

## Case Report

# Long segmental hyperplasia of interstitial cells of Cajal with giant diverticulum formation

Liyan Xue<sup>1</sup>, Tian Qiu<sup>1</sup>, Ying Song<sup>2</sup>, Ling Shan<sup>1</sup>, Xiuyun Liu<sup>1</sup>, Lei Guo<sup>1</sup>, Jianming Ying<sup>1</sup>, Shuangmei Zou<sup>1</sup>, Susheng Shi<sup>1</sup>, Alexandros D Polydorides<sup>3</sup>, Xinming Zhao<sup>2</sup>, Ning Lu<sup>1</sup>, Dongmei Lin<sup>1</sup>

Departments of <sup>1</sup>Pathology, <sup>2</sup>Imaging Diagnosis, Cancer Institute (Hospital), Peking Union Medical College, Chinese Academy of Medical Sciences, Beijing, China; <sup>3</sup>Department of Pathology, Icahn School of Medicine at Mount Sinai, New York, NY, USA

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**Abstract:** Sporadic gastrointestinal stromal tumors (GISTs) usually form a well-circumscribed mass. In contrast, diffuse interstitial cell of Cajal (ICC) hyperplasia along the Auerbach plexus without a discrete mass may occur in patients with germline mutations in the *NF1*, *c-KIT* or *PDGFRA* genes. However, sporadic, diffuse ICC hyperplasia without *c-KIT* or *PDGFRA* mutations has not been reported. We describe herein one such case, forming a giant diverticulum. A 63-year-old woman with no features of Neurofibromatosis 1 (NF1) presented with increasing abdominal pain for more than 30 years. A large, diverticulum-like mass in the ileum was resected. Microscopically, a diffuse proliferation of bland spindle cells was seen extending for 12 cm, replacing the muscularis propria and lined by intact mucosa. The spindle cells were CD117+/CD34+/DOG1+/SMA+/Desmin-/S100-. Mutation analyses did not reveal any mutations in *c-KIT* or *PDGFRA*. The lesion had two silent mutations in the *NF1* gene. It is rare of the diffuse form of sporadic ICC hyperplasia showing diffuse longitudinal microscopic growth completely replacing the muscularis propria, mimicking diffuse ICC hyperplasia in hereditary GIST syndromes, but without solid components and no *c-KIT* or *PDGFRA* gene mutations. This peculiar form of sporadic ICC hyperplasia may be related to intestinal dysmotility in this ileal segment and giant diverticulum formation.

