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 <i>n</i> = 11	4.0 (1.3–7.5) [3.5] <i>n</i> = 10	8/3/0 <i>n</i> = 11	1.8 (0–10) [1] <i>n</i> = 10	2/0/2/4/0 <i>n</i> = 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 <i>n</i> = 4	0/3/2/0	6.2 (1.2–10.0) [7.0]	4/0/1	1.3 (0–3) [1] <i>n</i> = 4	1/1/1/1/0 <i>n</i> = 4
No association with clinical syndrome								
Primary tumor	121	60.2 (19–98) [60] <i>n</i> = 119	56/61 <i>n</i> = 117	1/48/66/1 <i>n</i> = 116	5.5 (0.1–35.0) [5.0] <i>n</i> = 102	77/26/13 <i>n</i> = 116	4.2 (0–65) [1] <i>n</i> = 116	17/16/25/15/23 <i>n</i> = 96
Metastases/recurrences	6	51.7 (39–73) [48]	3/3	0/0/2/0 ^b <i>n</i> = 2	3.5 (2.5–5.0) [3.0] <i>n</i> = 3	5/1/0	30.4 (0–90) [23] <i>n</i> = 5	NA
Total	145	57.4 (8–98) [60] <i>n</i> = 143	65/75 <i>n</i> = 140	1/53/80/1 ^b <i>n</i> = 135	5.4 (0.1–35.0) [4.5] <i>n</i> = 121	95/30/14 <i>n</i> = 139	4.8 (0–90) [1] <i>n</i> = 136	20/18/28/20/23 <i>n</i> = 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)	<i>KIT</i> 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)	<i>KIT</i> 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 dyade. 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 centromere 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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References

- Hirota S, Isozaki K, Moriyama Y, *et al*. Gain-of-function mutations of c-kit in human gastrointestinal stromal tumors. *Science* 1998;279:577–580.
- Heinrich MC, Corless CL, Duensing A, *et al*. PDGFRA activating mutations in gastrointestinal stromal tumors. *Science* 2003;299:708–710.
- Lasota J, Miettinen M. Clinical significance of oncogenic KIT and PDGFRA mutations in gastrointestinal stromal tumours. *Histopathology* 2008;53:245–266.
- Miettinen M, Sobin LH, Lasota J. Gastrointestinal stromal tumors of the stomach: a clinicopathologic, immunohistochemical, and molecular genetic study of 1765 cases with long-term follow-up. *Am J Surg Pathol* 2005;29:52–68.
- Miettinen M, Makhoul H, Sobin LH, *et al*. Gastrointestinal stromal tumors of the jejunum and ileum: a clinicopathologic, immunohistochemical, and molecular genetic study of 906 cases before imatinib with long-term follow-up. *Am J Surg Pathol* 2006;30:477–489.
- Wardelmann E, Losen I, Hans V, *et al*. Deletion of Trp-557 and Lys-558 in the juxtamembrane domain of the c-kit protooncogene is associated with metastatic behavior of gastrointestinal stromal tumors. *Int J Cancer* 2003;106:887–895.
- Lasota J, Dansonka-Mieszkowska A, Sobin LH, *et al*. A great majority of GISTs with PDGFRA mutations represent gastric tumors of low or no malignant potential. *Lab Invest* 2004;84:874–883.
- Lasota J, Stachura J, Miettinen M. GISTs with PDGFRA exon 14 mutations represent subset of clinically favorable gastric tumors with epithelioid morphology. *Lab Invest* 2006;86:94–100.
- Antonescu CR, Sommer G, Sarraf L, *et al*. Association of KIT exon 9 mutations with nongastric primary site and aggressive behavior: KIT mutation analysis and clinical correlates of 120 gastrointestinal stromal tumors. *Clin Cancer Res* 2003;9:3329–3337.
- Corless CL, Schroeder A, Griffith D, *et al*. PDGFRA mutations in gastrointestinal stromal tumors: frequency, spectrum and *in vitro* sensitivity to imatinib. *J Clin Oncol* 2005;23:5357–5364.
- Debiec-Rychter M, Sciort R, Le Cesne A, *et al*. KIT mutations and dose selection for imatinib in patients with advanced gastrointestinal stromal tumours. *Eur J Cancer* 2006;42:1093–1103.
