Title

Duodenal-Jejunal Flexure GI Stromal Tumor Frequently Heralds Somatic NF1 and Notch Pathway Mutations

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abstract

Purpose GI stromal tumors (GISTs) are commonly associated with somatic mutations in *KIT* and *PDGFRA*. However, a subset arises from mutations in *NF1*, most commonly associated with neurofibromatosis type 1. We define the anatomic distribution of *NF1* alterations in GIST.

Methods We describe the demographic/clinicopathologic features of 177 patients from two institutions whose GISTs underwent next-generation sequencing of ≥ 315 cancer-related genes.

Results We initially identified six (9.7%) of 62 GISTs with *NF1* genomic alterations from the first cohort. Of these six patients, five (83.3%) had unifocal tumors at the duodenal-jejunal flexure (DJF). Two additional patients with DJF GISTs had non-*NF1* (*KIT* and *BRAF*) genomic alterations. After excluding one DJF GIST with an *NF1* single nucleotide polymorphism, four (57.1%) of seven sequenced DJF tumors demonstrated deleterious *NF1* alterations, whereas only one (1.8%) of 55 sequenced non-DJF GISTs had a deleterious *NF1* somatic mutation ($P < .001$). One patient with DJF GIST had a germline *NF1* variant that was associated with incomplete penetrance of clinical neurofibromatosis type 1 features along with a somatic *NF1* mutation. Of the five DJF GISTs with any *NF1* alteration, three (60%) had *KIT* mutations, and three (60%) had Notch pathway mutations (*NOTCH2*, *MAML2*, *CDC73*). We validated these findings in a second cohort of 115 GISTs, where two (40%) of five unifocal *NF1*-mutated GISTs arose at the DJF, and one of these also had a Notch pathway mutation (*EP300*).

Conclusion Broad genomic profiling of adult GISTs has revealed that *NF1* alterations are enriched in DJF GISTs. These tumors also may harbor concurrent activating *KIT* and/or inactivating Notch pathway mutations. In some cases, germline *NF1* genetic testing may be appropriate for patients with DJF GISTs.

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INTRODUCTION

GI stromal tumor (GIST) represents the most common type of sarcoma in the GI tract, with an annual incidence of 6.8 per million people in the United States.[1] GISTs arise from the interstitial cell of Cajal lineage within the enteric nervous system.[2] Approximately 70% to 80% of all sporadic GISTs have activating genomic alterations in *KIT*, whereas 5% to 10% have activating genomic alterations in *PDGFRA*.[3,4] Of the remaining GISTs, 10% to 15% have activation of the Ras pathway (*K/H/N-RAS*, *BRAF*, *NF1*); 2% arise from mutations or deficiencies in the succinate dehydrogenase (SDH) subunits (A, B, C, or D); and a subset occurs as a result of kinase fusions (*ETV6-NTRK3*, *FGFR1-TACC1*, or *FGFR1-HOOK3*) or additional mutations in genes, including *ARID1A* and *ARID1B*.[3-9]

Within sporadic and hereditary GISTs driven by Ras pathway alterations, a subset possesses *NF1* inactivating germline mutations that are associated with neurofibromatosis type 1 (NF-1 [ie, von Recklinghausen disease]).[10,11] NF-1 is an autosomal-dominant disorder with variable penetrance: Patients with NF-1 often present in childhood or adolescence[12] and have a 34-fold increased risk of developing GIST.[13] At the molecular level, the *NF1* gene encodes neurofibromin, which functions as a GTPase-activating protein that negatively regulates Ras family GTPases

(ie, KRAS, NRAS, HRAS) by increasing the catalytic conversion of the active form (RAS-GTP) to the inactive form (RAS-GDP).[14] Pathogenic *NF1* variants disrupt the normal function of neurofibromin and result in constitutive RAS activation.[15] This activation increases downstream signaling through BRAF/CRAF-mediated activation of the mitogen-activated protein kinase pathway and hence, facilitates tumor initiation and progression.[16] Until recently, *NF1* mutations in GIST were believed to be primarily of germline origin, associated with clinical NF-1, and combined with a second somatic mutation in the tumor. However, Belinsky et al[17] reported the first case of somatic inactivation of *NF1* in a patient with GIST without germline *NF1* mutation or clinical NF-1. Thus, our understanding of *NF1* in GIST continues to evolve.

