

A subset of gastrointestinal stromal tumors previously regarded as wild-type tumors carries somatic activating mutations in *KIT* exon 8 (p.D419del)

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About 10–15% of gastrointestinal stromal tumors (GISTs) carry wild-type sequences in all hot spots of *KIT* and platelet-derived growth factor receptor alpha (*PDGFRA*) (wt-GISTs). These tumors are currently defined by having no mutations in exons 9, 11, 13, and 17 of the *KIT* gene and exons 12, 14, and 18 of the *PDGFRA* gene. Until now, the analysis of further exons is not recommended. However, we have previously published a report on a *KIT* exon 8 germline mutation, which was associated with familial GIST and mastocytosis. We therefore investigated whether *KIT* exon 8 mutations might also occur in sporadic GIST. We screened a cohort of 145 wt-GISTs from a total of 1351 cases from our registry for somatic mutations in *KIT* exon 8. Two primary GISTs with an identical exon 8 mutation (p.D419del) were detected, representing 1.4% of all the cases analyzed. Based on all GISTs from our registry, the overall frequency of *KIT* exon 8 mutations was 0.15%. The first tumor originating in the small bowel of a 53-year-old male patient had mostly a biphasic spindled-epithelioid pattern with a high proliferative activity (14 mitoses/50 HPF) combined with a second low proliferative spindle cell pattern (4/50 HPF). The patient developed multiple peritoneal metastases 29 months later. The second case represented a jejunal GIST in a 67-year old woman who is relapse-free under adjuvant imatinib treatment. We conclude that about 1–2% of GISTs being classified as ‘wild type’ so far might, in fact, carry *KIT* mutations in exon 8. Moreover, this mutational subtype was shown to be activating and imatinib sensitive *in vitro*. We therefore propose that screening for *KIT* exon 8 mutations should become a routine in the diagnostic work-up of GIST and that patients with an exon 8 mutation and a significant risk for tumor progression should be treated with imatinib.

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Gastrointestinal stromal tumors (GISTs) are potentially malignant mesenchymal tumors of the gastrointestinal tract. *KIT* or — less often — *PDGFRA* (platelet-derived growth factor receptor alpha) mutations leading to a ligand-independent autoactivation

of one of two tyrosine kinases are the oncogenetic drivers of these tumors.^{1,2}

In GIST, primary *KIT* mutations do not occur arbitrarily but only in the hot spot regions, which are exons 9, 11, 13, and 17. These encode an extracellular domain, the juxtamembrane domain, and the kinase domains 1 and 2, respectively. Corresponding affected exons of the closely related *PDGFRA* gene are 12, 14, and 18.^{3–5} Activating mutations in the remaining exons of both the genes have not been described so far in sporadic tumors. It is now widely accepted that GISTs are characterized by a close genotype–phenotype correlation in terms of location within the gastrointestinal tract,

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morphology, clinical behavior, and response to treatment with imatinib, which is a potent tyrosine kinase inhibitor. Thus, the mutational status has a high clinical impact as: (1) it can confirm the diagnosis of GIST in selected cases that are hard to diagnose; (2) it has a prognostic value; and (3) it is predictive for the response to molecular-targeted therapies, eg, with imatinib. Concerning the prognostic value, specific mutations in *KIT* exon 11 were shown to be associated with a higher risk of metastatic disease,⁶ whereas most *PDGFRA*-mutated GISTs show a less aggressive behavior.^{7,8} In intestinal GISTs, *KIT* exon 9 mutations go along with aggressive behavior.⁹ In the context of targeted treatment, it has been recognized that the most common *PDGFRA* mutation (p.D842V), which nearly exclusively occurs in GISTs of the stomach, leads to resistance against imatinib.¹⁰ The detection of *KIT* exon 9 mutations in metastatic GISTs warrants doubling of the daily dose of imatinib to 800 mg/day in order to increase the response rate to treatment.^{11,12} Thus, mutational testing of GIST is recommended in the diagnostic work-up of all GIST specimens, eg, by NCCN guidelines¹³ and the ESMO Clinical Practice Guidelines,¹⁴ at least in GISTs with intermediate or high risk of recurrence.

