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Gastrointestinal Stromal Tumors associated with Neurofibromatosis Type I
– A Report of Two Cases –

Joo Heon Kim · In Ock Seong Seong-Kyu Lee[1] · Haing-Woon Baik[1] Seong Ho Kim · Dong Wook Kang Kyung Hee Kim · Mee Ja Park Yong Il Kim

Department of Pathology and [1]Biochemistry & Molecular Biology, Eulji University College of Medicine, Daejeon, Korea

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Corresponding Author
Joo Heon Kim, M.D.
Department of Pathology, Eulji University College of Medicine, 143-5 Yongdu-2dong, Jung-gu, Daejeon 301-070, Korea
Tel: 042-259-1657
Fax: 042-611-3459
E-mail: kjh2000@eulji.ac.kr

Gastrointestinal stromal tumor (GIST) is the most common non-epithelial neoplasm arising in the gastrointestinal tract, but this tumor is rarely seen in association with type 1 neurofibromatosis (NF-1). We report here on two cases of multiple GISTs of the small intestine that occurred in NF-1 patients. We also analyzed the mutations of *c-kit* exons 9, 11, 13 and 17 and the platelet-derived growth factor receptor-alpha (*PDGFRA*) exons 12 and 18 in two GIST patients. Histologically, the NF-1-associated GISTs were similar to those of non-the NF-1 GISTs, but they characteristically revealed hyperplastic interstitial cells of Cajal around the GISTs. Immunohistochemically, these tumors showed strong co-expressions of CD117 and CD34. The molecular genetic analysis of the GISTs showed that all of the *c-kit* and *PDGFRA* exons that were analyzed in the GISTs of the two patients were the wild-type, suggesting a limited role for the *c-kit* and *PDGFRA* mutations in the tumorigenesis of NF-1-associated GISTs.

Key Words : Gastrointestinal stromal tumor; Neurofibromatosis I; *c-kit*; *PDGFRA*

GISTs constitute the majority of the primary mesenchymal tumors of the gastrointestinal tract. Previous reports have pointed out that multiple GISTs arise preferentially in the small intestine and their multiplicity is frequently associated with NF-1 (von Recklinghausen's disease).[1-3] It has been reported that the clinical behavior of GISTs was associated with tumor size, a high rate of mitosis, *c-kit* mutations, and DNA ploidy.[4,5] We report here on two rare cases of GISTs that had PAS positive amorphous materials, hyperplastic lesions of the intestinal neural tissue and supporting structures from the myenteric plexus in the small intestine; these GISTs had the phenotypic clinical characteristics of NF-1. We also performed additional sequencing analysis for exons 9, 11, 13 and 17 of the *c-kit* gene and exons 12 and 18 of the *PDGFRA* gene.

CASE REPORT

Case 1

A 71-year old woman was admitted to our hospital with abdominal pain and anemia. She had no family history of NF-1. The physical examination revealed numerous cafe-au-lait patches and multiple cutaneous nodules on her upper extremities and trunk. We performed segmental resection of the small intestine. Several intramural masses of the small intestine were grayish tan, solid and well defined; they measured up to 2.2×1.6 cm in size for the largest mass. The remaining subserosal protruding nodules ranged from 0.3 to 1.4 cm in diameters.

Case 2

A 68-year old woman with no family history of NF-1 was

admitted with UGI bleeding and anemia. This patient had numerous cafe-au-lait patches and multiple cutaneous nodules on her upper extremities, trunk, abdomen and back. Gross examination of the surgical specimen of the small intestine showed a ulcerofungating mass that was grayish tan, solid and it measured 5.5×4.7 cm in size. The mass showed focal hemorrhage and necrosis, and it extended into the subserosal layer on the cut surface. Multiple intramural or subserosal protruding nodules were also shown. The nodules ranged from 0.6 to 1.2 cm in diameters.

Table 1. Summary of clinicopathologic data of NF-1-associated GISTs

	Case I		Case II	
	Small nodules	large tumor	Small nodules	large tumor
Total No. of GISTs	12	1	34	1
Tumor size (cm)	0.3-1.4	2.2×1.6	0.6-1.2	5.5×4.7
Histologic grading	Very low-risk	Low-risk	Very low-risk	Intermediate-risk
Cell shape	spindles	spindle	spindle	spindle
Mitoses/10 HPF	<1	<5	<1	<5
Necrosis/Hemorrhage	-	-	-	+
CD117/CD34 / Desmin / S100 protein	+/+/-/-	+/+/-/-	+/+/-/-	+/+/-/-
NF-1-associated lesion	Hyperplastic lesion		Hyperplastic lesion	
c-kit & PDGFRA gene mutation	No	No	No	No

