Seminars in Diagnostic Pathology (2006) 23, 91-102

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Seminars in
Diagnostic
Pathology

KIT and PDGFRA mutations in gastrointestinal stromal tumors (GISTs)

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KEYWORDS
Gastrointestinal stromal tumors;
KIT;
PDGFRA;
Mutation;
Deletion;
Missense mutation;
Duplication

Mutually exclusive KIT and PDGFRA mutations are central events in GIST pathogenesis, and their understanding is becoming increasingly important, because specific treatment targeting oncogenic KIT and PDGFRA activation (especially imatinib mesylate) has become available. KIT mutations in GIST are clustered in four exons. Most common are exon 11 (juxtamembrane domain) mutations that include deletions, point mutations (affecting a few codons), and duplications (mostly in the 3′ region). The latter mutations most often occur in gastric GISTs. Among gastric GISTs, tumors with deletions are more aggressive than those with point mutations; this does not seem to hold true in small intestinal GISTs. Exon 9 mutations (5-10%) usually are 2-codon 502-503 duplications, and these occur predominantly in intestinal versus gastric GISTs. Lesser imatinib sensitivity of these tumors has been noted. Kinase domain mutations are very rare; GISTs with such mutations are variably sensitive to imatinib. PDGFRA mutations usually occur in gastric GISTs, especially in the epithelioid variants; their overall frequency is approximately 30% to 40% of KIT mutation negative GISTs. Most common is exon 18 mutation leading Asp842Val at the protein level. This mutation causes imatinib resistance. Exon 12 and 14 mutations are rare. Most mutations are somatic (in tumor tissue only), but patients with familial GIST syndrome have consitutitonal KIT/PDGFRA mutations; >10 families have been reported worldwide with mutations generally similar to those in sporadic GISTs. GISTs in neurofibromatosis 1 patients, children, and Carney triad seem to lack GIST-specific KIT and PDGFRA mutations and may have a different disease mechanism. Secondary mutations usually occur in KIT kinase domains in patients after imatinib treatment resulting in resistance to this drug. Mutation genotyping is a tool in GIST diagnosis and in assessment of sensitivity to kinase inhibitors.

Because KIT and PDGFRA mutations are a driving force in GIST pathogenesis and specific treatment for oncogenic KIT/PDGFRA activation now exists, understanding of biology of these mutations is becoming increasingly importat in GIST management.

KIT maps to chromosome 4q12 and encodes for a 109870 D transmembrane glycoprotein. PDGFRA (platelet-derived growth factor receptor α) is located adjacent to KIT and encodes for a 122676 D transmembrane glycoprotein highly homologous to KIT.[1,2] Both KIT and PDGFRA belong to the type III receptor tyrosine kinase family and might have evolved, similar to CSF1R and PDGFRB at 5q31-33, from a common ancestral gene by gene duplication.[3,4] Platelet-derived growth factor receptor β (PDGFRB), a colony-stimulating factor-1 receptor (CSF1R), and FMS-related tyrosine kinase 3 (FLT3) are other members of type III receptor tyrosine kinase family.[5]

Members of the type III tyrosine kinase receptor family are transmembrane proteins with a characteristic structure (Figure 1). The extracellular/ligand-binding (EC) domain consists of five Ig-like loops. The cytoplasmic domain consists of juxtamembrane (JM) and tyrosine kinase (TK) domains. The latter is divided into an adenosine triphosphate (ATP) binding region (TK1) and a phosphotransferase region (TK2) by a hydrophilic kinase insert (KI). The ex-

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doi:10.1053/j.semdp.2006.08.006

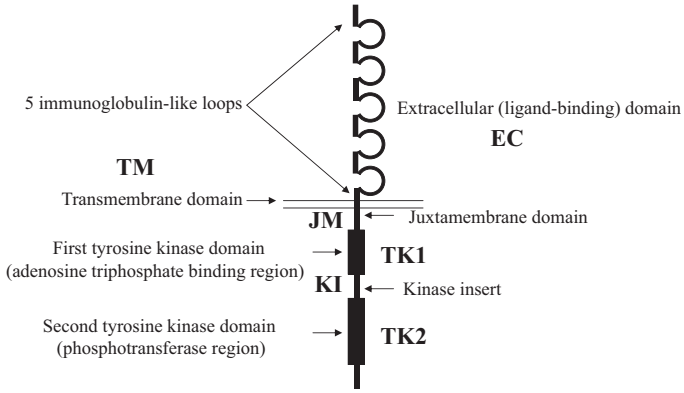

Figure 1 Schematic structure of the type III tyrosine kinase receptor family gene.