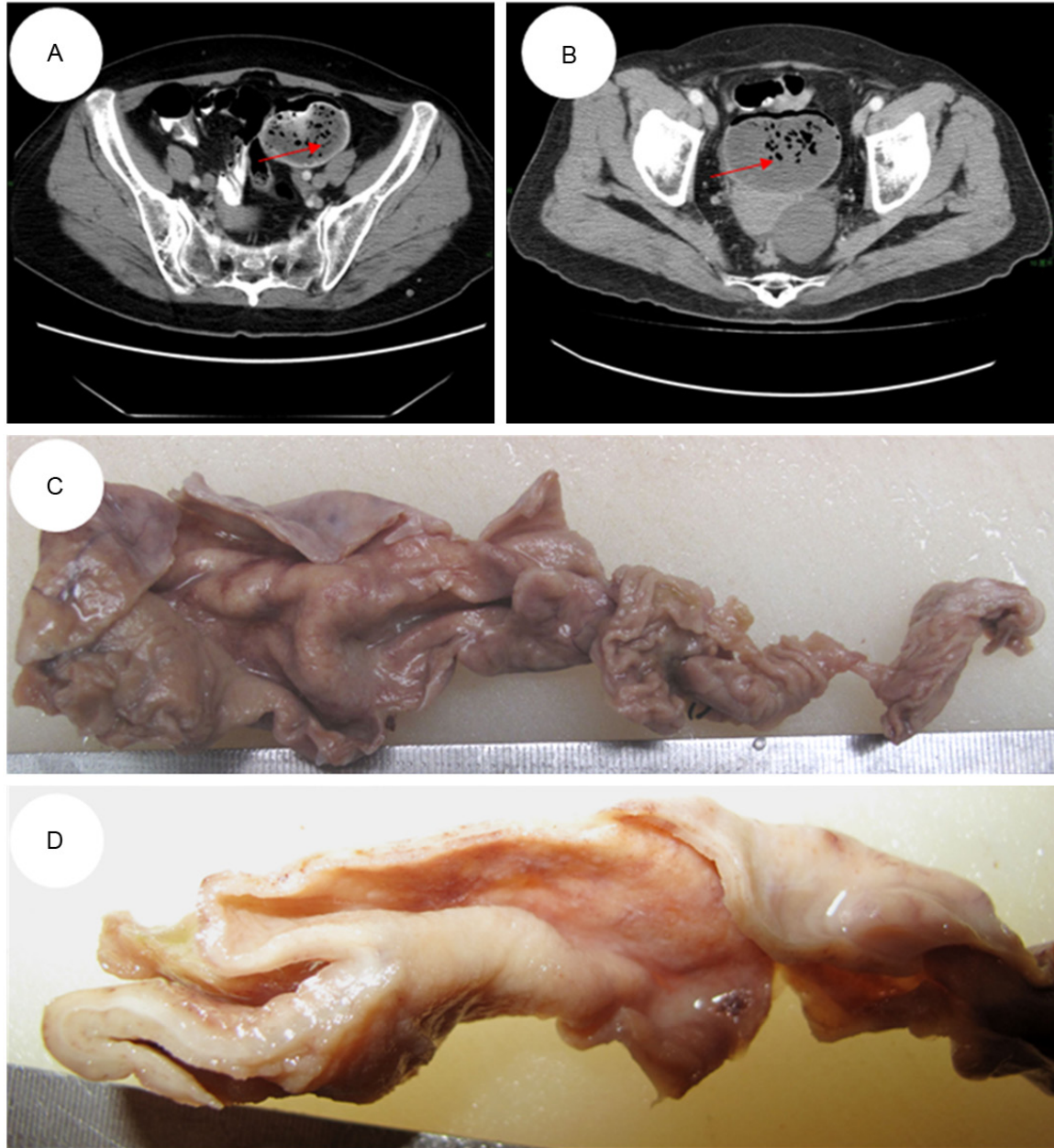
**Keywords:** ICC hyperplasia, giant diverticulum, *c-KIT*, *PDGFRA*, *NF1*

## Introduction

Gastrointestinal stromal tumors (GISTs) are the most common primary mesenchymal neoplasms of the tubular gastrointestinal tract. GISTs are KIT-positive and KIT-signaling-driven neoplasms caused by gain-of-function mutations in the *c-KIT* (80%) or Platelet-derived Growth Factor Receptor Alpha (*PDGFRA*, 10%) genes, leading to tumorigenesis through ligand-independent activation of type III receptor tyrosine kinases. About 10% of GISTs have no identifiable mutations in these two genes. Usually presenting as well-circumscribed lesions, GISTs are thought to arise from, or differentiate toward, interstitial cells of Cajal (ICCs), which form a network around the myenteric plexus of Auerbach and between the muscle fibers of the muscularis propria [1]. Most GISTs demonstrate an immunoprofile similar to that of ICCs (i.e., CD117+ and CD34+).

ICC hyperplasia refers to KIT-expressing, microscopic, focal or diffuse spindle cell proliferations involving the myenteric plexus and arising in conditions known to predispose to multiple GISTs, such as in patients with multiple familial GIST syndromes caused by germline mutations in the *c-KIT* or *PDGFRA* genes, or in patients with neurofibromatosis 1 (NF1, von Recklinghausen's disease), Carney's triad, and in a case of congenital intestinal neuronal dysplasia [2]. This syndromic ICC hyperplasia may either involve the myenteric plexus diffusely or form discrete micronodule that may coalesce to form grossly recognizable tumorlets. Diffuse ICC hyperplasia along the Auerbach plexus without forming a discrete mass may occur in patients with germline mutations in *NF1*, *c-KIT* or *PDGFRA* genes. Sporadic, diffuse ICC hyperplasia replacing the gut wall has only been described in few cases with *c-KIT* mutations [3]. To our knowledge, cases of long sporadic seg-

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**Figure 1.** A: Contrast CT scan showed a large diverticulum-like mass with a diameter of 6.5 cm in the ileum. B: Three years later, the diameter of the mass had enlarged to 8 cm. C and D: The ileal resection specimen was a diverticulum-like mass without prominent nodular areas and with thinner wall than the adjacent intestine. The muscularis propria is uniformly pale.

mental, diffuse ICC hyperplasia without *c-KIT* or *PDGFRA* mutations have not been reported. We describe herein one such case.