However, about 10–15% of GISTs carry wild-type sequences in all known hot spots of *KIT* and *PDGFRA* (wt-GISTs).¹⁵ In most cases, these wt-GISTs are known to have no or a poor response to tyrosine kinase inhibition. It now becomes apparent that wt-GISTs constitute an obviously heterogeneous group of different cancer subtypes. A subset of these tumors seem to be driven by other amplifications or oncogenic mutations of genes such as in *IGF1R*, *BRAF*, or *PIK3CA*.^{16,17} Another subset of wt-GISTs arises in the context of hereditary syndromes. In the autosomal-dominant disorder, neurofibromatosis type 1 multiple wt-GISTs can occur,^{18,19} arising predominantly in the small bowel. Patients with Carney–Stratakis syndrome suffer from gastric wt-GIST and paragangliomas.²⁰ This syndrome is caused by germline mutations in the succinate dehydrogenase (SDH) subunits B, C, or D, leading to dysfunction of complex II of the electron transport chain.^{20,21} However, it was shown that a subset of patients outside the Carney–Stratakis syndrome reveals mutations in SDH subtype B or C, pointing toward a pathogenetic role of SDH dysregulation in wt-GISTs.²² Paediatric GISTs, typically present in the stomach of young girls, display an epithelioid phenotype and can be multifocal. *KIT* and *PDGFRA* mutations are mostly absent²³ and a subgroup of them is again attributable to succinate dehydrogenase deficiency. Thus, the group of wt-GISTs is heterogeneous and further molecular characterization is needed to define subgroups, which might lead to a more specific therapy for these patients.

As stated earlier, wt-GISTs are currently defined by having wild-type sequences in exons 9, 11, 13,

and 17 of the *KIT* gene and exons 12, 14, and 18 of the *PDGFRA* gene. Until now, the analysis of additional exons is not recommended in the routine molecular diagnostics of GISTs. However, we have previously published a report on a *KIT* exon 8 germline mutation,²⁴ which was associated with systemic mastocytosis and familial GISTs.

The aim of this study was to investigate whether *KIT* exon 8 mutations also occur in sporadic GISTs. We therefore screened a cohort of 145 wt-GISTs for somatic mutations in *KIT* exon 8.

Materials and methods

Cases and Immunohistochemistry

A total of 145 wt-GISTs were extracted from our consultation files. All cases were wild type for *KIT* exons 9, 11, 13, and 17 and *PDGFRA* exons 12, 14, and 18. Baseline characteristics of the cases are displayed in Tables 1 and 2. The diagnosis of GISTs was made in all the presented cases by a senior pathologist specialized in soft tissue pathology (H-US, EW or RB). Immunohistochemistry for CD34 (clone QBEnd10, DAKO, 1:100), *KIT* (A4502, DAKO, 1:100), *PDGFRA* (SC-338, Santa Cruz, 1:50), *DOG1* (Spring Bioscience, Fremont, CA, USA; 1:100) and *Ki67* (Mib1, DAKO, 1:1000) was carried out as described earlier.²⁵

Mutational Analysis

For genomic DNA-extraction, the lesional areas were marked on a haematoxylin and eosin-stained slide by a senior pathologist (H-US, EW, or RB). Subsequently, the tissue was scraped from unstained slides and DNA was extracted using standard methods as described previously.²⁶ *KIT* exons 9, 11, 13, and 17 as well as *PDGFRA* exons 12, 14, and 18 DNA were amplified by PCR with specific primers and sequenced as described previously.^{26,27} *KIT* exon 8 DNA was amplified by PCR with the following specific primers: Forward: 5'-GAAGTGAA TGTGCTGAGG-3'; and Reverse: 5'-GTGAATTGCA GTCCTTCC-3'. PCR and cycle sequencing were repeated twice.

Fluorescence *In-Situ* Hybridization (FISH) Analysis

FISH was performed on interphase nuclei of 4- μ m sections of FFPE tissue. Locus-specific probe was derived from the BAC clone RP11-74L18 (imaGenes GmbH, Berlin, Germany) containing the full-length *KIT* gene using a Nick Translation Kit (Abbott Molecular, Germany) and spectrum orange-labelled dUTP (Abbott Molecular, Germany). *KIT* probe was used together with the chromosome 4 centromeric enumeration probe (CEP4, Abbott Molecular, Germany).

Table 1 Clinicopathological features of the wt-GIST cohort (n = 145)