- Heinrich MC, Owzar K, Corless CL, *et al*. Correlation of kinase genotype and clinical outcome in the North American Intergroup Phase III Trial of imatinib mesylate for treatment of advanced gastrointestinal stromal tumor: CALGB 150105 Study by Cancer and Leukemia Group B and Southwest Oncology Group. *J Clin Oncol* 2008;26:5360–5367.
- Demetri GD, Benjamin RS, Blanke CD, *et al*. NCCN Task Force report: management of patients with gastrointestinal stromal tumor (GIST)—update of the NCCN clinical practice guidelines. *J Natl Compr Cancer Netw* 2007;5(Suppl 2):S1–29; quiz S30.
- Casali PG, Blay JY. Gastrointestinal stromal tumours: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2010;21(Suppl 5):v98–102.
- Wardelmann E, Buttner R, Merkelbach-Bruse S, *et al*. Mutation analysis of gastrointestinal stromal tumors: increasing significance for risk assessment and effective targeted therapy. *Virchows Arch* 2007;451:743–749.
- Daniels M, Lurkin I, Pauli R, *et al*. Spectrum of KIT/PDGFRA/BRAF mutations and phosphatidylinositol-3-kinase pathway gene alterations in gastrointestinal stromal tumors (GIST). *Cancer Lett* 2011;312:43–54.
- Tarn C, Rink L, Merkel E, *et al*. Insulin-like growth factor 1 receptor is a potential therapeutic target for gastrointestinal stromal tumors. *Proc Natl Acad Sci USA* 2008;105:8387–8392.
- Mussi C, Schildhaus HU, Gronchi A, *et al*. Therapeutic consequences from molecular biology for gastrointestinal

- stromal tumor patients affected by neurofibromatosis type 1. *Clin Cancer Res* 2008;14:4550–4555.
- 19 Miettinen M, Fetsch JF, Sobin LH, *et al*. Gastrointestinal stromal tumors in patients with neurofibromatosis 1: a clinicopathologic and molecular genetic study of 45 cases. *Am J Surg Pathol* 2006;30:90–96.
 - 20 Carney JA, Stratakis CA. Familial paraganglioma and gastric stromal sarcoma: a new syndrome distinct from the Carney triad. *Am J Med Genet* 2002;108:132–139.
 - 21 Amar L, Bertherat J, Baudin E, *et al*. Genetic testing in pheochromocytoma or functional paraganglioma. *J Clin Oncol* 2005;23:8812–8818.
 - 22 Janeway KA, Kim SY, Lodish M, *et al*. Defects in succinate dehydrogenase in gastrointestinal stromal tumors lacking KIT and PDGFRA mutations. *Proc Natl Acad Sci USA* 2011;108:314–318.
 - 23 Miettinen M, Lasota J, Sobin LH. Gastrointestinal stromal tumors of the stomach in children and young adults: a clinicopathologic, immunohistochemical, and molecular genetic study of 44 cases with long-term follow-up and review of the literature. *Am J Surg Pathol* 2005;29:1373–1381.
 - 24 Hartmann K, Wardelmann E, Ma Y, *et al*. Novel germline mutation of KIT associated with familial gastrointestinal stromal tumors and mastocytosis. *Gastroenterology* 2005;129:1042–1046.
 - 25 Huss S, Wardelmann E, Goltz D, *et al*. Activating PDGFRA mutations in inflammatory fibroid polyps occur in exons 12, 14 and 18 and are associated with tumour localization. *Histopathology* 2012;61:59–68.
 - 26 Merkelbach-Bruse S, Dietmaier W, Fuzesi L, *et al*. Pitfalls in mutational testing and reporting of common KIT and PDGFRA mutations in gastrointestinal stromal tumors. *BMC Med Genet* 2010;11:106.
 - 27 Fassunke J, Blum MC, Schildhaus HU, *et al*. qPCR in gastrointestinal stromal tumors: evaluation of reference genes and expression analysis of KIT and the alternative receptor tyrosine kinases FLT3, CSF1R, PDGFRB, MET and AXL. *BMC Mol Biol* 2010;11:100.
 - 28 Miettinen M, Lasota J. Gastrointestinal stromal tumors: pathology and prognosis at different sites. *Semin Diagn Pathol* 2006;23:70–83.
 - 29 Cools J, DeAngelo DJ, Gotlib J, *et al*. A tyrosine kinase created by fusion of the PDGFRA and FIP1L1 genes as a therapeutic target of imatinib in idiopathic hypereosinophilic syndrome. *N Engl J Med* 2003;348:1201–1214.
