

Original Article



Genetic Characterization of Molecular Targets in Korean Patients with Gastrointestinal Stromal Tumors

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ABSTRACT

Purpose: Gastrointestinal stromal tumors (GISTs) frequently harbor activating gene mutations in either *KIT* or platelet-derived growth factor receptor A (*PDGFRA*) and are highly responsive to several selective tyrosine kinase inhibitors. In this study, a targeted next-generation sequencing (NGS) assay with an OncoPrint Focus Assay (OFA) panel was used for the genetic characterization of molecular targets in 30 Korean patients with GIST.

Materials and Methods: Using the OFA that enables rapid and simultaneous detection of hotspots, single nucleotide variants (SNVs), insertion and deletions (Indels), copy number variants (CNVs), and gene fusions across 52 genes relevant to solid tumors, targeted NGS was performed using genomic DNA extracted from formalin-fixed and paraffin-embedded samples of 30 GISTs.

Results: Forty-three hotspot/other likely pathogenic variants (33 SNVs, 8 Indels, and 2 amplifications) in 16 genes were identified in 26 of the 30 GISTs. *KIT* variants were most frequent (44%, 19/43), followed by 6 variants in *PIK3CA*, 3 in *PDGFRA*, 2 each in *JAK1* and *EGFR*, and 1 each in *AKT1*, *ALK*, *CCND1*, *CTNNA1*, *FGFR3*, *FGFR4*, *GNAI1*, *GNAQ*, *JAK3*, *MET*, and *SMO*. Based on the mutation types, majority of the variants carried missense mutations (60%, 26/43), followed by 8 frameshifts, 6 nonsense, 1 stop-loss, and 2 amplifications.

Conclusions: Our study confirmed the advantage of using targeted NGS with a cancer gene panel to efficiently identify mutations associated with GISTs. These findings may provide a molecular genetic basis for developing new drugs targeting these gene mutations for GIST therapy.

Keywords: Mutation; Next-generation sequencing; Gastrointestinal stromal tumors; *KIT* gene; Ion torrent sequencing

INTRODUCTION

Gastrointestinal stromal tumors (GISTs) are the most common stromal or mesenchymal subepithelial neoplasms originating in the gastrointestinal tract. Because of their relatively broad morphological distribution, GISTs were formerly called leiomyomas, leiomyosarcomas, and leiomyoblastomas of the gastrointestinal tract, until they were found to have clinical, histopathological, and molecular features that differentiated them from the other soft tissue tumors. GISTs have a characteristic morphology and are generally

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Author Contributions

Conceptualization: P.J., Y.H.M., K.J.G.; Data curation: P.J., Y.H.M.; Formal analysis: S.H.J., L.S.W., S.S.; Funding acquisition: Y.H.M., K.J.G.; Investigation: P.J., Y.H.M., K.J.G.; Methodology: P.J., S.H.J., S.S.; Writing - original draft: P.J., Y.H.M.; Writing - review & editing: P.J., K.J.G.

Conflict of Interest

No potential conflict of interest relevant to this article was reported.

positive for the CD117 antigen, an epitope of the KIT receptor tyrosine kinase (RTK). GISTs are primarily caused by oncogenic mutations in genes encoding either of 2 RTKs, *KIT* or platelet-derived growth factor receptor A (*PDGFRA*) [1]. *KIT* and *PDGFRA* are growth factor receptors that are activated by their respective ligands including stem cell factor and PDGF-AA to trigger cellular pathways that upregulate proliferation, downregulate apoptosis, and control cell differentiation, adhesion, and motility under normal conditions. Mutations in *KIT* and *PDGFRA* result in the constitutive activation of these cellular pathways, leading to spontaneous proliferation and uncontrolled tumor growth [2]. Different mutations, including point mutations, and deletions and insertions, have been found in the different exons or in different regions of a single exon in *KIT* (exons 9, 11, 13, and 17) and *PDGFRA* (exons 12, 14, and 18) genes in GISTs.

Identifying gene mutations in individual tumors is critical to improve the efficacy of cancer therapy by matching targeted drugs to specific mutations. Next-generation sequencing (NGS) technologies have revolutionized cancer genomics research by providing an unbiased and comprehensive method for detecting somatic cancer genome alterations. Targeted NGS with a gene panel is a powerful and practical approach as it enables the analysis of high-yield genes or genomic regions with relatively rapid turnaround time, low DNA input, and low cost [3-5].

Several targeted NGS panels are commercially available, although most of them are designed to cover the important alterations in various cancers. The Ion Torrent™ OncoPrint Focus Assay (OFA) is a targeted NGS assay that enables the analysis of over 1,000 biomarkers across 52 key solid tumor genes that are well characterized in published literature and associated with current oncology drugs [6,7]. This assay comprises 2 separate panels (DNA and RNA) that are designed to interrogate hotspot mutations (35 genes), copy number variations (19 genes), and gene fusions (23 genes). Together, these 2 panels can identify current actionable genetic variants and potential future targets for personalized therapy.