tracellular and cytoplasmic domains are connected by a transmembrane region.[5]

Tyrosine kinase receptors are activated by specific ligands. The binding of ligand induces dimerization of the receptor polypeptides, activates receptor kinase activity, and leads to trans-autophosphorylation of the dimer partners. Subsequently, intracellular adaptor proteins bind to the receptor phosphotyrosine residues and recruit other downstream signaling molecules activating networks of signal transduction pathways (Figure 2A), ultimately leading to modulation of nuclear regulatory proteins.[5,6]

KIT is normally activated by stem cell factor (SCF), previously also called Steel factor. Activation of KIT regulates important cell functions, including proliferation, apoptosis, chemotaxis, and adhesion, and is critical for the development and maintenance of mast cells, hematopoietic stem cells, melanocytes, gametocytes, and interstitial cells of Cajal (ICC), pacemaker cells involved in regulation of the gastrointestinal (GI) tract mobility and autonomous neural transmission.[7-14]

PDGFRs are normally activated by platelet-derived growth factors (PDGFs) and expressed on hematopoietic cells, including erythroid and myeloid bone marrow precursor cells, monocytes and megacaryocytes as well as glial cells, endothelial cells, fibroblasts, and osteoblasts.[6]

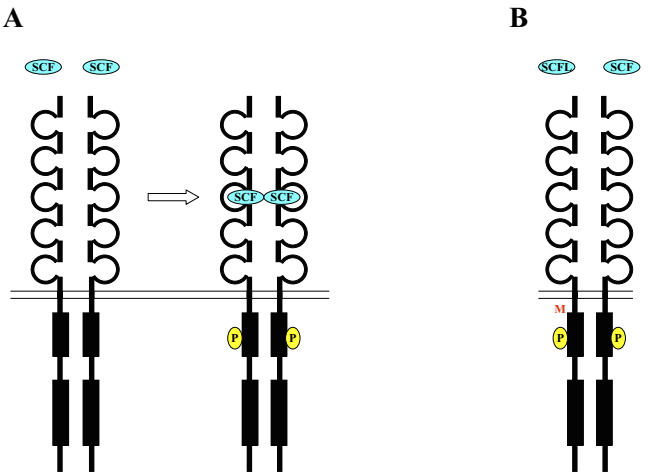

Figure 2 KIT activated by stem cell factor (SCF); the binding of ligand induces dimerization of the receptor polypeptides and leads to trans-autophosphorylation of the dimer partners (A). KIT activated by gain-of-function mutation (M) independently of ligand binding signal.

Overview of KIT and PDGFRA mutations in GISTs

The mutation nomenclature used in this review follows recommendations of the Human Genome Variation Society (http://www.hgvs.org). Nucleotide numbering is based on human KIT (X06182) and PDGFRA (M21574) mRNA sequences and dog KIT (AF044249) mRNA from GeneBank (at http://www.ncbi.nlm.nih.gov).

Gain-of-function KIT and PDGFRA mutations are considered to be a major driving force in the pathogenesis of sporadic, nonfamilial GISTs.[15,16] Based on location, these mutations can be divided in two classes: mutations of the regulatory domain including EC and JM, and mutations of the enzymatic domain including TK1 and TK2.[17] Mutations affecting the regulatory domain can lead to ligand-independent receptor dimerization and subsequent kinase activation (Figure 2B), whereas mutations affecting the enzymatic domain can lead to kinase activation, perhaps without receptor dimerization.[6,18] Mutational alteration of the regulatory or enzymatic domains has been shown to dysregulate tyrosine kinase activity and lead to continuous receptor activation independent of ligand binding signal. In vitro experiments documented that mutant KIT, when expressed in a cell line, elicited transforming ability.[15,19-21] Families with activating germ line KIT or PDGFRA mutations develop multiple GISTs, ICC hyperplasia, and variably hyperpigmentation and urticaria pigmentosa.[22-33] Recently developed transgenic mice carrying an inherited gain-of-function KIT exon 11 (Val558del) or KIT exon 13 (Lys642Glu) mutations and reproducing human familial GIST syndrome confirmed that mutational activation of KIT plays an essential role in oncogenesis.[34,35] However, a study based on HUMARA (human androgen receptor assay) showed that diffuse proliferations of interstitial cells of Cajal in a patient