Approximately 30% of all GISTs occur in the small intestine,[1] which measures approximately 5 to 6 m in length.[18] Most small bowel GISTs are associated with somatic *KIT* mutations, whereas a subset is associated with *NF1* mutations.[19] The small bowel is divided into three anatomically, histologically, and functionally distinct segments, namely the duodenum, jejunum, and ileum.[18] Although some studies distinguish duodenal GISTs from other small bowel GISTs, most combine them and do not characterize the biology of tumors on the basis of these distinct locations. Moreover, the exact transition from jejunum to ileum is somewhat arbitrary, but the duodenal-jejunal flexure (DJF), also known as the ligament of Treitz, represents a clear anatomic site that marks the transition from duodenum to jejunum. To date, no study of GISTs has specifically characterized tumors that arise from the DJF. We investigated the biology of GISTs at the DJF and report on an association with somatic and germline genomic alterations in *NF1*. Most published case series have focused primarily on GIST in patients with known clinical NF-1. The current study, however, started with presumably sporadic GISTs, which led to the unexpected finding that somatic *NF1* mutations in GISTs are more prevalent than previously suspected and that they are enriched in tumors that arise from the DJF.

METHODS

Primary Study Population

Patient demographic and tumor clinicopathologic data were retrospectively collected in an unbiased fashion from every patient with pathologically confirmed GIST seen within the University of California, San Diego (UCSD), health system from January 1, 2000, to April 30, 2017, under a UCSD institutional review board–approved protocol. Data collected were age, sex, race, ethnicity, primary GIST site, tumor size, TNM disease stage, and pathologic characteristics. Available operating notes or imaging reports were reviewed for these 165 patients to distinguish the primary site of origin of any small bowel GIST as duodenal, DJF, jejunal, or ileal.

Comprehensive Genomic Profiling

Of the 165 patients with GIST in the cohort, 62 underwent next-generation sequencing (NGS) of coding regions of cancer-related genes. NGS data from 61 of these patients were prospectively collected continuously as part of our institutional standard that began on May 1, 2014. One additional DJF GIST specimen from 2011 was retrospectively included in the NGS cohort (UCSD patient 1). Of note, 17 tumors in the NGS cohort, including two DJF GISTs (UCSD patients 3 and 5), were included in a prior study of pooled international data that aimed to identify novel deleterious genomic alterations in GISTs that lacked canonical driver mutations.[7] GIST tumor specimens were either submitted to Foundation Medicine (a Clinical Laboratory Improvement Act–certified and College of American Pathologists– and New York State–accredited laboratory) or sequenced internally by the UCSD Clinical Genomics Laboratory. DNA was extracted from formalin-fixed paraffin-embedded (FFPE) sections that contained a minimum of 20% tumor tissue and were used for comprehensive genomic profiling with hybridization-captured, adaptor ligation–based libraries. The FoundationOne assay is an NGS-based genomic test that sequences coding regions of cancer-related genes. The number of genes in the FoundationOne panel has evolved over time as new data on cancer-related genes have been published and currently includes 315 cancer-related genes plus select introns from 28 genes often rearranged or altered in solid tumor cancers. However, all versions of the assay simultaneously analyze the extracted DNA for base substitutions, short insertions and deletions, amplifications and homozygous deletions, and gene rearrangements with $\geq$ 99% sensitivity.[20] Specimens submitted to the UCSD Clinical Genomics Laboratory underwent NGS of the exons of 397 genes involved in pathways that control growth or differentiation and are known to be frequently mutated in solid tumors. The UCSD gene list includes all genes and select introns in the FoundationOne panel

in addition to 82 genes identified from the COSMIC (Catalog of Somatic Mutations in Cancer) database as being mutated in > 3% of breast, lung, colon, prostate, skin, or CNS tumors (Data Supplement). Coverage depth ranged from 283× to 830× across all sequenced tumor samples from both the FoundationOne and the UCSD assays.

Nontumor Tissue Sequencing

Patients found to have *NF1* mutations in their tumor tissue were also tested for germline *NF1* mutation. Four samples tested by using DNA extracted from FFPE sections of adjacent normal tissue or peripheral blood underwent NGS cancer-related mutation panel testing by the UCSD Clinical Genomics Laboratory as just described. One additional sample was tested by using commercially available targeted sequencing of the *NF1* gene from peripheral blood by ARUP Laboratories (Salt Lake City, UT).

Secondary Study Population

A confirmatory cohort of > 1,000 patients with pathologically confirmed GISTs from Memorial Sloan Kettering Cancer Center (MSKCC) with retrospectively collected patient demographic and tumor clinicopathologic data also were analyzed under an institutional review board–approved protocol. Data included age, sex, primary GIST site, tumor size, and pathologic characteristics. One hundred fifteen patients with GISTs had NGS results available in the MSK-IMPACT (Integrated Mutation Profiling of Actionable Cancer Targets) platform, which characterized frozen or FFPE tumor specimens for somatic DNA mutations, copy number alterations, and select rearrangements of 341 cancer-associated genes.[21]

Statistical Analysis

All statistical analyses were performed with Stata 9.0 software (StataCorp, College Station, TX). Comparisons between groups were performed by using the two-sample test of proportions. Statistical significance was accepted at the 5% level.