In this study, targeted NGS assay with an OFA panel was performed for the genetic characterization of molecular targets in 30 Korean patients with GIST. In addition, we assessed whether our molecular analysis could be considered as surrogate markers when compared to risk assessment criteria.

MATERIALS AND METHODS

Specimens

The study protocol was approved by the Institutional Review Board of The Catholic University of Korea, including written informed consent for clinical and molecular analyses (DC18SESI0113). A total of 30 formalin-fixed and paraffin-embedded (FFPE) specimens of GIST were included in the study. The specimens were obtained by surgical resection between April 2014 and August 2018 at the Daejeon St. Mary's Hospital, Republic of Korea. Specimens were selected based on the archival histopathological report and subsequent review by experienced pathologists. Tumor content was in the range of 50%–90%.

Risk assessment

Because the tumor site was located only in the stomach in all 30 patients with GIST, the National Institutes of Health (NIH) GIST Consensus Criteria was applied for GIST risk assessment. The criteria utilize 2 clinical pathological factors, tumor size and mitotic count,

and stratify recurrence risk as very low, low, intermediate, or high. Several reports of patients with localized GIST treated with surgery alone have confirmed the prognostic value of both tumor size and mitotic count [8,9].

DNA isolation and quantification

Genomic DNA was isolated from the FFPE samples using the RecoverAll Total Nucleic Acid Isolation Kit (ThermoFisher Scientific, Waltham, MA, USA) per the manufacturer's instructions after de-paraffinization and extraction of 1–2 mm thick paraffin sections in xylene. Amplifiable genomic DNA was quantitatively assessed using a Qubit 2.0 Fluorometer (ThermoFisher Scientific), a Qubit dsDNA High Sensitivity (HS) Assay Kit (ThermoFisher Scientific), and TaqMan RNase P Detection Reagent Kit (ThermoFisher Scientific) as appropriate.

Library preparation

DNA libraries were constructed using the Ion AmpliSeq Library Kit 2.0 (ThermoFisher Scientific) according to the manufacturer's recommendations. The OncoPrint Focus DNA Assay (ThermoFisher Scientific) was used to generate sequencing libraries using a total of 10 ng input genomic DNA per sample. This DNA panel is specifically optimized for detection of hotspots, single nucleotide variants (SNVs), insertion and deletions (Indels), and copy number variants (CNVs) across the following genes commonly implicated in human cancers and relevant to the targeted treatment of solid tumors: *AKT1*, *ALK*, *AR*, *BRAF*, *CDK4*, *CTNNB1*, *DDR2*, *EGFR*, *ERBB2*, *ERBB3*, *ERBB4*, *ESR1*, *FGFR2*, *FGFR3*, *GNA11*, *GNAQ*, *HRAS*, *IDH1*, *IDH2*, *JAK1*, *JAK2*, *JAK3*, *KIT*, *KRAS*, *MAP2K1*, *MAP2K2*, *MET*, *MTOR*, *NRAS*, *PDGFRA*, *PIK3CA*, *RAF1*, *RET*, *ROSL*, and *SMO* for hotspot mutations; *ALK*, *AR*, *BRAF*, *CCND1*, *CDK4*, *CDK6*, *EGFR*, *ERBB2*, *FGFR1*, *FGFR2*, *FGFR3*, *FGFR4*, *KIT*, *KRAS*, *MET*, *MYC*, *MYCN*, *PDGFRA*, and *PIK3CA* for focal CNV gains. The OncoPrint Focus RNA Assay is also available in the OncoPrint Focus Panel, however, gene fusions were not analyzed in the assay because of several actionable gene fusions such as *FGFR1-HOOK3*, *FGFR1-TACCL1*, and *ETV6-NTRK3* fusions for GIST were not included. Unique Ion Xpress Barcode 1–16 and Ion P1 Adapters (ThermoFisher Scientific) were ligated to the amplicons and subsequently purified to ensure that each individual sample had a unique ID. The final amplicon libraries were then amplified, purified, and equalized up to 100 pM using AMPure XP Reagent (Beckman Coulter, Indianapolis, IN, USA).

Semiconductor sequencing

Six uniquely barcoded library samples were pooled per run for sequencing on an Ion 318 v2 chip (ThermoFisher Scientific). The Ion Chef System (ThermoFisher Scientific) was used with the Ion PGM Hi-Q Chef Kit (ThermoFisher Scientific) for fully automated template preparation and Ion 318 v2 chip loading. Single-end sequence analysis was performed using the Ion PGM Hi-Q Sequencing Kit on the Ion Torrent Personal Genome Machine (Ion PGM) (ThermoFisher Scientific) for 200 base-read sequencing.

