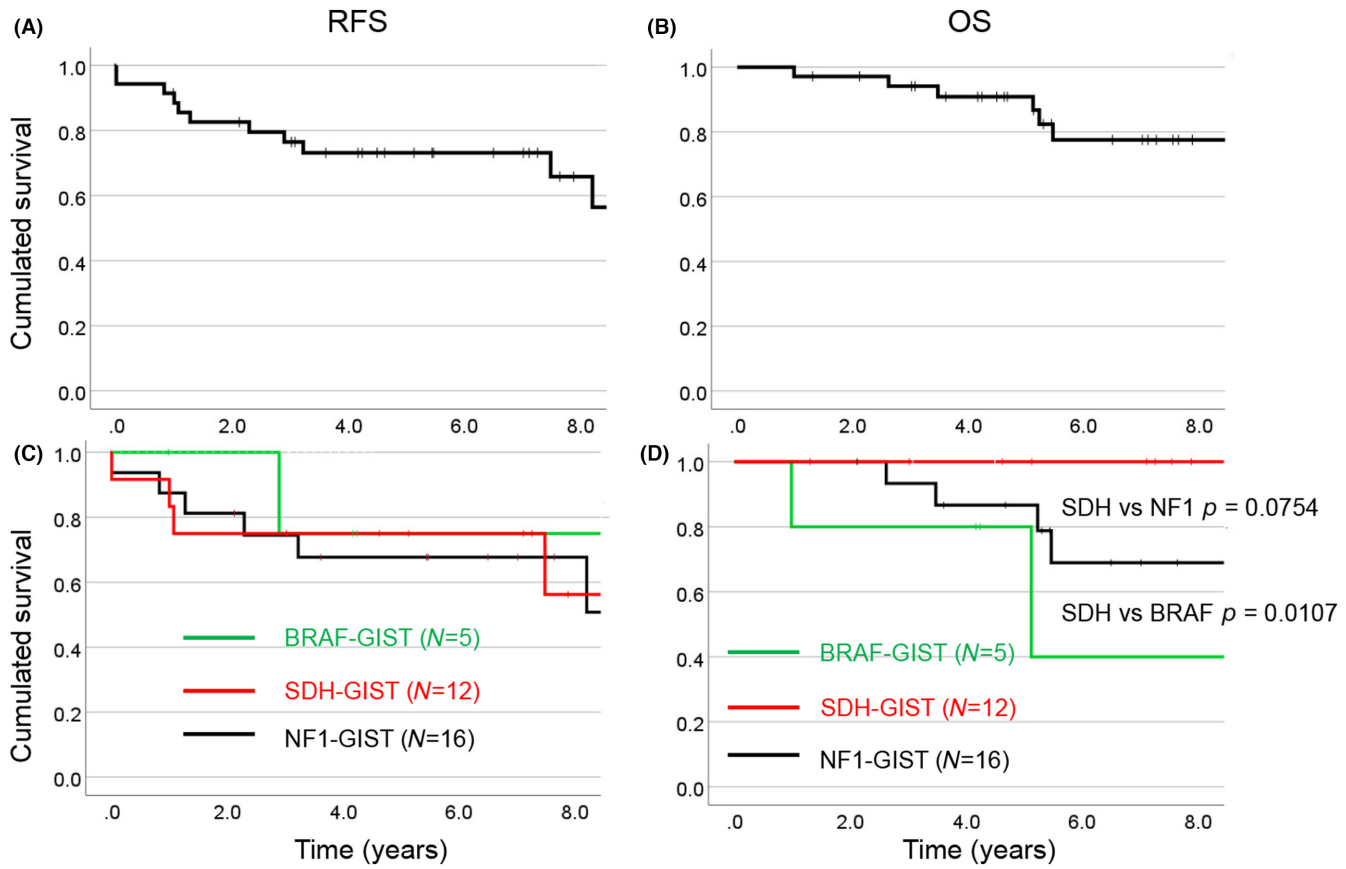

FIGURE 1 Prognostic outcomes of wild-type gastrointestinal stromal tumors (GISTs). Prognostic outcomes of 35 patients with wild-type GISTs are shown. (A) Recurrence-free survival (RFS) of 35 patients with wild-type GISTs. (B) Overall survival (OS) of 35 patients with wild-type GISTs. (C) RFS of BRAF-GISTs ($N=5$: green solid line), SDH-GISTs ($N=12$: red solid line), and NF1-GISTs ($N=16$: black solid line). There was no significant difference among the four groups. (D) OS of the above three groups is shown. The prognosis of patients with SDH-GISTs was significantly better than that of patients with NF1-GISTs ($p=0.0754$) or those with BRAF-GIST ($p=0.0107$).