RESULTS

NF1 Genomic Alterations in GIST Occur in the Absence of Clinical NF-1

From the UCSD institutional cohort of 165 consecutive adult patients (> 18 years old) with pathologically confirmed GISTs, 62 underwent NGS for coding regions of at least 315 cancer-related genes. We identified six (9.7%) of these 62 GISTs with *NF1* genomic alterations. None of these patients had previously known clinical manifestations of NF-1 (eg, café au lait spots; axillary/inguinal freckling; dermal neurofibromas; Lisch nodules; iris hamartomas; nervous system tumors, including malignant peripheral nerve sheath tumors [MPNSTs], pancreatic neuroendocrine tumors, pheochromocytomas, or other sarcomas]).[10] Therefore, the application of expanded NGS panels to characterize the mutational profile of GISTs identified an unappreciated subset of patients with *NF1* genomic alterations in their tumors but no clinical evidence of NF-1.

NF1 Genomic Alterations in GIST Frequently Occur at the DJF

Of the six patients with GIST for whom we identified *NF1* genomic alterations, five (83.3%) had unifocal tumors at the DJF, and one (16.7%) had a unifocal tumor in the stomach (Fig 1A). In the remaining cohort of 103 patients without available NGS data, two additional patients had GISTs at the DJF. Finally, in the 56 patients with GIST and NGS data that indicated non-*NF1* mutations, two GISTs arose at the DJF. The demographic, clinicopathologic, and genomic characteristics of these nine patients with DJF tumors are listed in Table 1 (n = 7 NGS GISTs) and the Data Supplement (n = 2 non-NGS GISTs). Taken together, within the entire cohort of 165 patients, DJF GISTs (n = 9) represented an infrequent tumor site (5.5%). Given the infrequency of *NF1* genomic alterations in GIST, the DJF appears to be an over-represented focus of *NF1* mutant tumors.

NF1 Genomic Alterations in GIST Are Primarily Somatic but Can Herald Mild Neurofibromatosis

NF1 genomic alterations for each patient are listed in Table 2. Of the 62 patients with GISTs and NGS data, four (57.1%) of seven DJF GISTs had known deleterious *NF1* genomic alterations, but only one (1.8%) of 55 tumors outside the DJF had a deleterious *NF1* mutation ($P < .001$). Although none of the patients had known prior clinical evidence of NF-1, we next obtained nontumor DNA from blood or nontumor tissue to determine whether any of these patients had germline *NF1* mutations. UCSD patient 2 (Table 2) had two *NF1* variants noted on his tumor profile; one was classified as a mutation and the other as a variant of unknown significance, which had been previously established as a pathogenic low-penetrance germline *NF1* mutation seen in patients with mild NF-1 manifestations.[22] The patient underwent genetic counseling and confirmatory germline