| Clinical presentation | n | Age (years) | Gender (male/female) | Localization (esophagus/stomach/small bowel/colon) | Size (cm) | Morphological subtype (spindle/mixed/epithelioid) | Mitotic count (per 50 HPF) | Risk classification ^a (no/very low/low/intermediate/high risk) |
|---------------------------------------|-----|------------------------------|----------------------|--|---------------------------------|---|----------------------------|---|
| Neurofibromatosis type 1 (NF1) | 12 | 53.1 (34–65) [55] | 5/7 | 0/1/10/0 n = 11 | 4.0 (1.3–7.5) [3.5] n = 10 | 8/3/0 n = 11 | 1.8 (0–10) [1] n = 10 | 2/0/2/4/0 n = 8 |
| Carney dyade | 1 | 34 | 0/1 | 0/1/0/0 | 4.5 | 1/0/0 | 1 | 0/1/0/0/0 |
| Pediatric (aged < 18 years) | 5 | 12.8 (8–15) [14] | 1/3 n = 4 | 0/3/2/0 | 6.2 (1.2–10.0) [7.0] | 4/0/1 | 1.3 (0–3) [1] n = 4 | 1/1/1/1/0 n = 4 |
| No association with clinical syndrome | | | | | | | | |
| Primary tumor | 121 | 60.2 (19–98) [60] n = 119 | 56/61 n = 117 | 1/48/66/1 n = 116 | 5.5 (0.1–35.0) [5.0] n = 102 | 77/26/13 n = 116 | 4.2 (0–65) [1] n = 116 | 17/16/25/15/23 n = 96 |
| Metastases/recurrences | 6 | 51.7 (39–73) [48] | 3/3 | 0/0/2/0 ^b n = 2 | 3.5 (2.5–5.0) [3.0] n = 3 | 5/1/0 | 30.4 (0–90) [23] n = 5 | NA |
| Total | 145 | 57.4 (8–98) [60] n = 143 | 65/75 n = 140 | 1/53/80/1 ^b n = 135 | 5.4 (0.1–35.0) [4.5] n = 121 | 95/30/14 n = 139 | 4.8 (0–90) [1] n = 136 | 20/18/28/20/23 n = 109 |

HPF, high-power field. Range is indicated in brackets, median in square brackets. In the event that not all cases were evaluable for a clinicopathological parameter, n indicates the number of available cases.

^aAccording to the NCCN-AFIP criteria.²⁸

^bAdditional four cases presented as liver metastasis.

Table 2 Summary of two gastrointestinal stromal tumors with sporadic KIT exon 8 mutations

| Case No. | Age/gender | Localization | Size (cm) | Morphological subtype | Risk classification ^a (mitoses) | Mutation | Adjuvant Treatment | DFS (months) | Follow up (months) |
|----------|------------|--------------|-----------|-------------------------------------|--|---|-------------------------|--------------|--|
| 1 | 53/M | Small bowel | 5.4 | Biphasic (epithelioid and spindled) | High (14/50 HPF) | KIT exon 8 c.1255_1257delGAC p.D419del heterozygous in primary tumor homozygous in metastasis | None | 29 | Peritoneal metastases (29) lost to follow up with progressive disease (34) |
| 2 | 67/F | Small bowel | 10.0 | Spindled | Moderate (1/50 HPF) | KIT exon 8 c.1255_1257delGAC p.D419del heterozygous | Imatinib (400 mg daily) | 24 | No evidence of disease (24) |

DFS, disease-free survival; HPF, high power field.

^aAccording to the NCCN-AFIP criteria.²⁸

Deparaffinized slides were incubated in 0.2 M HCl for 20 min, followed by washing and 80 °C heat pretreatment in pretreatment solution (Abbott Molecular, Germany) for 30 min and further washing. Tissue was digested for 1.5 h with protease, slides were washed again, and fixed in 4% buffered formalin. All pretreatment steps were carried out in a prehybridization processor (VP2000, Abbott Molecular). After washing, dehydration, and air

drying, an appropriate amount of fluorochrome-labeled probe mixture was applied to the centre of the tissue section. Tissue DNA and probe mixture were co-denatured at 85 °C for 10 min, cooled down to 37 °C, and hybridized overnight in a Hybrite Hybridizer (Abbott Molecular, Germany). After stringent washing and dehydration, sections were counterstained using fluorescence mounting media containing de-amidino-phenyl-indole (DAPI, Zyto-

vision GmbH, Germany). FISH slides were evaluated with a fluorescence microscope (DM 5500, Leica, Germany) using appropriate filter sets. Orange (*KIT*) and green (CEP4) signals were counted in 60 non-overlapping tumor cell nuclei from the three tumor areas.

Results

Clinicopathological Features

A total of 145 patients with proven wild-type sequences in all known mutational hot spots of *KIT* and *PDGFRA* were included in our study. Detailed clinicopathological data are provided in Table 1.

A comparison of these data with established clinicopathological features of GISTs (including wt-GISTs) demonstrates that our wt-GIST cases display a more or less representative cohort. For instance, small bowel GISTs ($n=80$) outnumbered GISTs of the stomach ($n=53$) and of the oesophagus and colon ($n=1$ each). Most cases displayed a spindle or mixed cell morphology ($n=125/139$) and only 14 cases revealed a pure epithelioid phenotype. In all, 12 patients were known to fulfill clinical criteria of neurofibromatosis type 1 and one patient presented with a Carney dyad. The tumor size was known in 121 cases and ranged from 0.1 to 35.0 cm in diameter (mean: 5.4 cm, median: 4.5 cm). Mitotic count was assessable in 136 cases and ranged from 0 to 90 (mean: 4.8; median: 1), with most cases having a low mitotic count. Therefore, according to the NCCN-AFIP criteria,²⁸ only 43 cases were classified as being at 'intermediate' or 'high' risk for progressive disease among 120 classifiable cases. Four other cases presented as liver metastasis. The overall patients' mean age was 57.4 years (median: 60 years, range: 8–98 years; $n=143$), including five pediatric patients. Clinicopathological data were not available for all the patients, because most samples had been evaluated in the setting of a referral center with limited access to external clinical parameters.