Variant calling and data analysis

Raw data from the DNA panel were collected and processed to generate sequence reads and trimmed using the Ion Torrent platform-specific pipeline software. Removal of polyclonal and poor signal-profile reads as well as 3' quality trimming of the reads was performed using Torrent Suite Assay Development Mode v5.0 (ThermoFisher Scientific). The reads were aligned to the reference genome (human genome hg19) and Ion Reporter v5.1 software package (ThermoFisher Scientific) was used for data analysis of DNA panel. ThermoFisher recommends 500x coverage to detect somatic mutations using their AmpliSeq technology; a 500x coverage cut off was applied to all analyses in this study. As a result, the target regions

with >500× demonstrated sufficient and uniform amplification and sequencing coverage, with mutant alleles detected at 5% allele frequency. Briefly, the 'OncoPrint™ Focus - 520 - w2.4 - DNA - Single Sample' automatic workflow in Ion Reporter was used to identify and annotate the SNVs, Indels, and CNVs from the OFA. This workflow has preconfigured Torrent Variant Caller (TVC) parameter settings: Min Allele Freq (frequency cutoff for supporting a variant), SNV 0.04, InDel 0.07, Hotspot 0.03; Min Coverage (total coverage required for reads or no-call), SNV 15, InDel 15, Hotspot 15; Strand Bias (proportion of variant alleles comes overwhelmingly from one strand) SNV 0.96, InDel 0.9, Hotspot 0.96 for SNVs and Indels calling, and 5% CI CNV ploidy \geq gain of 2 over normal was used for CNV calling.

Bioinformatic analysis and experimental validation

To distinguish between somatic and germline mutations, the detected mutations were compared to the variants in the Exome Aggregation Consortium (<http://exac.broadinstitute.org/>), 6,500 exomes of the National Heart, Lung and Blood Institute (NHLBI) GO Exome Sequencing Project (<http://evs.gs.washington.edu/EVS/>), and 1,000 Genomes Project [10]. To estimate recurrent mutations in GIST, ClinVar (<https://www.ncbi.nlm.nih.gov/clinvar/>) and the COSMIC (version 88) (<https://cancer.sanger.ac.uk/cosmic>) databases were used. Additionally, direct sequencing was performed to confirm some of the detected mutations (mutant allele burden >20%) other than hotspot mutations with no COSMIC ID.

Statistical analysis

Fisher's exact test was used to compare the mutation profiles and histopathological findings by NIH risk assessment. Multivariable logistic regression was used to calculate adjusted odds ratio for NIH risk assessment for these variables including mutation profiles. Survival and disease-free survival analyses were not performed because all patients were alive and disease-free during the study period. All statistical analyses were performed using MedCalc Statistical Software Version 17.6 (MedCalc software, Ostend, Belgium). Statistical significance was set at $P < 0.05$.

RESULTS

The cohort comprised of 50% (15/30) male and 50% (15/30) female Korean patients with a median age at 62 years (range, 41–79 years) with GIST. During the study period (median follow-up period 865 days) there was no recurrence or death from any cause. Four out of the 30 patients received adjuvant imatinib treatment. Immunohistochemistry (IHC) results for c-kit or discovered on GIST-1 (DOG1) were available for 28 out of the 30 GISTs: All 28 were positive for c-kit (28/28) and DOG1 (22/22), whereas there was no negative result for IHC of c-kit or DOG1. The clinicopathologic features of the 30 GIST patients are summarized in **Supplementary Table 1**. After applying stringent parameters for reliable variant calling (allele burden >4%, coverage depth >500×) and after filtering out potential raw base calling errors, 43 hotspot/other likely pathogenic variants (26 hot spots and 17 other variants; 33 SNVs, 8 Indels, and 2 amplifications) in 16 genes were identified in 26 out of the 30 GISTs. Other than hotspot mutations with >allele burden of 20% were confirmed by direct sequencing. Only one patient out of the remaining 4 GISTs without any alterations received the adjuvant imatinib treatment. *KIT* variants were most frequent (44%, 19/43), followed by 6 variants in *PIK3CA*, 3 in *PDGFRA*, 2 each in *JAK1* and *EGFR*, and one 1 in *AKT1*, *ALK*, *CCND1*, *CTNNB1*, *FGFR3*, *FGFR4*, *GNAI1*, *GNAQ*, *JAK3*, *MET*, and *SMO*. Based on the mutation types, most of the variants were missense mutations (60%, 26/43), followed by 8 frameshifts, 6 nonsense, and

1 stop-loss, and 2 amplifications (**Fig. 1**). Previous studies showed that functional SNVs may contribute to the development of GISTs [1,2,11,12]. **Fig. 2** illustrates the incidence of SNVs in combination with other mutations in this study. The results showed that 81% (21/26) of samples had at least 1 or more SNVs, 38% (10/26) had at least 2 or more SNVs, while 69% (18/26) of samples incurred no Indels. A detailed list of frameshift, stop-loss, nonsense, and missense mutations, and amplifications identified in this study is provided in **Tables 1 and 2**.