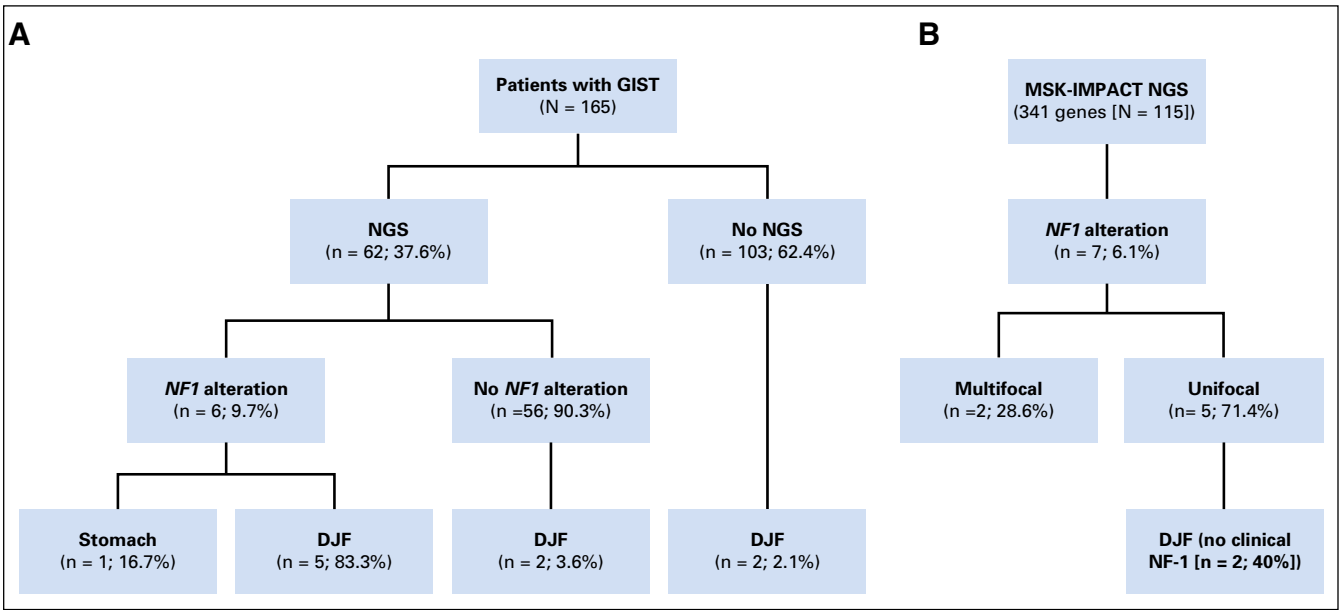

Fig 1. Schematic of patients with *NF1*-altered GI stromal tumors (GISTs). (A) From a total of 165 patients (University of California, San Diego [UCSD]) with pathologically confirmed GISTs, 62 had available next generation sequencing (NGS). Of these 62 patients, six had *NF1* genomic alterations. (Seventeen tumors in the UCSD NGS cohort, including two duodenal-jejunal flexure [DJF] GISTs, were previously included in an earlier study of pooled international data that aimed to identify novel deleterious genomic alterations in GISTs that lack canonical driver mutations.[7]) (B) From a validation cohort (Memorial Sloan Kettering Cancer Center) of 115 patients with pathologically confirmed GISTs and available NGS, seven had *NF1* genomic alterations. Five of these tumors were unifocal, and from two of these five, unifocal *NF1*-mutated GISTs arose from the DJF. MSK-IMPACT, Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets.

testing; his c.2970_2972delAAT (M992del) mutation was confirmed to be germline in origin. A follow-up dermatology examination revealed no evidence of neurofibromas, but several café au lait spots and mild axillary freckling were identified. This finding is consistent with the mild phenotype/low penetrance reported in other patients with this germline *NF1* mutation.[22]

NF1 Genomic Alterations in GISTs Are Associated With Other Cancer-Related Mutations

At the genomic level, the UCSD cohort NGS data generally parallel the known distribution of driver mutations in GISTs (Fig 2). Of the 65 driver mutations identified in the 62 GISTs, oncogenic *KIT* (63%), *PDGFRA* (11%), and SDH subunit (11%) mutations were most frequent, whereas *BRAF* and *KRAS* mutations were less common. Moreover, *NF1* mutations (six of 65) represented 9% of total genomic alterations. The loss of the *NF1* tumor suppressor gene often leads to additional chromosomal alterations, but second-hit mutations and/or loss of heterozygosity are necessary to promote tumorigenesis.[23] Therefore, we analyzed a cohort of five patients with *NF1* mutant DJF GISTs for additional cancer-related gene mutations. Within these GISTs, three (60%) also had oncogenic *KIT* mutations (exons 9 or 11). Moreover, three (60%) had inactivating Notch pathway mutations (*NOTCH2*, *MAML2*, *CDC73*). In comparison, only two (3.6%) of the 55 non-DJF GISTs had a Notch pathway alteration (*NOTCH2*; $P < .001$).

NF1 Genomic Alterations Are Associated With DJF GIST in a Validation Cohort

To ensure that the findings were not limited to a single institutional experience, we validated these observations with another cohort of 115 patients with GISTs with available NGS data from the MSKCC. Seven (6.1%) had identifiable *NF1* genomic alterations. These tumors were unifocal in five (71%). Two of the unifocal *NF1* mutant GISTs were located within the DJF (Fig 1B), of which neither patient had clinical NF-1. Demographic and clinicopathologic characteristics of these two patients are listed in Table 3, with the characteristics of the remaining five patients without DJF and/or multifocal GISTs listed in the Data Supplement. In addition to an *NF1* mutation, one patient had a concomitant mutation in *EP300*, which encodes a histone acetyltransferase/transcriptional coactivator that has been previously reported to interact with *MAML1* and *MAML2* to potentiate Notch signaling.[24] This supports our earlier observation that unifocal *NF1* mutant DJF GISTs also can have impaired Notch signaling. In summary, 40% of unifocal *NF1* mutant GISTs in the MSKCC cohort were localized at the DJF, which corroborates the high rate (83.3%) of similar tumors in the UCSD cohort. Taken together, these data demonstrate that *NF1* mutant GISTs frequently occur at the DJF in adults and that these tumors also harbor concurrent activating *KIT* and/or inactivating Notch pathway mutations (Fig 3).