The clinicopathologic features of NF-1-associated GISTs are summarized in Table 1. Characteristically, sequential morphologic features of the hyperplastic interstitial lesions with Cajal cells to the well defined tumorous mass of the GISTs were observed (Fig. 1). The histologic features of the NF-1-associated GISTs were almost the same as those of the non-NF-1 GISTs. The tumors were composed of interlacing fascicles of the uniform spindle cells with elongated cytoplasm. The hyperplastic intestinal neural tissue and its supporting structures were noted in the regions of the myenteric plexuses around some of the GISTs. The tumor cells lacked pleomorphism, and mitotic figures were

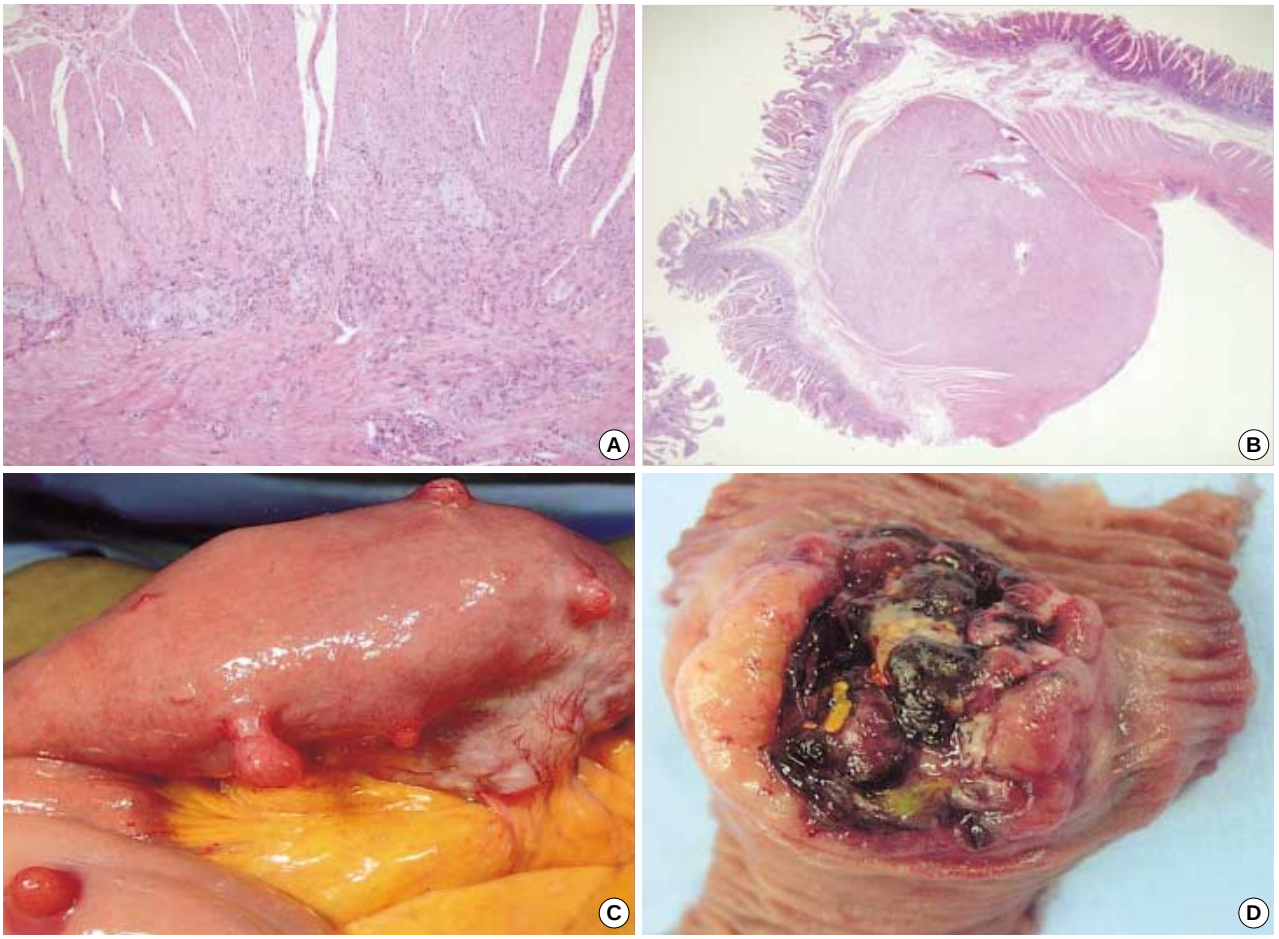

Fig. 1. A sequential morphologic feature from hyperplastic interstitial lesions of Cajal cells to the well defined tumorous mass of GISTs is noted; proliferating aggregates of hyperplastic interstitial cells of Cajal (A), an intramural nodule (B), subserosal protruding nodules (C), and an ulcerofungating tumorous mass (D).