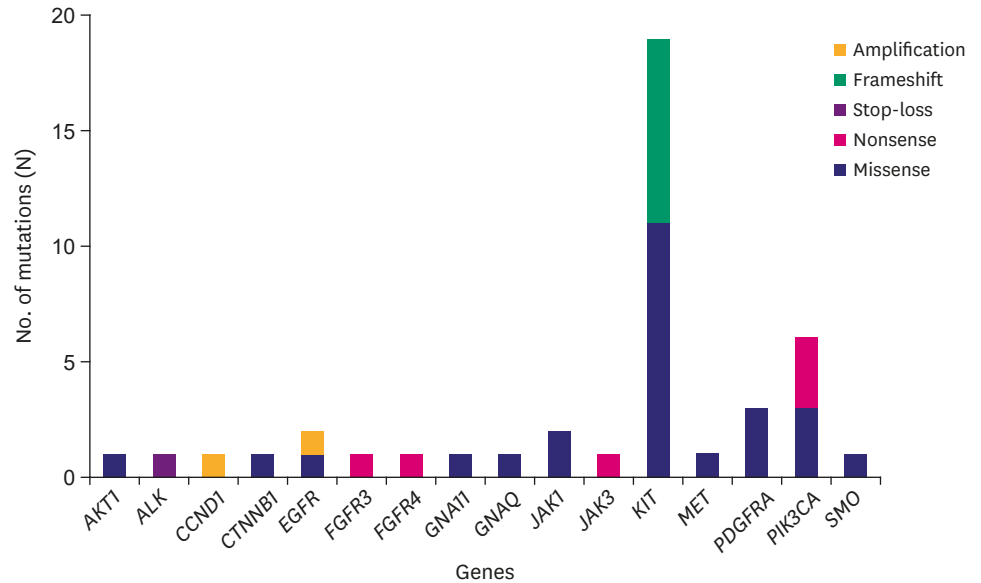


Fig. 1. Frequencies of somatic mutation types detected in the various genes by the OncoPrint Focus DNA Assay in 26 GISTs. Gene identities are depicted on the x-axis, and frequency of mutations on the y-axis. GIST = gastrointestinal stromal tumor.

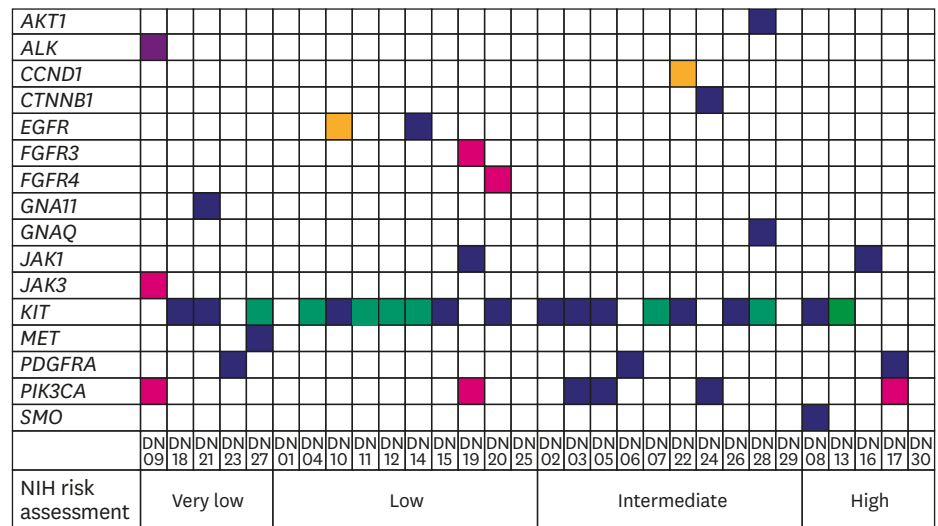


Fig. 2. Distribution of somatic mutation types detected for each patient by OncoPrint Focus DNA Assay in 26 GISTs. Individual patients are depicted on the x-axis, and identity of genes on the y-axis. Yellow box, amplification called; green box, frameshift mutation called; violet box, stop-loss mutation called; red box, nonsense mutation called; indigo box, missense mutation called; white box, no mutation called. GIST = gastrointestinal stromal tumor; PDGFRA = platelet-derived growth factor receptor A; NIH = National Institutes of Health.