Table 1. Characteristics of Patients With Duodenal-Jejunal Flexure GI Stromal Tumors

Characteristic	No.	%
Age, years		
< 39	2	22.2
40-49	1	11.1
50-59	3	33.3
> 60	3	33.3
Median (range)	55 (36-80)	
Mean ± SD	55.9 ± 15	
Sex		
Male	4	44.4
Female	5	55.6
Race		
White	7	77.8
African American	1	11.1
Asian or Pacific Islander	1	11.1
Ethnicity		
Non-Hispanic white	5	55.6
Hispanic/Latino	4	44.4
Stage		
Localized	6	66.7
Regional	0	0.0
Distant	1	11.1
Unknown	2	22.2
Mitotic index		
Low	4	44.4
High	3	33.3
Unknown	2	22.2
Cell morphology		
Spindle	5	55.6
Epithelioid	0	0.0
Mixed	3	33.3
Unknown	1	11.1
Genomic alterations		
Somatic *KIT*	4	44.4
Somatic *NF1*	4	44.4
Somatic *KIT* and *NF1* (concurrent)	3	33.3
Other somatic alterations	5	55.6
Germline *NF1*	2	22.2
Miettinen/AFIP risk assessment		
High	3	33.3
Low	3	33.3
No	1	11.1
Unknown	2	22.2
Joensuu/NIH risk assessment		
High	2	22.2
Intermediate	1	11.1
Low	3	33.3
Very low	1	11.1
Unknown	2	22.2

Abbreviations: AFIP, Armed Forces Institute of Pathology; NIH, National Institutes of Health; SD, standard deviation.

DISCUSSION

GISTs associated with *NF1* genomic alterations usually are believed to be multifocal and have been presumed to arise only in the setting of clinical NF-1.[25] Before the widespread use of NGS, clinical NF-1 was believed to occur in only 1.5% of GISTs (6% of duodenal GISTS; 4% of jejunal-ileal GISTs).[19] A recent Italian study reported *NF1* mutations in 13 (59%) of 22 quadruple-negative (ie, *KIT/PDGFRA/BRAF* mutation–negative/SDH-intact) GISTs,[11] but the frequency of *NF1* mutations in the entire Italian GIST population was not reported. Moreover, we recently analyzed NGS data from an international cohort of 186 patients with GISTs that included some patients from our institution (17 of whom have NGS data within the current study).[7] In that study, the frequency of *NF1* mutations was 9.7% (18 of 186). We now show in two single-institution cohorts of patients with GISTs that 6.1% (MSKCC, seven of 115) to 9.7% (UCSD, six of 62) have *NF1* alterations. If we exclude the 17 patients who overlap with our previous study (two of whom have *NF1* mutations [UCSD patients 3 and 5 in the current study]), we show that 8.9% (four of 45) of patients with GISTs have *NF1* alterations. Taken together, *NF1* mutation frequencies range from 6% to 10% in GIST and are higher than previously appreciated but similar to our recent analysis.[7] For the first time in our knowledge, we show that broad genomic profiling of DJF or ligament of Treitz GISTs in adults reveals frequent *NF1* alterations (somatic and/or germline) that occur even in the absence of clinical NF-1. This discovery suggests a unique mechanism of oncogenesis whereby both acquired and germline *NF1* gene alterations can lead to GIST development at this specific anatomic location. Moreover, it represents a previously unappreciated presentation of clinical NF-1 in adults who may have a low-penetrance germline mutation.

Table 2. Clinicopathologic Data of Seven Patients With Duodenal-Jejunal Flexure GI Stromal Tumors in the First Cohort With Next-Generation Sequencing