 - 30 Schildhaus HU, Cavlar T, Binot E, *et al*. Inflammatory fibroid polyps harbour mutations in the platelet-derived growth factor receptor alpha (PDGFRA) gene. *J Pathol* 2008;216:176–182.
 - 31 Went PT, Dirnhofer S, Bundi M, *et al*. Prevalence of KIT expression in human tumors. *J Clin Oncol* 2004;22:4514–4522.
 - 32 Beghini A, Larizza L, Cairoli R, *et al*. c-kit activating mutations and mast cell proliferation in human leukemia. *Blood* 1998;92:701–702.
 - 33 Lantermier F, Cohen-Akenine A, Palmerini F, *et al*. Phenotypic and genotypic characteristics of mastocytosis according to the age of onset. *PLoS One* 2008;3:e1906.
 - 34 Lasota J, vel Dobosz AJ, Wasag B, *et al*. Presence of homozygous KIT exon 11 mutations is strongly associated with malignant clinical behavior in gastrointestinal stromal tumors. *Lab Invest* 2007;87:1029–1041.
 - 35 Shih LY, Liang DC, Huang CF, *et al*. Cooperating mutations of receptor tyrosine kinases and Ras genes in childhood core-binding factor acute myeloid leukemia and a comparative analysis on paired diagnosis and relapse samples. *Leukemia* 2008;22:303–307.
 - 36 Molderings GJ, Meis K, Kolck UW, *et al*. Comparative analysis of mutation of tyrosine kinase kit in mast cells from patients with systemic mast cell activation syndrome and healthy subjects. *Immunogenetics* 2010;62:721–727.
 - 37 Bodemer C, Hermine O, Palmerini F, *et al*. Pediatric mastocytosis is a clonal disease associated with D816V and other activating c-KIT mutations. *J Invest Dermatol* 2010;130:804–815.
 - 38 Markova J, Trnkova Z, Michkova P, *et al*. Monitoring of minimal residual disease in patients with core binding factor acute myeloid leukemia and the impact of C-KIT, FLT3, and JAK2 mutations on clinical outcome. *Leuk Lymphoma* 2009;50:1448–1460.
 - 39 Goemans BF, Zwaan CM, Cloos J, *et al*. FLT3 and KIT mutated pediatric acute myeloid leukemia (AML) samples are sensitive *in vitro* to the tyrosine kinase inhibitor SU11657. *Leuk Res* 2010;34:1302–1307.
 - 40 Beghini A, Ripamonti CB, Cairoli R, *et al*. KIT activating mutations: incidence in adult and pediatric acute myeloid leukemia, and identification of an internal tandem duplication. *Haematologica* 2004;89:920–925.
 - 41 Fuster O, Barragan E, Bolufer P, *et al*. Rapid detection of KIT mutations in core-binding factor acute myeloid leukemia using high-resolution melting analysis. *J Mol Diagn* 2009;11:458–463.
 - 42 Goemans BF, Zwaan CM, Miller M, *et al*. Mutations in KIT and RAS are frequent events in pediatric core-binding factor acute myeloid leukemia. *Leukemia* 2005;19:1536–1542.
 - 43 Paschka P, Marcucci G, Ruppert AS, *et al*. Adverse prognostic significance of KIT mutations in adult acute myeloid leukemia with inv(16) and t(8;21): a Cancer and Leukemia Group B Study. *J Clin Oncol* 2006;24:3904–3911.
 - 44 Nanri T, Matsuno N, Kawakita T, *et al*. Mutations in the receptor tyrosine kinase pathway are associated with clinical outcome in patients with acute myeloblastic leukemia harboring t(8;21)(q22;q22). *Leukemia* 2005;19:1361–1366.
 - 45 Gari M, Goodeve A, Wilson G, *et al*. c-kit proto-oncogene exon 8 in-frame deletion plus insertion mutations in acute myeloid leukaemia. *Br J Haematol* 1999;105:894–900.
 - 46 Park SH, Chi HS, Min SK, *et al*. Prognostic impact of c-KIT mutations in core binding factor acute myeloid leukemia. *Leuk Res* 2011;35:1376–1383.
 - 47 Ahmad F, D'Souza W, Mandava S, *et al*. Molecular analysis of WT1 and KIT mutations in patients from an Indian population with de novo acute myeloid leukemia: determination of incidence, distribution patterns, and report of a novel KIT mutation. *Leuk Lymphoma* 2011;52:865–876.

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