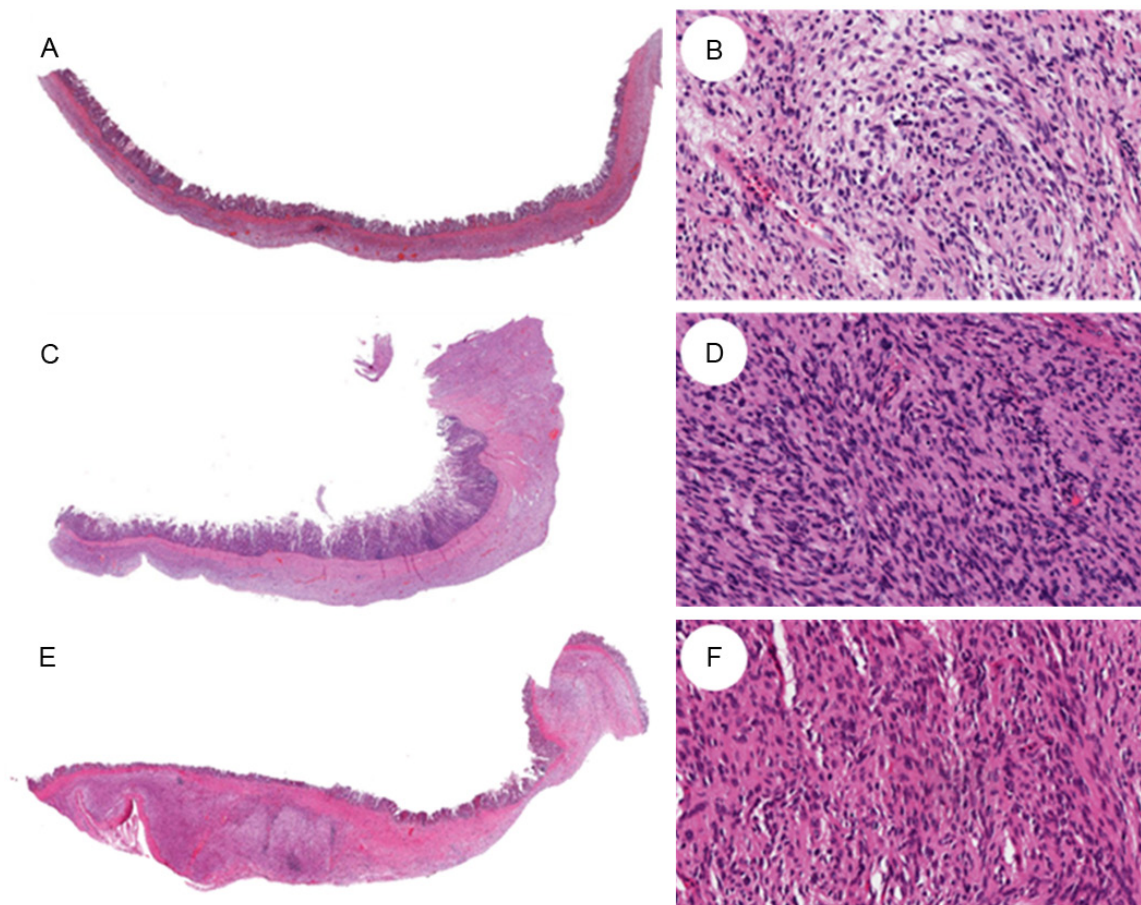
### Case presentation

#### *Clinical history*

A 63-year-old woman presented with increasing abdominal pain for more than 30 years.