Pathologic Feature	Patient 1	2	3*	4	5*	6	7
Tumor resection	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Tumor size, cm	13	1.5	15	5.3	3	2.1	2.5
Mitotic index/ 5 mm^2	High	High	High	Low	Low	Low	Low
Cellular morphology	Mixed spindle/ epithelioid	Spindle	Unknown	Spindle	Spindle	Spindle	Mixed spindle/ epithelioid
Histologic grade	G2	G1	G2	G1	G1	G1	G1
Immunostain positivity							
KIT	Yes	Yes	Yes	Yes	Yes	Yes	Yes
DOG-1	Yes	Yes	Yes	Yes	Yes	Unknown	Yes
Genomic alterations							
Somatic *KIT* (AF)	S501_A502insCL (30%)	W557R (20%)	Y570_L576del (59%)	None	None	V559D (25%)	None
KIT exon	9	11	11	None	None	11	None
Somatic *NF1* (AF)	None	S1407fs*21 (17%)	G910fs*8 (91%)	T2631fs*13 (43%)	3974delG (10%) 3975-2A>G (8%)	None	None
Other somatic alterations (AF)	None	*NOTCH2* P6fs*27 (50%)	*MEN1* R98* (90%) *ASXL1* D855fs*11 (46%) *CDC73* R268* (35%) *ARID1A* loss exons 5-20	*MALM2* K768fs*23 (21%)	*BCOR* R1131L (51%)	None	*BRAF* V600E (25%)
Germline *NF1* (AF)	D176E (43%)†	M992del (45%)	None	None	None	None	None
Clinical NF-1							
Diagnostic criteria	No	No	No	No	No	No	No
Risk assessment							
Mietinnen/ AFIP	High	No risk	High	High	Low	Low	Low
Joensuu/NIH	High	Very low	High	Intermediate	Low	Low	Low
History of cancer							
Personal	No	No	No	No	No	No	No
Family	No	No	Gallbladder	Cervical	No	Ovarian	No

Abbreviations: AF, allelic frequency; AFIP, Armed Forces Institute of Pathology; NIH, National Institutes of Health.

**NF1* previously reported in an international pooled cohort of patients.[7]

†Single nucleotide polymorphism variant D176E is classified as a benign variant (Single Nucleotide Polymorphism database rs112306990).

The data support investigations of *NF1* testing (somatic and/or germline) in all patients with DJF GISTs, the rationale for which is threefold: characterization of GISTs with *NF1* genomic alterations may have implications for imatinib resistance in the absence of *KIT* mutations and may lead to potential alterations in personalized therapy; diagnosis of an otherwise clinically silent, heritable condition (ie, patients with NF-1 who present with isolated DJF GIST) provides earlier

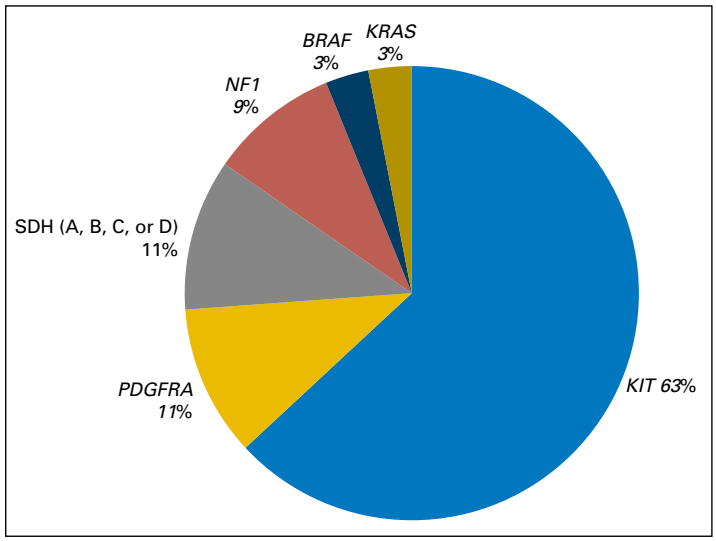

Fig 2. Driver mutations are representative of the genomic landscape of GI stromal tumors. Of the 62 patients with GI stromal tumors with available next generation sequencing, 65 known driver mutations were identified: 41 in *KIT*, seven in *PDGFRA*, six in *NF1*, seven in succinate dehydrogenase (SDH) subunits (A, B, C, or D), two in *BRAF*, and two in *KRAS*.

awareness of the risk of additional tumors (eg, dermal neurofibromas, nervous system tumors [including MPNSTs, pancreatic neuroendocrine tumors, pheochromocytomas, and other sarcomas])[10] and allows for earlier screening of these NF-1–associated tumors, although whether different malignancies and GISTs have unique presentation patterns remains to be determined; and diagnosis of NF-1 allows for genetic counseling and testing of potentially affected family members. Of note, individuals can have segmental mosaicism of NF-1, which occurs when an *NF1* somatic mutation occurs early in embryonic development such that only the tissues derived from the *NF1*-mutated cell carry the mutation while the remaining tissues are wild type.[26] As with UCSD patient 2, a clinical genetics work-up may be indicated for patients with *NF1* mutations on tumor profiling that could indicate a germline rather than a somatic origin.