Mutational Analysis

Two primary GISTs (cases 1 and 2) with an identical *KIT* exon 8 mutation (c.1255_1257delGAC) leading to the deletion of an aspartic acid on residue 419 (p.D419del) were detected, representing 1.4% of all the cases investigated. The mutation was also found in a peritoneal metastasis of case 1, which was sequenced independently. No activating mutations in *KIT* exon 8 could be identified in the remaining 143 wild-type cases. However, silent mutations were found in *KIT* exon 17 (p.I798I, $n=7$, 4.8%) and *PDGFRA* exon 18 (p.V824V, $n=24$, 16.6%). Based on evaluation of the mutational status of 1351 well-characterized GIST samples from the Bonn–Cologne

GIST registry, the overall frequency of *KIT* exon 8 mutations was 0.15%. In our referral center cohort, *KIT* exon 9 mutations were detected in 126 cases (9.33%), exon 11 mutations in 810 cases (59.96%), exon 13 mutations in 25 cases (1.85%), and exon 17 mutations in 23 cases (1.70%). *PDGFRA* mutations in exon 12 were found in 25 cases (1.85%), exon 14 mutations in 8 cases (0.59%), and exon 18 mutations in 189 cases (13.99%) (Figure 1).

Clinicopathological Characteristics of *KIT* Exon 8 Mutant GISTs

One GIST with a sporadic *KIT* exon 8 mutation (p.D419del) was found in a 53-year-old male patient. The tumor originated in the small bowel and measured 5.4 cm in diameter. The lesion showed a biphasic spindle-epithelioid histological appearance. Large tumor nodules consisted of epithelioid tumor cells with eosinophilic to clear cytoplasm, which were aggregated in an alveolar fashion. Multinucleated giant cells were not observed. High proliferative activity with numerous mitoses was noted and tumor necrosis was seen. The mitotic count was 13/50 HPF in this area. Furthermore, a second spindle cell pattern was noted. In this area, the mitotic count was 4/50 HPF (Figure 2, Table 2). According to the NCCN-AFIP criteria,²⁸ the patient was classified as being at high risk of progressive disease. The patient developed multiple peritoneal metastases 29 months after surgical removal of the primary tumor. Mutational analysis of the metastatic lesions revealed the same deletion in *KIT* exon 8. Notably, a heterozygous mutation was detected in the primary tumor, whereas a homozygous pattern was detected in the metastatic disease. After 34 months, the patient was lost to

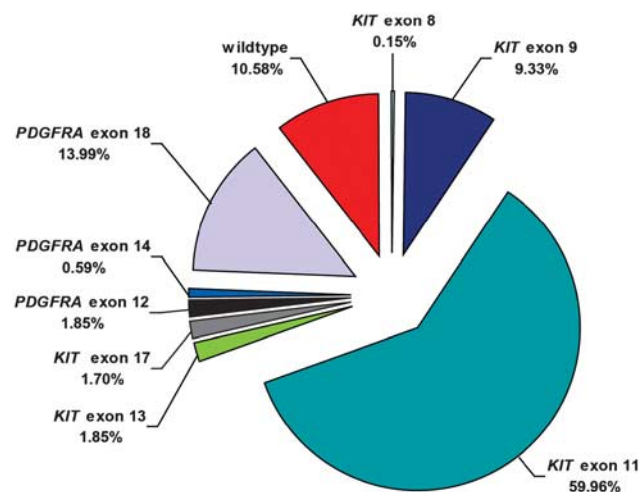


Figure 1 Mutational status of 1351 GIST cases in the Bonn–Cologne GIST registry ($n=1351$). *KIT* mutations were found in 72.99% and *PDGFRA* mutations in 16.43% of cases. The remaining 10.58% were classified as wt-GISTs. The overall frequency of *KIT* exon 8 mutations is 0.15%.