Contrast CT scan showed a large diverticulum-like mass with a diameter of 6.5 cm in the ileum (**Figure 1A**). Three years later, contrast CT scan showed the mass to have enlarged to 8 cm (**Figure 1B**). A tumor in the left ovary with a diameter of 6 cm was also found. She underwent oophorectomy and at the same time, the diverticulum-like mass was resected. The patient was alive and well without any recurrent

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**Figure 2.** A diffuse proliferation of spindle cells is seen replacing the full thickness of muscularis propria and lined by intact mucosa, both in the diverticulum-like thinner areas (A) and in the adjacent thicker areas (C and E). Higher magnification (B, D, and F) shows the spindle cells to be sparse in some areas, both in the diverticulum-like thinner areas and in the adjacent thicker areas, and dense in other areas, mainly in the adjacent thicker areas (B, D, and F) (A, C, E,  $\times 10$ ; B, D, F,  $\times 200$ , H&E) (B is the higher power of A, D is the higher power of C and F is the higher power of E, respectively).

mass on CT scan 20 months after surgery. She had no features of Neurofibromatosis 1 (NF1) or other relevant familial history.

A written consent was obtained from the patient to the use of her biological samples for research purposes.

### *Specimen processing and immunohistochemistry*

Specimens were routinely processed (formalin-fixed, paraffin-embedded, and H&E-stained) for histological examination. Immunohistochemical staining was performed using an automated immunostainer (Ventana Benchmark XT; Ventana Medical Systems, Inc, Tucson, AZ). Antibodies against CD117 (*c-KIT*) (1:600; Dako, Glostrup, Denmark), S-100 protein (1:700;

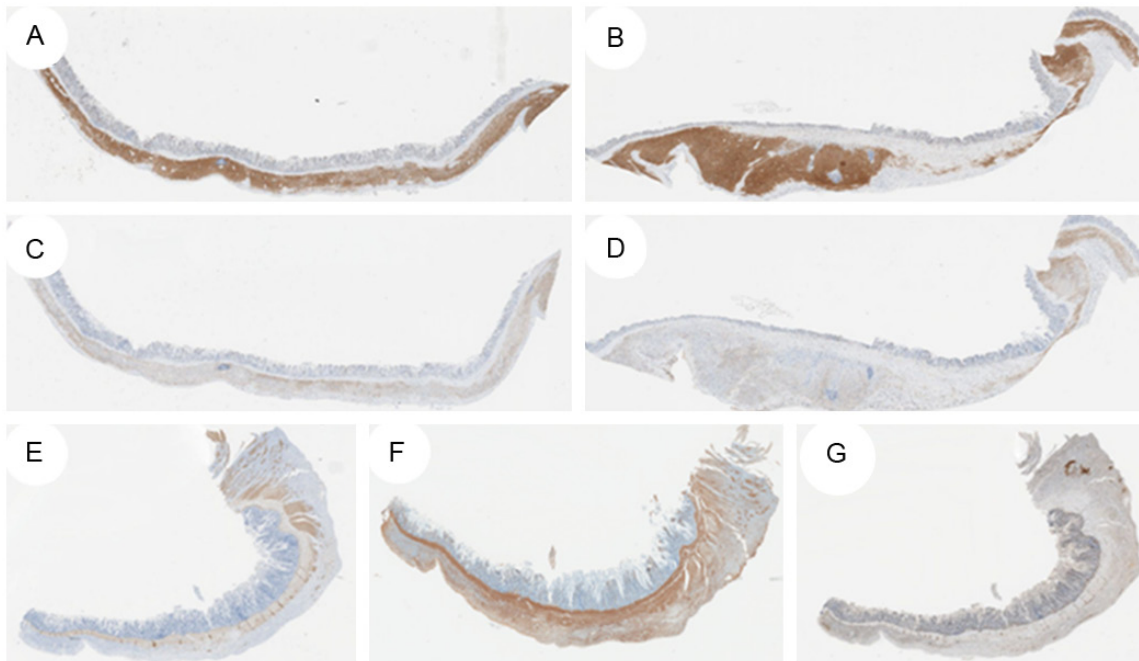
Dako, Glostrup, Denmark), DOG1, CD34, SMA and Desmin working solution (Maxim Biological Technology Co. Ltd., Fuzhou, China) were used. Antigen recovery was conducted using moderate heat retrieval according to manufacturer's recommendation. Slides were incubated with the primary antibody for 30 minutes at room temperature.