Genetic Characterization in GIST

Table 1. Results of somatic mutations identified by OncoPrint Focus DNA Assay in 26 gastrointestinal stromal tumors

Patient	Genes	Transcript	Base change	Amino acid change	Allele burden (%)	Coverage (x)	Effect	COSMIC ID
DN02	<i>KIT 11</i>	NM_000222.2	c.1669T>G	p.Trp557Gly	29	1959	Missense	COSM1221
DN03	<i>KIT 11</i>	NM_000222.2	c.1676T>C	p.Val559Ala	43	1957	Missense	COSM1255
	<i>PIK3CA</i>	NM_006218.3	c.112C>T	p.Arg38Cys	33	761	Missense	COSM744
DN04	<i>KIT 11</i>	NM_000222.2	c.1724_1726del	p.Gln575del	39	1737	Frameshift	COSM96875
DN05	<i>KIT 11</i>	NM_000222.2	c.1676T>A	p.Val559Asp	42	1957	Missense	COSM1252
	<i>PIK3CA</i>	NM_006218.3	c.3140A>G	p.His1047Arg	45	1684	Missense	COSM775
DN06	<i>PDGFRA</i>	NM_006206.5	c.2525A>T	p.Asp842Val	40	1962	Missense	COSM736
DN07	<i>KIT 11</i>	NM_000222.2	c.1674_1679del	p.Lys558_Val560delinsAsn	44	1971	Frameshift	COSM27069
DN08	<i>KIT 11</i>	NM_000222.2	c.1676T>A	p.Val559Asp	43	1963	Missense	COSM1252
	<i>SMO</i>	NM_005631.4	c.869G>A	p.Arg290His	50	1997	Missense	COSM1699347
DN09	<i>ALK</i>	NM_004304.4	c.4861T>C	p.*1621Argext*41	5	502	Stop-loss	
	<i>JAK3</i>	NM_000215.3	c.1600A>T	p.Lys534*	5	1631	Nonsense	
	<i>PIK3CA</i>	NM_006218.3	c.1656G>A	p.Trp552*	5	535	Nonsense	
DN10	<i>KIT 11</i>	NM_000222.2	c.1727T>C	p.Leu576Pro	42	597	Missense	COSM1290
DN11	<i>KIT 11</i>	NM_000222.2	c.1813_1824dup	p.Glu605_Ala608dup	31	735	Frameshift	
DN12	<i>KIT 11</i>	NM_000222.2	c.1727_1729de	p.Leu576del	32	1226	Frameshift	COSM1289
DN13	<i>KIT 11</i>	NM_000222.2	c.1668_1676del	p.Gln556_Val559delinsHis	47	1448	Frameshift	COSM133641
DN14	<i>KIT 9</i>	NM_000222.2	c.1507_1508insTTGCCT	p.Ala502_Tyr503insPheAla	30	1488	Frameshift	COSM53306
	<i>EGFR</i>	NM_005228.4	c.886C>T	p.Pro296Ser	7	737	Missense	
DN15	<i>KIT 11</i>	NM_000222.2	c.1670G>C	p.Trp557Ser	48	1311	Missense	COSM1227
DN16	<i>JAK1</i>	NM_002227.3	c.2218C>T	p.Pro740Ser	8	535	Missense	
DN17	<i>PDGFRA</i>	NM_006206.5	c.2543A>C	p.Asn848Thr	39	1616	Missense	
	<i>PIK3CA</i>	NM_006218.3	c.1656G>A	p.Trp552*	5	666	Nonsense	
DN18	<i>KIT 11</i>	NM_000222.2	c.1676T>A	p.Val559Asp	24	608	Missense	COSM1252
DN19	<i>FGFR3</i>	NM_000142.4	c.343C>T	p.Gln115*	5	1273	Nonsense	
	<i>JAK1</i>	NM_002227.3	c.1972G>A	p.Val658Ile	8	669	Missense	COSM41757
	<i>PIK3CA</i>	NM_006218.3	c.1271G>A	p.Trp424*	6	573	Nonsense	
DN20	<i>KIT 11</i>	NM_000222.2	c.1676T>A	p.Val559Asp	31	545	Missense	COSM1252
	<i>FGFR4</i>	NM_213647.2	c.426C>A	p.Tyr142*	7	536	Nonsense	
DN21	<i>KIT 11</i>	NM_000222.2	c.1676T>A	p.Val559Asp	50	1488	Missense	COSM1252
	<i>GNAI1</i>	NM_002067.4	c.583G>C	p.Asp195His	11	817	Missense	
DN22	<i>KIT 17</i>	NM_000222.2	c.2464A>T	p.Asn822Tyr	35	619	Missense	COSM19109
DN23	<i>PDGFRA</i>	NM_006206.5	c.2525A>T	p.Asp842Val	23	841	Missense	COSM736
DN24	<i>CTNNB1</i>	NM_001904.3	c.122C>T	p.Thr41Ile	4	514	Missense	COSM5676
	<i>PIK3CA</i>	NM_006218.3	c.1633G>A	p.Glu545Lys	6	564	Missense	COSM763
DN26	<i>KIT 11</i>	NM_000222.2	c.1669T>G	p.Trp557Gly	28	624	Missense	COSM1221
DN27	<i>KIT 11</i>	NM_000222.2	c.1708_1728del	p.Tyr570_Leu576del	53	658	Frameshift	COSM1285
	<i>MET</i>	NM_001127500.2	c.3262G>A	p.Val1088Met	48	783	Missense	
DN28	<i>KIT 11</i>	NM_000222.2	c.1724_1726del	p.Gln575del	24	597	Frameshift	COSM96875
	<i>AKT1</i>	NM_001014431.1	c.152C>T	p.Pro51Leu	11	582	Missense	
	<i>GNAQ</i>	NM_002072.4	c.668T>C	p.Val223Ala	5	634	Missense	