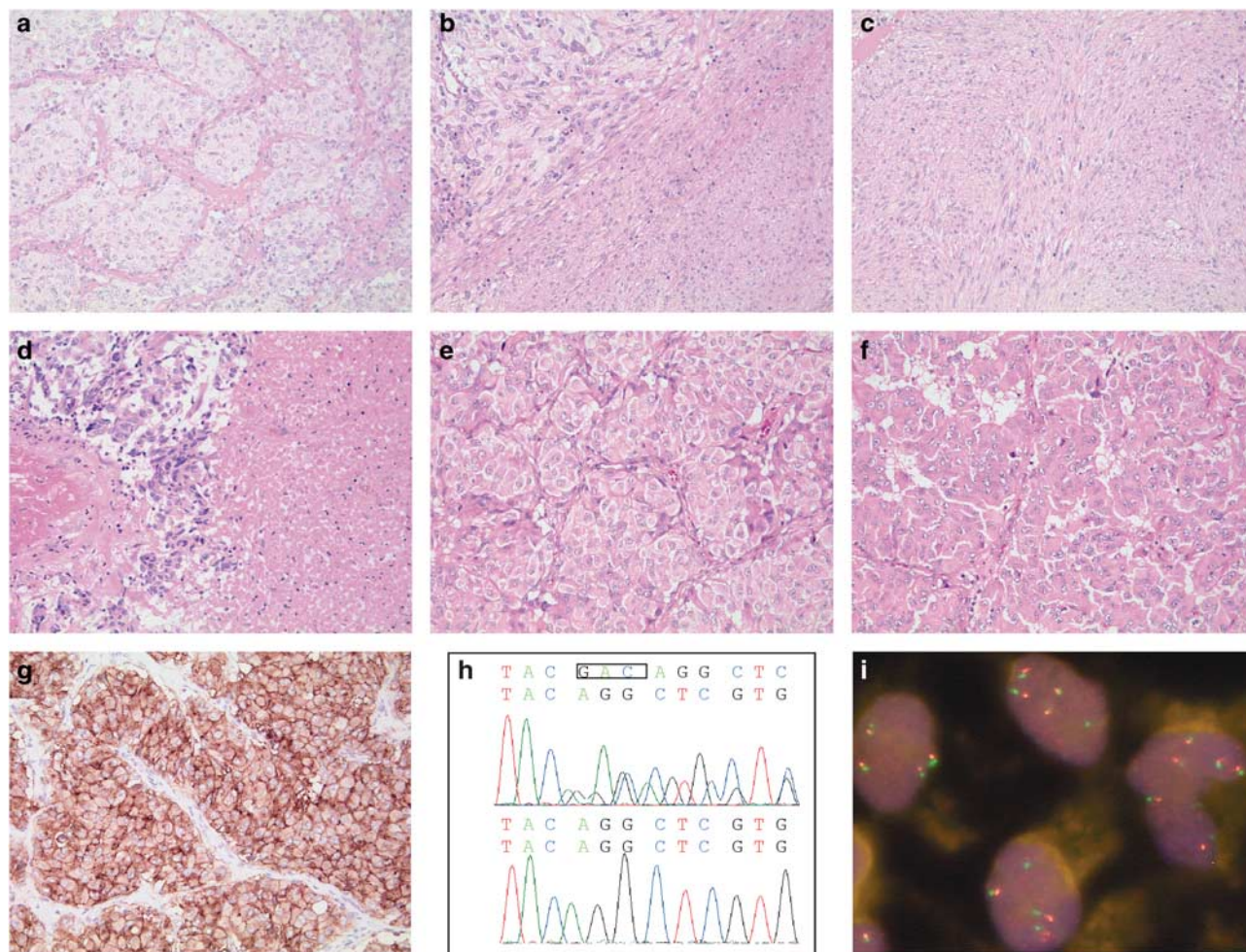


Figure 2 Histopathological and molecular features of a GIST with *KIT* exon 8 mutation. In case 1, epithelioid tumor cells with eosinophilic to clear cytoplasm were aggregated in an alveolar fashion ((a), $\times 200$, H&E). A second tumor cell component showed a spindle cell appearance and was situated in close proximity to the epithelioid areas ((b,c), $\times 200$, H&E). Abundant necrosis was noted ((d), $\times 200$, H&E). Metastatic tissue revealed epithelioid tumor cells ((e,f), $\times 200$, H&E). Strong expression of the KIT receptor ((g), $\times 200$, KIT). The p.D419del mutation occurred heterozygously in the primary tumor ((h), top) and homozygously in the metastasis ((h), bottom). FISH analysis showed polysomy by presenting multiple orange *KIT* signals as well as multiple green CEP4 signals in both the primary tumor and metastasis (i).

follow up with progressive metastatic disease. As the patient was diagnosed in the pre-imatinib era, no adjuvant treatment was administered. Instead, the patient was treated with adriamycin and ifosfamide for his metastatic disease.