The ovarian tumor was a thecoma-fibroma, composed of abundant collagen and spindle-shaped cells with pale cytoplasm which were negative for CD117, CD34, DOG1, SMA, Desmin, and S100.

The large diverticulum-like mass of the ileum had a thinner wall than the adjacent intestine, no prominent nodular areas and a uniformly pale muscularis propria (**Figure 1C** and **1D**).



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**Figure 3.** The spindle cells were CD117+ (A and B), and CD34+ (C and D), both in the diverticulum-like thinner area (A and C) and in the adjacent thicker area (B and D). The spindle cells were Desmin- (E), SMA+ (F) and S100- (G) (Immunohistochemistry,  $\times 10$ ).

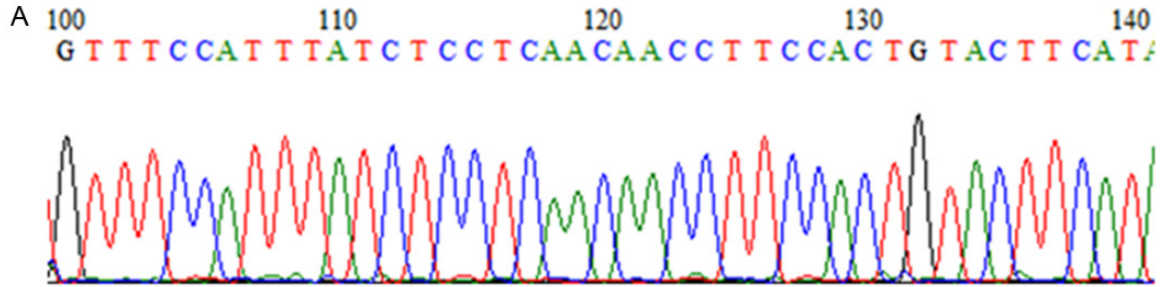
Microscopically, a diffuse, band-forming proliferation of bland spindle cells was seen extending for 12 cm and replacing the full thickness of the muscularis propria. Intact mucosa lined both the diverticulum-like thinner area and the adjacent thicker area. The lesional spindle cells, which were sparse in some areas and dense in others, had elongated to plump nuclei and fibrillar eosinophilic cytoplasm (**Figure 2**). One resection margin was involved by the lesion. There was no significant atypia and the mitotic rate was less than 1 per 50 high power fields. By immunohistochemistry, the spindle cells were CD117+/CD34+/DOG1+/SMA+/Desmin-/S100-, both in the diverticulum-like thinner area and in the adjacent thicker area (**Figure 3**). The elements of the enteric nervous system were absent.

### DNA extraction and sequencing

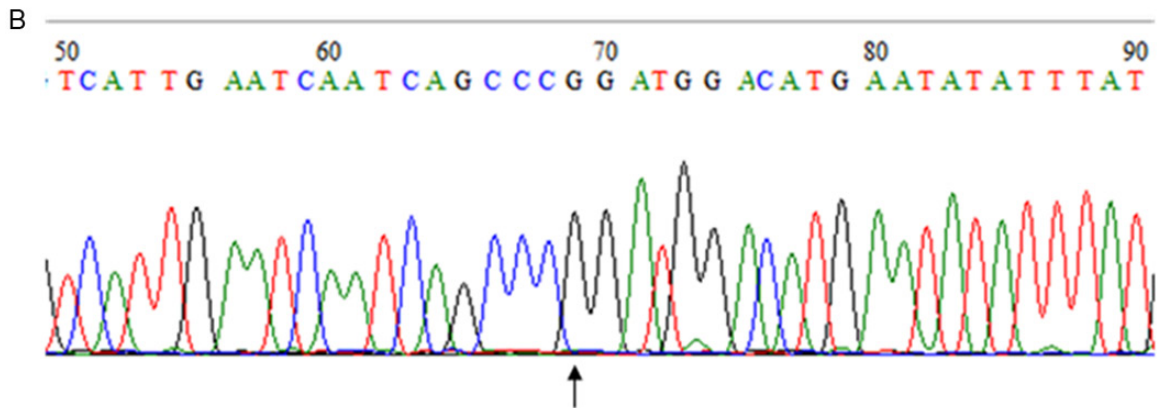
Genomic DNA was extracted from paraffin-embedded tissues using QIAamp® DNA and blood mini kit (Qiagen, Germany), according to the instructions of the manufacturer. Quality and concentration of the DNA samples were examined by Nano Drop. Genomic DNA was then diluted to a working concentration of 100 ng/ $\mu$ l.