Table 2. Results of copy number variations identified by OncoPrint Focus DNA Assay in 26 gastrointestinal stromal tumors

Patient	Genes	Length (Kb)	Variant class	CytoBand
DN10	<i>EGFR</i>	60.6	Amplification	7p11.2(55198956-55259538)x8.98
DN22	<i>CCND1</i>	10.1	Amplification	11q13.3(69456942-69467039)x11.43

***KIT* and *PDGFRA* mutations in GISTs**

Accumulating evidence indicates that activating mutations of *KIT* or *PDGFRA* are the initiating event in GISTs. Activation of *KIT* or *PDGFR* leads to downstream signaling in the PI3K, Ras, and JAK/STAT pathways, resulting in increased cell proliferation and inhibition of apoptosis [13,14]. Accordingly, 19 (63%) of the 30 GISTs sequenced in this study had *KIT* gene mutations; mutations in exons 9 (n=1), 11 (n=17), and 17 (n=1) were found located in regions corresponding to the transmembrane and cytoplasmic domain but not along the extracellular

Table 3. Correlation of NIH risk assessment with select histopathological features and mutation profiles

Variables	NIH risk assessment		P-value
	Very low and low	Intermediate and high	
Cellularity			0.215
Low	6	2	
Moderate and severe	9	13	
Cellular pleomorphism			0.215
Low	13	9	
Moderate and severe	2	6	
Necrosis			0.002
Negative	15	7	
Positive	0	8	
Any mutations identified by OFA			1.000
Negative	2	2	
Positive	13	13	
<i>KIT</i> or <i>PDGFRA</i> mutations			1.000
Negative	4	4	
Positive	11	11	

NIH = National Institutes of Health; OFA = OncoPrint Focus Assay; *PDGFRA* = platelet-derived growth factor receptor A.

domain. Mutations in exon 11 (89%, 17/19), which encodes the regulatory domain of the enzyme, were found to carry missense mutations in 10 patients (59%, 10/17) and frameshift mutations in 7 patients (41%, 7/17). Exon 9, which encodes the immunoglobulin-like C2-type 5 domain, was mutated only in one patient (5%, 1/19) with an insertion of c.1507_1508insTTGCCT resulting in p.Ala502_Tyr503insPheAla. Exon 17, which encodes the kinase activation loop, was also mutated only in one patient (5%, 1/19) with a substitution of c.2464A>T resulting in p.Asn822Tyr. These data support the critical role of *KIT* mutations in the development and progression of GISTs.

Our results also showed that 3 (10%) of the 30 GISTs sequenced in this study had *PDGFRA* gene mutations: exon 18 (n = 3) was found to be mutated in the cytoplasmic domain. Mutations in exon 18 (100%, 3/3), which encodes the kinase domain of the enzyme, included missense mutations (2 p.Asp842Val and 1 p.Asn848Thr) in the 3 patients (59%, 10/17).

Influence of mutation profiles on NIH risk assessment

To estimate the correlation of NIH risk assessment with selected histopathological findings and mutation profiles, the 4 criteria in the NIH risk assessment were categorized into 2 distinct groups: ‘very low & low’ and ‘intermediate & high’ (Fig. 2). Based on Fisher’s exact test, there was no statistical significance between NIH risk assessment and selected histopathological findings or mutation profiles except for necrosis. Patients in the ‘intermediate & high’ (53%, 8/15) group had an increased number of necrotic cells, compared to the patients in the ‘very low & low’ (0%, 0/15; odds ratio, 2.143; 95% confidence interval, 1.247–3.681; P=0.002) group. However, multivariable logistic regression analysis demonstrated that the ‘intermediate & high’ criteria for NIH risk assessment were not associated with necrosis or the histopathological findings and mutation profiles (Table 3).

DISCUSSION

GISTs are mesenchymal neoplasms that arise primarily from within the muscular wall of the stomach and small intestines; they rarely occur in extra-intestinal locations. The aggressiveness of the tumor is correlated with tumor size, mitotic activity, and anatomical

origin [15]. GISTs frequently harbor activating gene mutations in either *KIT* or *PDGFRA* genes and thus are highly responsive to several selective tyrosine kinase inhibitors [12,16]. Surgical resection is currently the only curative treatment for localized GIST and constitutes over half of potentially curative GIST treatments. However, the clinical course of GIST ranges from that of a tumor cured by surgical resection to that of a locally advanced or even widely metastatic, and ultimately fatal, disease. This clinicopathologic heterogeneity is paralleled by an underlying molecular diversity: the majority of GISTs are associated with spontaneous activating mutations in *KIT*, *PDGFRA*, or *BRAF* genes, while additional subsets are driven by genetic lesions (often inherited) in *NF1* or components of the succinate dehydrogenase enzymatic complex [17,18]. Routine genotyping has become an integral part of the management of GISTs undergoing tyrosine kinase inhibitor therapy [15].