In case 2, the same heterozygous mutational subtype (p.D419del) was found in a GIST arising in the proximal jejunum. The 57-year-old female patient followed, however, a benign clinical course under adjuvant treatment (400 mg imatinib/day). The tumor measured 10 cm in size and a spindle cell phenotype of the entire tumor was observed (Figure 3, Table 2). One mitosis was found in 50 HPF. According to the NCCN-AFIP criteria,²⁸ the patient was classified as being at moderate risk of progressive disease. Twenty-four months after removal of the GIST, there is no evidence of recurrence, while the patient is currently under adjuvant treatment with imatinib (400 mg/day).

FISH Analysis

In case 1, the primary tumor showed the deletion in exon 8 of the *KIT* gene in a heterozygous pattern, whereas a homozygous status was detected in the relapse. In order to further elucidate the development of this homozygous pattern, we performed FISH analysis of the *KIT* gene locus in primary tumor and in the relapse. Spindle cell and epithelioid areas of the primary tumor did not differ in terms of *KIT* and centromere 4 copy numbers. Both the primary lesion and metastasis tumor cells showed polysomy by presenting multiple orange *KIT* signals as well as multiple green CEP4 signals (Figure 2). Thus, FISH analysis revealed neither a monosomy of chromosome 4 nor a mono-allelic deletion of the *KIT* gene locus as the cause for the homozygous mutational pattern.