Polymerase chain reaction (PCR) and sequencing of *c-KIT* and *PDGFRA* were carried as follows. *c-KIT* exons 9, 11, 13, 17, and *PDGFRA* exons 12, 18 were amplified using the following primers. *c-KIT* exon 9, forward primer, 5'-TTTCTAGAGTAAGCCAGGG-3'; *c-KIT* exon 9, reverse primer, 5'-GACTGATATGGTAGACAGAGC-3'; *c-KIT* exon 11, forward primer, 5'-AGGTGATC-TATTTTTCCCTTTC-3'; *c-KIT* exon 11, reverse primer, 5'-CTTAAAGTCACTGTTATGTGTA-3'; *c-KIT* exon 13, forward primer, 5'-CTTGACATCAGTT-TGCCAGTTGT-3'; *c-KIT* exon 13, reverse primer, 5'-GACAGACAATAAAAGGCAGCTTG-3'; *c-KIT* exon 17, forward primer, 5'-TGGTTTTCTTTCTCC-TCCAA-3'; *c-KIT* exon 17, reverse primer, 5'-GC-AGGACTGTCAAGCAGAGA-3'; *PDGFRA* exon 12, forward primer, 5'-AAGCTCTGGTGCCTGGGAC-TT-3'; *PDGFRA* exon 12, reverse primer, 5'-ATTGTAAAGTTGTGTGCAAGGGA-3'; *PDGFRA* exon 18, forward primer, 5'-CTTGCAAGGGTGA-TGCTATT-3'; *PDGFRA* exon 18, reverse primer, 5'-AGAAGCAACACCTGACTTTAGAGATTA-3'. A final volume of 25  $\mu$ l containing purified genomic DNA (100 ng/ $\mu$ l) 1  $\mu$ l, 10 $\times$ ABI buffer 2.5  $\mu$ l, MgCl<sub>2</sub> (25 mM) 1.5  $\mu$ l, dNTP (2.5 mM) 2  $\mu$ l, ABI AmpliTaq Gold DNA Taq polymerase 0.125  $\mu$ l (5 U/ $\mu$ l), forward primer and reverse primer (10  $\mu$ M) 1  $\mu$ l. After denaturation at 95° for 7 minutes, 40 amplification cycles at 95° for 30 s,