counted in <5 per 50 HPF in the largest masses of both cases. All of the other small sized tumors were relatively benign with hypocellularity, inconspicuous mitotic activity and bland nuclear features. The results of the immunohistochemical staining revealed that the tumor cells were diffusely positive for CD117 (polyclonal rabbit, Dako, Japan), CD34 (QBEnd-10, Novocastra, USA), and vimentin (V9, Novocastra, USA); they were negative for smooth muscle actin (α sm-1, Novocastra, USA), S100 protein (polyclonal rabbit, Novocastra, USA), and desmin (DE-R-11, Novocastra, USA) (Fig. 2A-C). There were prominent amor-

Table 2. KIT and PDGFRA primer sequences with PCR annealing conditions

Primer	Sequence	Product size (bp)	T_A (°C)
KIT exon 9-F	5′-ATTTATTTTCCTAGAGTAAGCCAGGG-3′	305	55
KIT exon 9-R	5′-ATCATGACTGATATGGTAGACAGAGC-3′		
KIT exon 11-F	5′-CCAGAGTGCTCTAATGACTG-3′	191	58
KIT exon 11-R	5′-ACTCAGCCTGTTTCTGGGAAACTC-3′		
KIT exon 13-F	5′-GCTTGACATCAGTTTGCCAG-3′	193	55
KIT exon 13-R	5′-AAAGGCAGCTTGGACACGGCTTTA-3′		
KIT exon 17-F	5′-TGTGAACATCATTCAAGGCGTAC-3′	331	56
KIT exon 17-R	5′-CAGGACTGTCAAGCAGAGAATGG-3′		
PDGFRA exon 12-F	5′-TCCAGTCACTGTGCTGCTTC-3′	260	58
PDGFRA exon 12-R	5′-GCAAGGGAAAAGGGAGTCTT-3′		
PDGFRA exon 18-F	5′-ACCATGGATCAGCCAGTCTT-3′	260	54
PDGFRA exon 18-R	5′-TGAAGGAGGATGAGCCTGACC-3′		