In this study, we aimed to identify and evaluate genetic mutations in GIST for selective molecular targets using targeted NGS with the OFA panel. This approach identified 19 *KIT* and 3 *PDGFRA* mutations as missense or frameshift mutations in 22 different patients with GIST. Most *KIT* mutations (89%, 17/19) were in exon 11 of the *KIT* gene; only one mutation each was detected in exon 9 and 17 (Fig. 3). Gain-of-function mutations in *KIT* result in a growth advantage by constitutive, ligand-independent activation of the RTK [19]. While most GISTs are heterozygous for a given mutation, in around 15% of the tumors, the remaining wild-type *KIT* allele is lost, and this is associated with malignant behavior, increased mitotic activity, and topoisomerase II expression [20]. The most frequent hotspot mutation in *KIT* is detected in exon 11; other mutations are less frequently detected in exon 9 and rarely in exon 13 and exon 17 [21]. Importantly, the presence of *KIT* del-ins557/558 is an important prognostic factor for poor outcome in comparison with other *KIT* exon 11 mutations, *KIT*

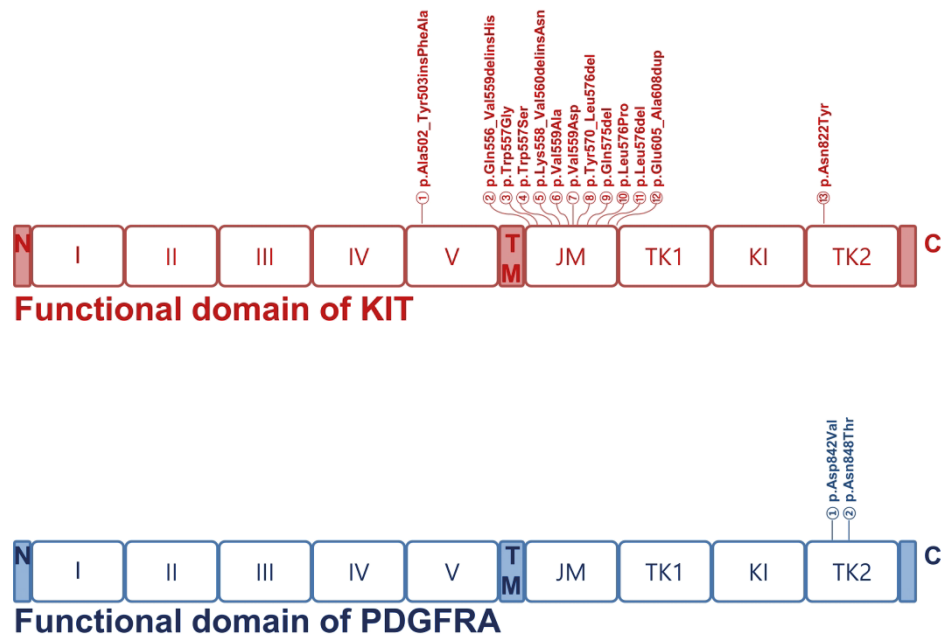


Fig. 3. Distribution of mutations in *KIT* and *PDGFRA* functional domains. Somatic mutations in *KIT* and *PDGFRA* identified in our study are shown. Boxes represent functional domains: I–V, 5 immunoglobulin-like domain; TM, transmembrane domain; JM, juxtamembrane domain; TK1, tyrosine kinase domain 1; KI, kinase insert domain; TK2, tyrosine kinase domain 2. The type of mutation detected within the domains are indicated above. GIST = gastrointestinal stromal tumor; *PDGFRA* = platelet-derived growth factor receptor A.

exon 9 and *PDGFRA* exon 18 mutations, even in tumors classified as non-high-risk ([very]low and intermediate) that originate from the stomach [22]. In this study, 2 *KIT* del-ins557/558 and 2 missense mutation at the position 557 were identified. Prognostic evaluations were difficult because the patients were alive during the study period.

PDGFRA and *KIT* mutations are mutually exclusive and activate similar downstream signal transduction pathways. However, *PDGFRA*-mutant GISTs are almost exclusively of gastric origin (90%–93%), which is prognostically favorable [12]. The most prevalent genotype is the p.D842V substitution involving the second kinase domain (which corresponds to exon 17 of *KIT*), which is detected in 60%–65% of all *PDGFRA* mutated tumors [23]. In this study, 2 patients (DN06 and DN23) carried an exon 18 D842V substitution of the *PDGFRA* only and were classified as very low and intermediate by NIH risk assessment, respectively. A previous study suggested that GISTs with *PDGFRA* exon 14 mutations represent a subset of clinically favorable gastric tumors (exclusively gastric location) with almost exclusively epithelioid morphology [24]. However, there was no exon 14 alteration of the *PDGFRA* in this study.