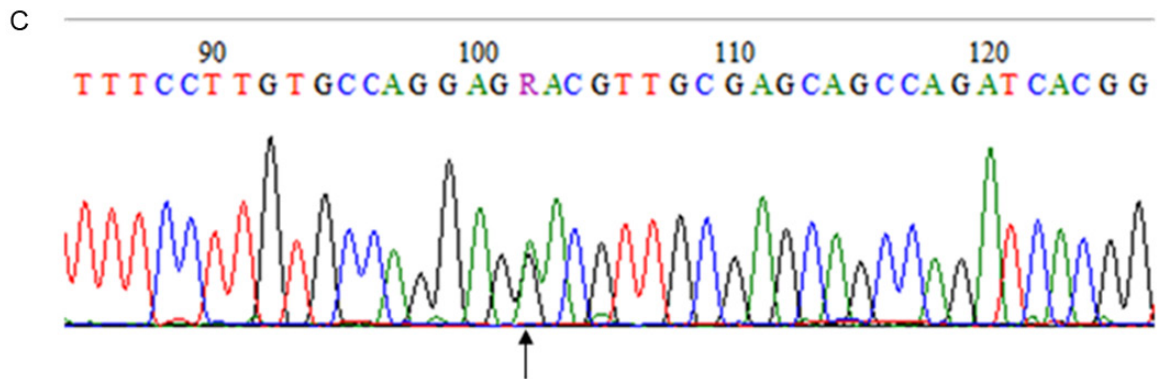
## C11-R



## P12-F



## P18-R



**Figure 4.** Wild type *c-KIT* exon 11 (A). SNPs: exon 12 c.1701A>G (p.P567P) and exon 18 c.2472C>T (p.V824V) in *PDGFRA* (B and C). R, reverse; F, forward.

56° for 30 s, 72° for 45 s, and elongation at 72° for 10 minutes. The PCR products sequencing was performed with ABI BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems) according to the manufacturer's instructions. Sequencing reactions were electrophoresed on an ABI3500XL genetic analyzer (Applied Biosystems). All sequencing reactions were performed in both the sense and antisense

directions. Mutations, single nucleotide polymorphisms (SNPs) and silent mutations were verified in a second independent PCR and sequencing reaction.

Sequencing of *NF1* was performed by Beijing Genomics Institute (BGI)-Shenzhen, a genomics company. The genomic DNA was used for library construction according to illumina stan-

standard PEI library construction protocol, then *NF1* gene sequence was enriched by array. The enriched DNA was sequenced via HiSeq 2000.

Mutation analyses did not reveal any mutations in *c-KIT* exons 9, 11, 13 and 17 and revealed a single nucleotide polymorphism (SNP) in *PDGFRA* exon 12 c.1701A>G (p.P567P) and in *PDGFRA* exon 18 c.2472C>T (p.V824V), respectively (**Figure 4**). In addition, mutation analyses revealed silent mutations in *NF1* exon (CDS) 7 c.702G>A (p.L234L) (rs1801052) (heterozygous) and exon 18 c.2034G>A (p.P678P) (rs2285892) (heterozygous). Moreover, there were 12 mutations in introns, 3 mutations in the 3'-UTR, 4 insertions in introns and 1 deletion in the 3'-UTR of the *NF1* gene.

### Results and discussion

The nomenclature of incidental, diminutive gross and/or microscopic GIST lesions has been a matter of controversy. Agaimy et al. suggested the classification of benign microscopic proliferative ICC lesions/minute incidental GISTs into four types: ICC hyperplasia-localized type, ICC hyperplasia-diffuse type, GIST tumorlets, and incidental, benign GISTs [3]. The term ICC hyperplasia would best fit microscopic lesions that do not form a grossly recognizable tumor mass irrespective of the clinical settings in which they occur.

ICC hyperplasia may occur either as a sporadic (incidental) lesion or present in a syndromic setting known to predispose to multiple GISTs at different sites. Most of the cases of sporadic incidental ICC hyperplasia reported in previous studies were of localized type [4, 5]. Diffuse type ICC hyperplasia is microscopically found, rarely causing thickening of the muscularis propria, and demonstrates confluent, nodular, or multifocal growth patterns. It is usually hereditary, rarely seen together with neuronal intestinal dysplasia (very rare Carney triad). It can occur in any part of the gastrointestinal tract; in *NF-1*, it is found mostly in the small intestine. Diffuse ICC hyperplasia has been encountered in a variety of gastrointestinal motility disorders. A few cases have reported GISTs arising within a true diverticulum, but there was no diffuse ICC hyperplasia described [6-8]. Agaimy et al. reported two cases. One had a large diverticulum-like pattern associated with diffuse microscopic ICC hyperplasia as well as a large