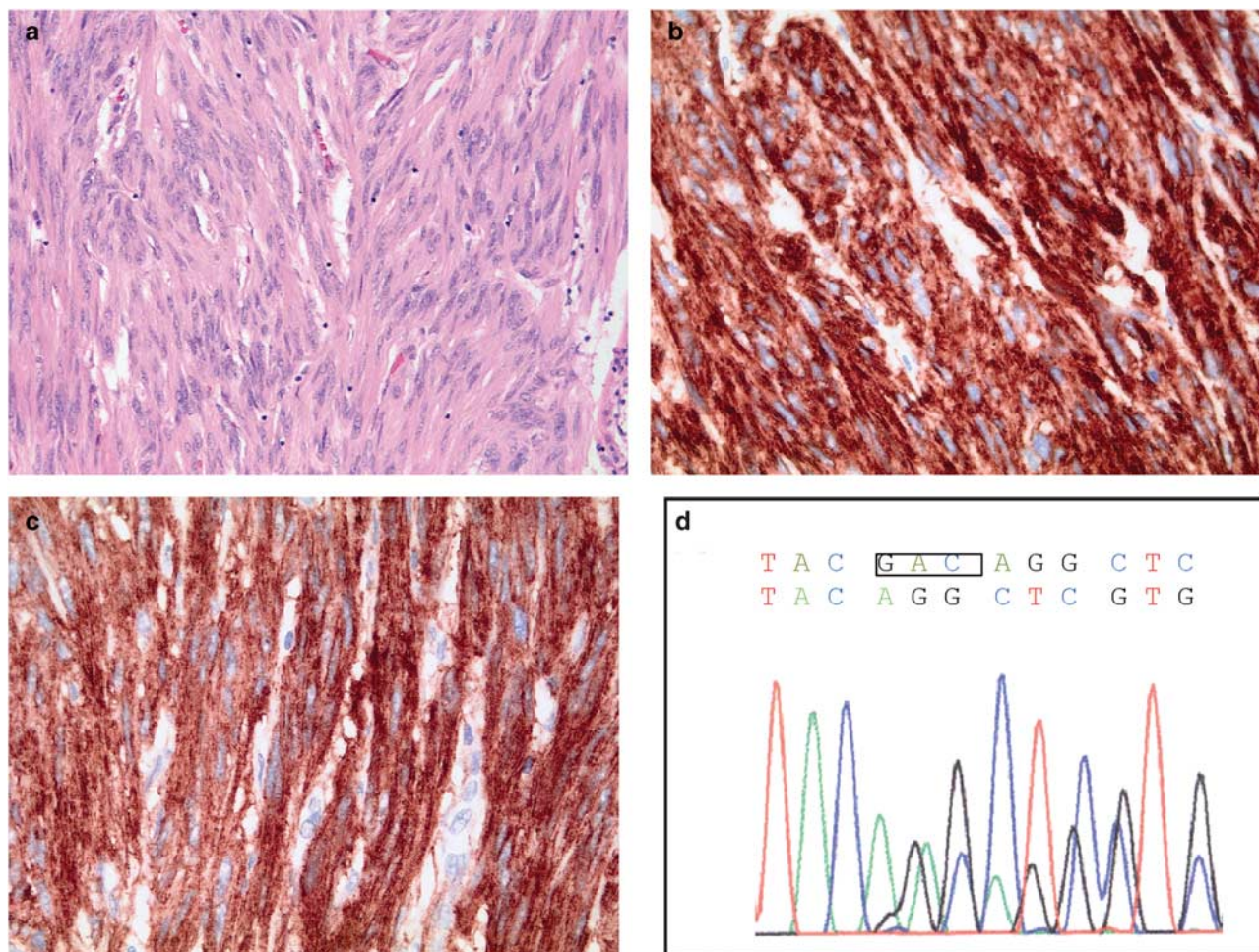


Figure 3 Histopathological and molecular features of a GIST with *KIT* exon 8 mutation. In case 2, a spindled cell morphology was noted (a), $\times 200$, H&E). Immunohistochemistry revealed strong positivity for DOG1 (b, $\times 200$) and KIT (c). Sanger sequencing showed a heterozygous deletion in *KIT* exon 8 (p.D419del) (d).

Discussion

We have previously reported familial GISTs caused by germline mutation in *KIT* exon 8.²⁴ This led us to the hypothesis that *KIT* exon 8 mutations might also represent driver mutations in a subset of sporadic GISTs. We therefore screened a well-defined cohort of 145 wt-GISTs for mutations in *KIT* exon 8.

On the molecular level, GISTs are characterized by mutations in the *KIT* or *PDGFRA* genes, both encoding for closely related tyrosine kinase proteins, which consist of regulatory and catalytic domains. The regulatory domain consists of an extracellular ligand-binding domain (EC) and a juxtamembrane domain (JM). Main parts of the catalytic domain are the two tyrosine kinase domains (TK1 and TK2), which are separated by a kinase insert.³ Each exon of *KIT* and *PDGFRA* corresponds to a specific molecular structure of the receptor. In sporadic GISTs, primary *KIT* mutations were hitherto found in EC and JM domains (exons 9 and 11, respectively) as well as in both kinase

domains (exons 13 and 17); corresponding affected exons of *PDGFRA* are 12 (JM), 14 (TK1) and 18 (TK2).³ Beside GISTs, *PDGFRA* translocations or mutations are associated with various human neoplasms such as myeloproliferative diseases associated with hypereosinophilia²⁹ or inflammatory fibroid polyps.^{25,30} *KIT* mutations have also been reported in other malignancies, such as melanomas or seminomas.³¹ Some of these tumors share the same mutational hot spots and affected exons with GISTs.

KIT exon 8 mutations are located in the EC domain of the receptor and are frequently found in hematopoietic disorders, such as acute myeloid leukemia and/or mastocytosis.^{32,33} Several mutational subtypes have been described that mostly affect codons 417–421 (Table 3). Up to now, *KIT* exon 8 mutations have not been systematically investigated in sporadic GISTs, and we report, for the first time, two cases with somatic mutations. In our cohort of wt-GISTs, which obviously appears to be representative, the frequency was 1.4%.