Tumor development in the setting of *NF1* genomic alterations is associated with additional second-hit mutations.[23] We now have shown that DJF tumors with *NF1* genomic alteration also can harbor concurrent *KIT* mutations. In general, most *KIT* mutations portend imatinib sensitivity, whereas *NF1* mutations do not.[3,27] Consistent with this aforementioned drug sensitivity, tumors with a high risk of recurrence are recommended for adjuvant imatinib on the basis of the American College of Surgeons Oncology Group Z9001[28] and Scandinavian Sarcoma Group XVIII/AIO[29] trials where cumulative results demonstrated improved relapse-free survival (RFS) and overall survival with 36 months of adjuvant imatinib in patients with high-risk disease. In our cohort, three patients with DJF GISTs had a high risk of recurrence by validated risk assessment models.[30,31] UCSD patient 4 lacked a *KIT* mutation and was predicted to not respond to imatinib therapy; instead, this patient could experience toxicity. On the basis of the tumor mutation, this patient was not offered adjuvant imatinib. UCSD patient 1 had a *KIT* exon 9 mutation and was treated with adjuvant imatinib 800 mg daily for 5 years. This approach was supported by EORTC-62005 and Southwest Oncology Group S0033/Cancer and Leukemia Group B 150105 phase III trials that collectively demonstrated improved response rates and RFS in patients with advanced GISTs and *KIT* exon 9 mutations treated with high-dose imatinib (800 mg daily) compared with standard-dose imatinib (400 mg daily).[32] The patient also had a germline *NF1* variant and ultimately developed a local recurrence after 5 years of adjuvant imatinib therapy. UCSD patient 3 had a known *KIT* exon 11 mutation and was treated with dose-escalated imatinib followed by the multikinase inhibitor sunitinib for metastatic disease. NGS tumor profiling revealed the presence of both a somatic *NF1* mutation and an *ARID1A* loss. A brief trial of the mammalian target of rapamycin inhibitor everolimus in combination with imatinib was attempted to block parallel signaling pathways, but the patient rapidly succumbed to the disease. Much like patients with metastatic colorectal cancer who are tested for pan-*RAS* mutations before treatment targeting upstream cell surface receptors (ie, epidermal growth factor receptor),[33,34] we propose that patients with DJF GISTs be tested for *NF1* (which would activate RAS) and *BRAF* V600E genomic alterations (which two of the patients with DJF GISTs also harbored) before being offered imatinib therapy, which targets upstream KIT.

We have recently shown that *NOTCH1* can be mutated in both wild-type and non–wild-type GISTs.[7] We now add evidence that implicates the Notch signaling pathway in GISTs. The Notch receptors (*NOTCH1-4*) bind to canonical ligands on adjacent cells (eg, Delta-like and Jagged). In turn, Notch undergoes sequential proteolytic cleavage, which releases the Notch intracellular domain (NICD). The NICD then translocates to the nucleus and leads to activation of a transcriptional complex (including mastermind-like proteins 1 and 2 [*MAML1* and *MAML2*] and E1A binding protein p300 [*EP300*]) that regulates expression of target genes, such as *HES1*.[35] Herein, we found inactivating mutations in both *NOTCH2* and *MAML2* that co-occur with *NF1*

Table 3. Clinicopathologic Data of Two Duodenal-Jejunal Flexure GI Stromal Tumors in the Second Cohort

	Patient	
Clinicopathologic Feature	**10**	**11**
Tumor size, cm	1	8
Mitotic index/5 mm^2	Unknown	High
Cellular morphology	Spindle	Spindle
Immunostain positivity		
KIT	Yes	Yes
DOG-1	Yes	Yes
Genomic alterations		
Somatic *KIT* (AF)	None	None
KIT exon	None	None
Somatic *NF1* (AF)	M2031fs (35%)	Q83fs (34%), F1275fs (38%)
Other somatic alterations	None	*BRAF* V600E (24%) *ERBB4* 2q34 intragenic deletion *EP300* T151A (69%) *RB1* 2212-2_2212-1delAG (52%) *TSC2* L658del (31%)
Germline *NF1* (AF)	Unknown	Unknown
Clinical NF-1		
Diagnostic criteria	No	No
Risk assessment		
Mietinnen/AFIP	Unknown	High
Joensuu/NIH	Unknown	High
History of cancer		
Personal	Ovarian	No

Abbreviations: AF, allelic frequency; AFIP, Armed Forces Institute of Pathology; NIH, National Institutes of Health.