high-risk cohort was low. This may indicate that the clinicopathological features of wild-type GISTs in clinical practice may be less aggressive than those of *KIT*-mutated GISTs and that the prognosis of patients with wild-type GISTs is better, as reported previously.[24,25] In this study, we analyzed genomic alterations in *KIT* and *PDGFRA* wild-type GISTs from primary and high-risk GIST cohorts using targeted NGS analysis. Among potential genomic alterations in wild-type GISTs,[1,2,5] we only detected mutations in the *NF1*, *SDH*, and *BRAF* genes. Indeed, we found no mutations in *RAS* and *PIK3CA* or no fusion genes involving *NTRK1/2* or FGFR2/3. In this study, genetic mutations in *KIT*, *PDGFRA*, *NF1*, *SDH*, and *BRAF* genes account for more than 99.7% of primary GISTs subjected to mutational research. The number of patients analyzed was limited, and alterations in genes other than *NF1*, *SDH*, and *BRAF* may be considered very infrequent in GISTs.[1,6,26,27] These results indicate that commercially available CGP test, such as the FoundationOne CDx, may cover most mutations found in primary GISTs. Furthermore, additional alterations were infrequent in primary GISTs. Two GIST patients carried additional mutations in *TP53*, and mutations in *CREBBP*, *CDKN2A*, or *CHEK2* were found in the 33 wild-type GISTs analyzed by targeted NGS analysis. This is probably because of the included treatment-naive GISTs, contrasting with the results for heavily treated metastatic/advanced GISTs.[27–29] Moreover, we did not identify any genetic alterations in two wild-type GISTs in our analysis (6% of wild-type GISTs and 0.3% of primary GISTs underwent genetic analysis), indicating that these GISTs may harbor rare driver mutations or alterations, which are difficult to detect by our targeted NGS analysis, such as exon-level deletion, or mutations or alterations other than those listed in Table 1 and Table S1.[6,26,29]

NF1-GISTs and SDH-GISTs are the most frequent wild-type GISTs without *KIT* and *PDGFRA* mutation in both adults and children, including adolescent and young adults (AYAs), respectively.[1,6,14,15] According to previous reports,[15–17] NF1-GISTs are predominantly found in the small intestine, are sometimes multicentric, and show spindle cell features, consistent with this report. NF1-GISTs are considered primarily refractory to all approved tyrosine kinase inhibitors, including imatinib, sunitinib, and regorafenib.[15] Approximately 7% of patients with NF1 may develop GISTs over their lifetime.[15] In the present study, only 3 patients had a family history of NF1 and 10 had no family history, though 4 patients of these 10 did have subcutaneous multiple neurofibromas and café au lait spots (Table 1). Although we did not evaluate germline mutations, germline mutations may occur de novo in *NF1*.[17,30] In fact, 50% of individuals with NF1 reportedly have no family history of the disease. Taken

together, when patients have multiple NF1-GISTs associated with features of café au lait spots and subcutaneous multiple neurofibromas, germline mutations may be present. NF1 is a cancer predisposition syndrome, and the lifetime risk of malignancy in NF1 patients is estimated to be 60%.[30] In this study, two patients died from other cancers, indicating that cancer surveillance is important for NF1-GIST patients.