On the other hand, GISTs without activating mutations in *KIT* and/or *PDGFRA* genes tend to have 2 or more mutations (75%, 3/4), compared to GIST with *KIT* and/or *PDGFRA* mutations (45%, 10//22). Among them, co-occurrence with *PIK3CA* mutations was dominant: Detection of *PIK3CA* mutations in large or metastatic *KIT*-mutant GISTs may suggest that *PIK3CA*-mutant clones have a proliferative advantage during disease progression. Tyrosine kinase inhibitors have been successfully used in GIST treatment. However, resistance frequently develops due to secondary *KIT* mutations or activation of downstream signaling pathways, such as the PI3K/AKT/mTOR pathway. Genotyping of *PIK3CA* in GISTs may help to differentiate between primary and metastatic tumors with the potential to develop resistance to tyrosine kinase inhibitors and guide therapies with PI3K inhibitors [25].

Interestingly, 2 amplifications, one each in the *CCND1* and *EGFR* genes were identified in GISTs with *KIT* missense mutation. In previous studies, amplifications were reported for *CMYC* in 3 of 90 (3.3%), for *MDM2* in 5 of 94 (5.3%), for *EGFR1* in 5 of 94 (5.3%), and for *CCND1* in 7 of 79 (8.9%) evaluable cases. Among them, *MDM2* and *CCND1* amplifications were associated with clinical and histological malignancy [26]. On the other hand, there was no correlation between *EGFR* gene amplification or EGFR protein overexpression with GIST [27]. In our study, the *EGFR* amplification was detected in a low risk, whereas the *CCND1* amplification was detected in an intermediate by NIH risk assessment case.

Identifying single or combinations of mutations with the aim of delivering individualized treatment with a single or combination of target agents has been an effective strategy for cancer therapy [2]. Approximately 85%–90% of GISTs harboring *KIT* or *PDGFRA* mutations benefit from imatinib treatment before or after surgery and in the setting of unresectable/metastatic disease [28], except for tumors with some specific mutations such as *PDGFRA* exon 18 D842V [29]. The remaining 10%–15% of GISTs without *KIT* or *PDGFRA* mutations are classified as wild-type GIST. These tumors do not respond to imatinib. In this group, several mutations have been identified in genes including those encoding succinate dehydrogenase (SDH) complex subunits, neurofibromatosis type 1, *BRAF*, and other genes [30]. European Organisation for Research and Treatment of Cancer (EORTC) and Scandinavian Sarcoma Group (SSG) trials further suggested that adjuvant imatinib treatment should be carefully applied to high-risk patients and that the tumor genotype should also be taken into consideration. For example, in the advanced/metastatic setting, the *PDGFRA* exon 18 D842V

mutated GISTs do not benefit from imatinib [29] and a higher dose of imatinib (800 mg daily) is recommended by some institutions for *KIT* exon 9-mutated GISTs [31]. Evaluation of specific mutations can provide information for a specific tailored therapy. Further trials led to the approval of 2 more drugs by the FDA, sunitinib and regorafenib, which expands options for GIST treatment following failure of imatinib.

Unfortunately, there are 2 major limitations in our study. Due to the inherent underrepresentation of genes in the OFA, critical genes could not be investigated. The OFA does not cover the presence of significantly mutated driver genes that were previously identified (*TP53*, *ARID1A*, and *CDHI*) and some new ones (*MUC6*, *CTNNA2*, *GLI3*, *RNF43*, and others) associated with gastrointestinal tumor/carcinoma [32]. In fact, the OFA used in this study was too small gene panel for excavation of the complicated genetic alterations in GIST. Thus, a combination of the oncoPrint comprehensive panel and the Ion S5XL platform (ThermoFisher Scientific) would be better suited for application in routine clinical NGS test for solid tumors [33]. Second, it is possible that no calls of mutations in 4 GISTs with wild-type were due to low tumor purity. Both regular prospective and retrospective quality management processes and adequate designation to enrich tumor cellularity by pathologists in the molecular diagnostics laboratory can reduce the risk for a false negative result. The NGS platform with higher analytic sensitivity can detect mutations in specimens with lower tumor cellularity [34]. Finally, in our study, the relapse-free or disease-free survival analysis was not applicable due to the small sample size (n=30) and short median follow-up duration.

In conclusion, our study confirms the utility of the Ion Torrent sequencing platform with an OFA panel to efficiently identify *KIT* and *PDGFRA* mutations associated with GISTs, and other gene mutations associated with solid tumors. These findings may provide a genetic basis for developing new GIST therapeutic agents specifically targeting these gene mutations. As more experience and information are gained from the NGS, it is necessary to expand our understanding of the sensitivity of individualized therapies to specific mutations.

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SUPPLEMENTARY MATERIAL

Supplementary Table 1

Clinicopathologic findings in 30 Korean patients with gastrointestinal stromal tumors

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