mutations. We identified another tumor with a mutation in *CDC73*, which encodes the RNA polymerase II–associated factor complex protein parafibromin and was recently reported to potentiate Notch signaling by binding to the NICD.[36] We also found an *EP300* mutation in the validation cohort that could abrogate signaling of the Notch transcriptional complex. Of note, Notch signaling was reported to have a tumor suppressor function in GIST by downregulating *KIT* mRNA expression.[37] This prior study also demonstrated that patients with GISTs with low *HES1* expression had shorter RFS times than those with high *HES1* expression. Whether loss-of-function mutations in Notch pathway genes that lead to decreased *HES1* expression contribute to GIST tumorigenesis remains to be determined. Moreover, data in neural stem-cell differentiation suggest that the Notch pathway intersects with NF1 signaling. NF1 regulates MEK/Smad3/Jagged1/Hes1–dependent glial and neuronal differentiation.[38] Notch has also been reported to mediate transformation of benign plexiform neurofibromas into MPNSTs in the setting of NF-1,[39] which suggests an alternate oncogenic role of Notch in NF-1. More studies are required to elucidate the biologic significance of Notch pathway mutations in GIST, including NF-1–associated GIST.

Finally, the mechanism by which *NF1* mutations lead to GISTs that specifically arise at the DJF remains unclear. Expression of the *NF1* gene product is ubiquitous throughout all tissue types,[40] but an analysis of open reading frames adjacent to the *NF1* locus on chromosome 17q reveals that the *SLC6A4* gene that encodes a high-affinity, sodium-dependent serotonin (5-hydroxytryptamine-3) transporter may have some tissue specificity for the small intestine, particularly the dudodenum.[41] In addition, interstitial cells of Cajal, which are the precursor cells to GIST, express 5-hydroxytryptamine-3 receptors, and their activation enhances gut

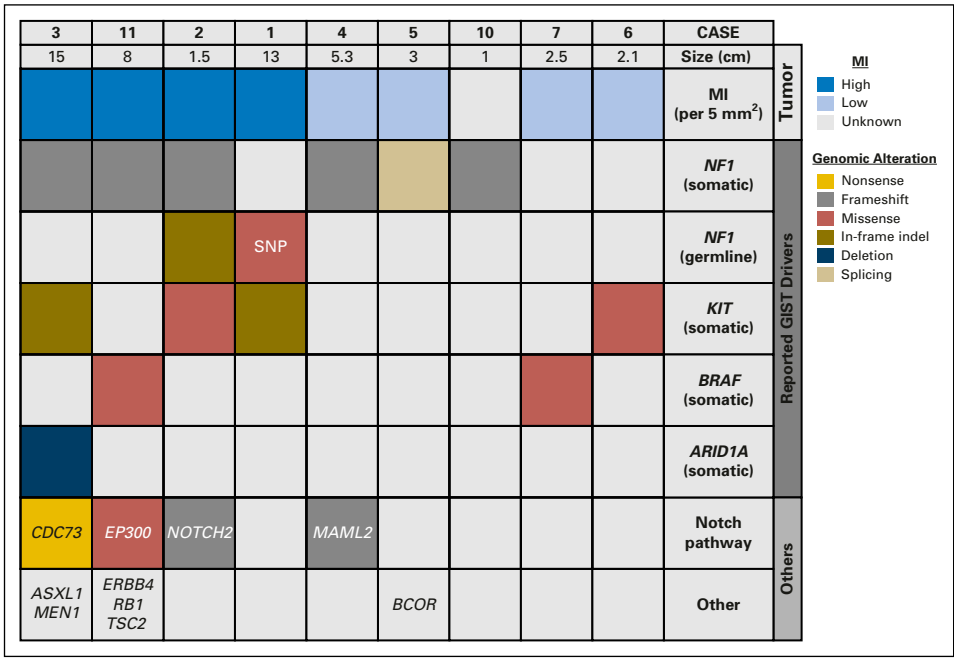

Fig 3. Summary of duodenal-jejunal flexure genomic alterations. Tumor size, mitotic index (MI), and genomic alterations for patients with duodenal-jejunal flexure GI stromal tumors (GISTs) from both cohorts are presented and organized by known driver genes and Notch pathway alterations. Somatic and germline mutations are annotated by color for mutation type. SNP, single nucleotide polymorphism.

pacemaker activity.[42] One could postulate that this region of chromatin on 17q is open and transcriptionally active at the DJF. Therefore, *NF1* may be more susceptible to mutations at this site. However, the exact mechanism behind the specific association of *NF1* genomic alterations with DJF GIST will require additional investigation.

In conclusion, this work provides new insights into the pathobiology of *NF1* mutant GISTs. We continue to stratify patients with GISTs into distinct groups on the basis of their tumor genomics and now provide evidence that anatomic location at the DJF may have genetic and clinical implications. The data support *NF1* genetic testing in all patients with DJF GISTs. Taken together, this study demonstrates how implementation of genomically driven precision oncology care can guide personalized treatments and counseling for patients with GISTs as well as for their family members.

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