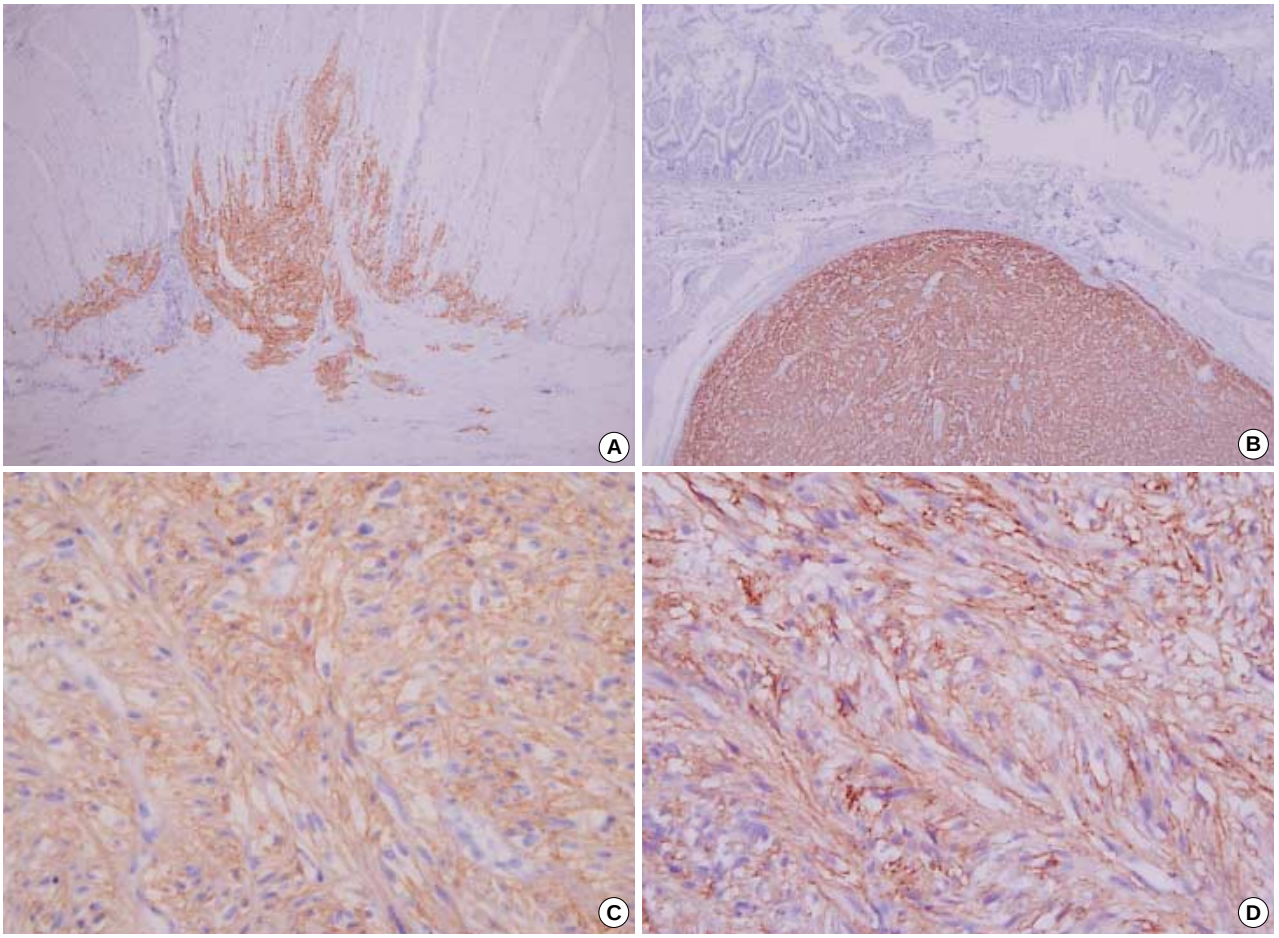

Fig. 2. Immunostaining for CD117 shows strong positivity in the hyperplastic interstitial lesion of Cajal cells and the well defined GIST (A-C). CD34 is expressed in the cell membrane and cytoplasm of the tumor cells in the largest GIST (D).