In contrast, SDH-GIST patients are younger, and it is more common in females; the tumors are mainly found in the stomach, as reported previously.[6–14] SDH-GISTs frequently show multifocal nodular growth and epithelioid cell features. Although the proliferation activities of SDH-GISTs were relatively high in this study of wild-type GISTs (eight were high risk GISTs and three were treated with R2 surgery), the patients with SDH-GISTs showed relatively good oncologic outcomes compared with those with other wild-type GISTs and *KIT*-mutated GISTs. Recurrences occurred in four patients, and during a median follow-up of 7.4 years, no SDH-GIST patients died. Previous reports suggest that most SDH-GISTs have indolent clinical behavior, even though they frequently have lympho-vascular involvement and occasional lymph node metastasis as well as hepatic metastasis.[6,9,11,12]

In this study, we did not examine germline mutations because informed consent was not obtained in either registry study and the analysis of germline mutations was not planned in the present study approved by the IRB. The previous reports have suggested that most of mutations found in gene-encoding components of the SDH complex are associated with germline mutation, known as Carney–Stratakis syndrome, and the remainder may be associated with hypermethylation of the *SDHC* promoter, known as Carney triad.[6,13,14] These syndromic GISTs may be associated with paraganglioma and paraganglioma as well as pulmonary chondroma, respectively.[13,14] Although SDH-GIST is considered to have either germline mutations in SDH subunits or hypermethylation, no cases in this series had these associations at the date of data cutoff. In two GIST patients with negative SDHB staining and no mutations in four subunits of the SDH complex, *SDH* may have been hypermethylated, but we did not perform methylation analysis. Most SDH-GISTs examined were negative for SDHB immunostaining except one, and the other wild-type GISTs were positive (Table 1). One SDH-GIST (patient No. 19 in Table 1) with an R94T mutation in *SDHB*, was diagnosed SDHB positive in pathology (Figure S3) because a small portion was positive for SDHB immunostaining, whereas most tumor cells in the rest were negative for SDHB immunostaining. It can be assumed that a DNA extracted portion, a large part of the tumor, was SDHB negative and harbored this *SDHB* mutation.

There are some limitations in this study. First, the number of patients with each mutation type was relatively small to evaluate prognostic outcomes. However, most reports on wild-type GISTs are case series, and registry-based reports are rare—this study may be one of the largest.[6–12,15–17,20,21] Second, mutations found in the study included three *SDH* variants not reported in the ClinVar and COSMIC databases, indicating their unknown pathogenicity. However, these wild-type GISTs did not harbor other apparent potential driver mutations, which should be addressed by future studies. Third, we did not distinguish somatic mutations from germline mutations.

In summary, we recruited 35 patients with wild-type GISTs based on prospective registries and conducted CGP-based targeted NGS analysis. Most NF1-GISTs were multifocal spindle cell tumors in the small intestine. There may be a significant number of patients with de novo germline mutations in *NF1*. This cancer predisposition syndrome may require careful follow-up after diagnosis of NF1-GIST. Patients with SDH-GISTs were young, and the tumor was clinically indolent despite clinicopathological features. NGS-based analysis may reveal oncogenic mutations for most (94%) of wild-type GISTs, but driver mutations in few wild-type GISTs could not be identified by our analysis. Thus, further genomic analysis or whole-exome or whole-genome analysis may be warranted.

AUTHOR CONTRIBUTIONS

Toshirou Nishida: Conceptualization; formal analysis; funding acquisition; investigation; supervision; visualization; writing – original draft; writing – review and editing. **Yoichi Naito:** Conceptualization; project administration; writing – review and editing. **Tsuyoshi Takahashi:** Resources; writing – review and editing. **Takuro Saito:** Resources; writing – review and editing. **Shigeo Hisamori:** Resources; writing – review and editing. **Dai Manaka:** Resources; writing – review and editing. **Katsuhiro Ogawa:** Resources; writing – review and editing. **Seiichi Hirota:** Data curation; investigation; methodology; validation; writing – review and editing. **Hitoshi Ichikawa:** Data curation; investigation; methodology; validation; writing – review and editing.

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DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are available within the article and its supplementary information files (Figures (S1–S3) and Table S1). Raw sequence data are not publicly available in online repository due to informed consent.

ETHICS STATEMENT

Approval of the research protocol by an Institutional Reviewer Board: Ethical approval was initially obtained from the institutional review board (IRB) of the National Cancer Center (No. 2016–404).
Informed Consent: Written informed consent was obtained from all participants.
Registry and the Registration No. of the study/trial. N/A.
Animal Studies: N/A.

CONSENT FOR PUBLICATION

All authors have confirmed the final version of the submitted manuscript and have approved it.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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