solid-type GIST. The other case was a patient who underwent segmental sigmoid colon resection for adenocarcinoma in a villous adenoma. Random sections from grossly unremarkable colonic wall showed a diffuse proliferation of CD117+/CD34+ spindle cells completely replacing the muscularis propria for a length of 6 mm [3]. Our case is similar to those two cases with a unique, diffuse microscopic growth pattern of sporadic ICC hyperplasia, different from both the sporadic (incidental) and the diffuse (hereditary) ICC hyperplasia. Although the ICC hyperplasia pattern we describe herein may superficially mimic the diffuse hereditary form in patients with germline mutations in *NF-1*, *c-KIT* and *PDGFRA*, the findings in our case are different in terms of extent (diffuse but segmental) and involvement of the entire thickness of the muscularis propria. ICC hyperplasia in syndromic settings usually presents as a thin layer of spindle cell proliferation along the Auerbach plexus occupying the intermuscular space between the inner circular and outer longitudinal muscle layers. Some of these hereditary ICC hyperplasia lesions may grow further to form discrete GIST nodules (tumorlets) [3]. The elements of the enteric nervous system were absent in our present case.

Unlike the cases reported by Agaimy et al. [3], the present case has no mutations in *c-KIT* or *PDGFRA* (we evaluated *c-KIT* exons 9, 11, 13, 17, and *PDGFRA* exons 12, 18, which were the sites most mutations occurs, and we did not evaluate *PDGFRA* exon 14, in which less mutations occurs.) and no clinical features of *NF1* and other syndromic disease. Although the uniform spindled histology and immunoprofile would rather suggest a hyperplastic nature, the tendency of these lesions to infiltrate the surrounding muscularis propria argues for a neoplastic lesion. About 10% of GISTs have no mutations of *c-KIT* or *PDGFRA* and mutations in these genes are very rare events in *NF-1* GISTs as compared with sporadic GISTs [9, 10]. Mutation analysis in our case revealed two silent mutations in the exons of *NF1* as well as multiple intronic and 3'-UTR mutations, insertions, and deletions. Kimchi-Sarfaty et al. reported that silent mutations could, under certain circumstances, determine how well a final protein performs. Perhaps, silent mutations reside in rarely used nucleotide triplets, which could slow down the protein-making machinery. Protein folding is somewhat speed-dependent,

and slower synthesis could cause the protein to take an altered final form. The cell might be able to compensate for one silent mutation but not for multiple, rarely used triplets [11]. It would seem that the silent mutations or the alterations in introns or the 3'UTR of the *NF1* gene caused ICC hyperplasia in our case.

The finding of ICC hyperplasia may explain the development of multiple GISTs in some conditions, possibly because ICC hyperplasia represents a preneoplastic lesion [12]. Although patients with familial GIST syndrome have germline mutations in *c-KIT* or *PDGFRA*, associated ICC hyperplasia has been found to be polyclonal in nature [13]. Supporting this, it has been reported that ICC hyperplasia in *NF1* lacked LOH at 14q and 22q, unlike small, but apparent nodule-forming GIST (<1 cm), which exhibited LOH. In addition, although *KIT* activation due to *c-KIT* gene mutation or *NF1* gene inactivation seems to be sufficient to result in polyclonal expansion of ICC, additional gene alterations associated with 14q and 22q losses are presumably required to generate the neoplastic proliferations recognized as GIST [9]. Our present case may or may not have progressed into a solid GIST, but the risk seems low due to the long duration of symptoms. There is also the possibility that an underlying muscle disorder led to the diverticulum and over the years resulted in compensatory ICC hyperplasia.

In summary, we describe a diffuse form of sporadic ICC hyperplasia showing diffuse longitudinal microscopic growth completely replacing the muscularis propria, mimicking diffuse ICC hyperplasia in hereditary GIST syndromes, but without solid components and no *c-KIT* or *PDGFRA* gene mutations. The lesion had two silent mutations in the *NF1* gene, but the patients did not exhibit features of *NF1* syndrome. This peculiar form of sporadic ICC hyperplasia may be related to intestinal dysmotility in this ileal segment and giant diverticulum formation.

### Acknowledgements

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### Disclosure of conflict of interest

None.

**Address correspondence to:** Dr. Ning Lu or Dr. Dongmei Lin, Department of Pathology, Cancer Institute (Hospital), Peking Union Medical College, Chinese Academy of Medical Sciences, Beijing 100021, China. Tel: 86-10-87788435; 86-10-87788548; Fax: 86-10-67702630; E-mail: nlu03@126.com (Ning Lu); lindm3@163.com (Dongmei Lin)

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