Table 3 Reported *KIT* exon 8 mutations

| | Mutation | | Clinical presentation | | | Reference (selected) |
|------------------|------------------------|--|-----------------------|--------------------|------|----------------------------|
| | HGVS | Literature | AML | Mast cell neoplasm | GIST | |
| Point mutations | p.Y418N | Y418N | X | | | 35 |
| | p.D419H | D419H | | X | | 36 |
| | p.C443Y | C443Y | | X | | 37 |
| Insertions | p.Y418_D419insFF | Y418_D419insFF | X | | | 35 |
| | p.D419_R420insFL | ins 419 TTC CTC | X | | | 38 |
| | p.D419_R420insFF | ins 419 TTT TTC | | X | | 38 |
| Deletions | p.T417_Y418del | TY417-418 deletion | X | | | 39 |
| | p.Y418_D419del | del 418–419 TAC GAC | X | | | 38 |
| | p.D419del | D419del | X | X | X | 37,38,40,41, Current study |
| Delins mutations | p.T417_Y418delinsH | Deletion TY417-418H | X | | | 42 |
| | p.T417_D419delinsF | T417FΔ2AA | X | | | 40 |
| | p.T417_D419delinsG | T417_D419delinsG | X | | | 41 |
| | p.T417_D419delinsI | TYD 417-419I | X | | | 43 |
| | p.T417_D419delinsIP | T417_D419delinsIP | X | | | 43 |
| | p.T417_D419delinsKS | del CTTACGA inst AATC | X | | | 40 |
| | p.T417_D419delinsL | 1249–1256 del ACTTACGA ins CT | X | | | 44 |
| | p.T417_D419delinsN | del CTTACGAC ins AT | X | | | 45 |
| | p.T417_D419delinsNG | T417_D419delinsNG | X | | | 43 |
| | p.T417_D419delinsRA | TYD417-419RA | X | | | 42 |
| | p.T417_D419delinsRG | T417_D419delinsRG | X | | | 43 |
| | p.T417_D419delinsS | T417_D419delinsS | X | | | 41 |
| | p.T417_D419delinsV | del ACTTACGAC ins GTG | X | | | 45 |
| | p.T417_D419delinsW | T417_D419delinsW | X | | | 43 |
| | p.[T417del(;):D419del] | T417del and D419del | X | X | | 43 |
| | p.T417_R420delinsHG | T417 R420delinsHG | X | | | 46 |
| | p.T417_D419delinsRG | del CTTACGA ins GAGG | X | | | 45 |
| | p.T417_R420delinsSVIVG | 1250–1260 del CTTACGACA ins GCGTCATTGTGG | X | | | 44 |
| | p.T417_V422delinsSRIL | T417_V422delinsSRIL | X | | | 41 |
| | p.Y418_D419delinsG | YD418-419G | X | | | 42 |
| | p.Y418_D419delinsS | Deletion YD418-419S | X | | | 42 |
| | p.Y418_D419delinsT | del 418–420 TAC GAC AGG, ins 418 ACC AGG | X | | | 38 |
| | p.T418_R420delinsV | T418_R420del insV | X | | | 47 |
| | p.Y418delinsGFF | Y418delinsGFF | X | | | 38 |
| | p.D419_R420delinsFLNM | del 419, 420 GAC AGG, ins TTC CTC AAC ATG | X | | | 38 |
| | p.D419_R420delinsFFDG | del GACA ins TCCTCCGACG | X | | | 45 |
| | p.D419_L421delinsF | del GACAGGC ins T | X | | | 45 |
| | p.D419_L421delinsVHV | D419_L421delinsVHV | X | | | 46 |
| | p.D419_V422delinsWSL | D419 R420delinsWS and V422del | X | | | 46 |
| | p.D419_Y422delinsYSP | del 419–422 GAC AGG CTC GTG, ins 419 TAC TCT CCG | X | | | 38 |

AML, acute myeloid leukemia, GIST, gastrointestinal stromal tumor.

Mutations are displayed according to the Human Genome Variation Society (HGVS) standard as well as originally reported in the literature.

However, they represent very rare mutational events, contributing to <0.2% of all GISTs.

In both our cases, we observed the same deletion (p.D419del), which was also shown to be associated with pediatric-onset cutaneous mastocytosis (urticaria pigmentosa) and acute myeloid leukemia (Table 3). The first case differed substantially from the second case in terms of morphology and clinical behavior: it had a peculiar biphasic and partially alveolar epithelioid growth pattern and followed an aggressive clinical course. A heterozygous mutation was found in the primary tumor, shifting to a homozygous mutation in the metastatic disease,

which might reflect tumor progression. FISH analysis showed polysomy with more than three copies both of centromer 4 and the *KIT* locus in all the analyzed tumor cells. This finding rules out mono-allelic deletion of the wild-type *KIT* locus and is consistent with a duplication of the *KIT* mutant allele. This mechanism was previously described for *KIT* exon 11-mutated tumors by Lasota *et al.*³⁴ They showed that a loss of heterozygosity was attributed to the loss of the wild-type allele with subsequent gain of one or multiple copies of the mutated one. As in our study, the phenomenon was associated with a malignant course of disease.

Our second case was an intermediate risk tumor of spindled subtype, which did not progress under adjuvant tyrosine kinase inhibition with imatinib. The 67-year-old female patient suffered from a small bowel tumor but is well with no evidence of disease 24 months after her surgery.

Exactly the same type of mutation was once reported as p.D419del germline mutation in familial GISTs. We could identify a kindred with both familial GISTs and mastocytosis.²⁴ These familial cases were typical low-risk GISTs with a spindled morphological subtype. By *in vitro* experiments, we could previously show that this particular mutation results in constitutively activated *KIT*, which can be inhibited by imatinib.²⁴ In our present cases, no clue for neither a familial background nor an associated mastocytosis was found, based on information from the patients' files and oral consultation with their physicians.

We conclude that sporadic *KIT* exon 8 mutations in GISTs represent rare events. A small proportion (about 1–2%) of GISTs being formerly reported as 'wild type' might, in fact, carry a mutation in *KIT* exon 8. Only one mutational subtype (p.D419del) was identified so far that was shown to be activating and imatinib sensitive *in vitro*. We therefore propose that the screening for *KIT* exon 8 mutations should be implemented in the routine molecular diagnostics of GIST. In addition, patients with proven *KIT* exon 8 mutations and significant risk for tumor progression should be treated with imatinib.

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Disclosure/conflict of interest

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