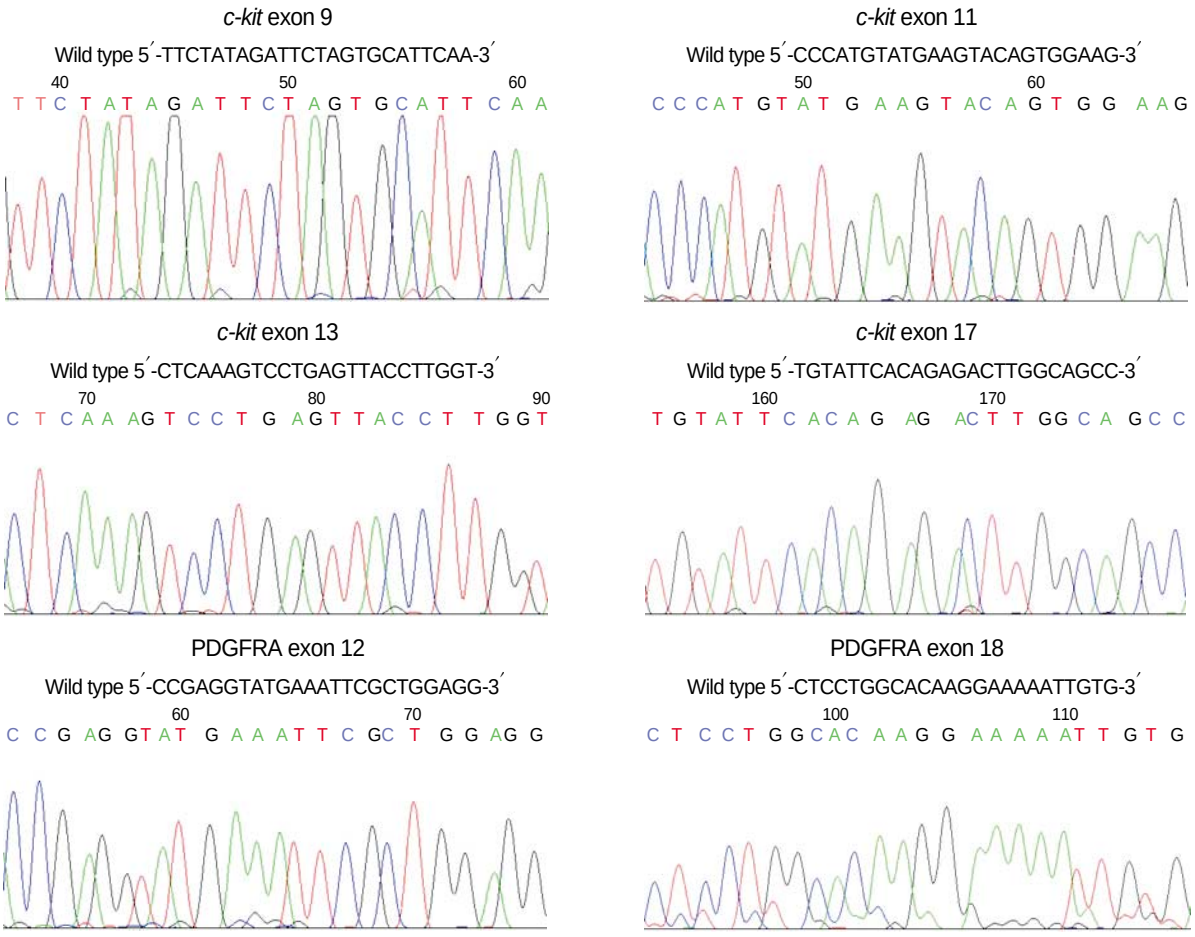

Fig. 3. Sequence analysis of exons 9, 11, 13, and 17 of the *c-kit* gene and exons 12, 18 of the *PDGFRA* gene reveals wild-type.

phous and eosinophilic materials on the hematoxylin-eosin stain and positive staining with periodic acid Schiff stain was noted in the tumorous stroma (Fig. 2D).

We performed molecular analysis on the small and large GISTs of two NF-1 patients. Exons 9, 11, 13 and 17 of the *c-kit* gene and exons 12 and 18 of the *PDGFRA* gene were amplified by polymerase chain reaction (PCR), and then they were directly sequenced. The primers used for the PCR reactions are shown in Table 2.[6] All of the exons analyzed in the GISTs of the two NF-1 patients were the wild-type (Fig. 3).

DISCUSSION

GISTs are the most frequent mesenchymal tumors of the gastrointestinal tract. Most of them are thought to be sporadic, but some arise in the settings of NF-1. NF-1-associated GISTs occur in older adults and nearly all of them are multiple tumors in the small intestine, while sporadic GISTs are most frequently found in the stomach; this is followed by the small intestine, colorectum and esophagus.[7]

We have described multiple small intestinal GISTs with NF-1 in two patients. Our two cases of GISTs grossly showed multiple variable sized intramural or subserosal nodules and large ulcerofungating tumorous masses. Also, the microscopic findings revealed several irregular proliferating aggregates of hyperplastic interstitial cells of Cajal around some of the GISTs, which expressed CD117 and they were imperceptibly merged with the smooth muscle fibers of the muscularis propria. These sequential morphologic features range from the hyperplastic interstitial lesions of Cajal to the well defined GISTs; this suggests that GISTs are derived from interstitial cells of Cajal (ICCs) or they differentiate toward ICCs.[8]

Most GISTs have mutations in the proto-oncogene *c-kit*[9-11] or the related tyrosine kinase platelet-derived growth factor receptor-alpha (*PDGFRA*),[12] this results in ligand-independent activation of the these genes. *c-kit* gene mutations have been detected in up to 70-90% of GISTs, whereas *PDGFRA* gene mutations

are observed in about 5%.[13] However, in GISTs associated with NF-1, *c-kit* gene mutations are detected in approximately 8%, and *PDGFRA* gene mutations in approximately 6%, suggesting that they play only a limited role in the tumorigenesis of GISTs in NF-1 patients.[8] In the present study, we performed molecular analysis for *c-kit* exons 9, 11, 13 and 17 and for *PDGFRA* exons 12 and 18. All of the two cases did not exhibit any of the *c-kit* and *PDGFRA* mutations that are known to occur in sporadic GISTs. The reports that have been assessed the molecular abnormalities of multiple GISTs arising in NF-1 are as of yet limited, and so additional studies are needed to analyze the pathophysiologic mechanism of GISTs arising in NF-1 patients.

In summary, we report here on two unusual cases of multiple GISTs associated with NF-1. These cases showed the sequential histologic features ranging from hyperplastic interstitial foci of Cajal to the well defined GISTs; this suggests that GISTs are derived from the interstitial cells of Cajal. The molecular genetic results suggest a limited role for the *c-kit* and *PDGFRA* mutations in the tumorigenesis of NF-1-associated GISTs.

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