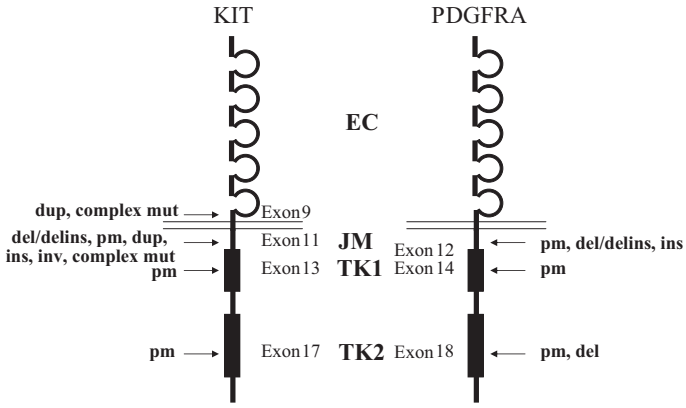

Figure 3 Distribution and types of KIT and PDGFRA mutations identified in sporadic GISTs.

with familial GISTs represented polyclonal nonneoplastic hyperplasia, whereas their GISTs were monoclonal.[36] This suggests that the growth of a GIST might require additional genetic changes beyond activating KIT or PDGFRA mutations. A recent study showed frequent KT mutations in ICCs from normal tissue surrounding gastric GISTs, whereas counterpart ICCs in gastric cancer harbored no such mutations.[37] This suggests that KIT-mutated ICCs represent precancerous cells; however, more studies should be done to confirm such a hypothesis.

In sporadic GISTs, a great majority of mutations (>90%) have been identified in KIT-JM domain encoded by exon 11.[38] In addition, mutations in KIT-EC (exon 9) and -TK1 (exons 13) and -TK2 (exon 17) domains have been reported in a smaller number of cases.[39-41] Subsequently, subset of KIT-wild type (WT) GISTs has been shown to have activating PDGFRA mutations.[16] A great majority of these mutations were found in PDGFRA-TK2 (exon 18) domain; however, in a few cases mutations in PDGFRA-JM (exon 12) and -TK1 (exon 14) domains also have been reported.[42-44]

The following mutation types have been identified in KIT and PDGFRA: deletions (del), deletion–insertion (delins), point mutations (pm), duplications (dup), insertions (ins), and inversion (inv). The latter two types are extremely rare, and only a few GISTs with such mutations have been reported. Figure 3 summarizes distribution and types of mutations affecting different KIT and PDGFRA domains. For comparison, data on other human and canine tumors with documented KIT mutations are shown in Figure 4. These tumors include acute myeloid leukemia (AML),[45,46] mast cell leukemia/mastocytosis,[47,48] sinonasal NK/T-cell lymphoma,[49] and seminoma.[50-52]

KIT and PDGFRA mutations reported in familial GIST syndrome[22-33] are structurally similar to those found in sporadic GISTs (Figure 5). However, members of a recently described family with GIST and mastocytosis carried constitutional inherided KIT-EC (exon 8) domain mutation 1276_1278delGAC (Asp419del), never reported in sporadic GISTs.[53] Identical Asp419del was previously reported in the patients with acute myeloid leukemia.[44,45]

In GISTs, KIT and PDGFRA mutations are believed to be mutually exclusive, and only one type of either KIT or PDGFRA mutation can be present in primary tumor and its recurrent or metastatic lesions.[16,54] The presence of two different KIT or PDGFRA mutations affecting the same or different exons has been reported in a few cases.[42,55-58] More recently, double KIT exon 11 mutations have been found in as many as 9% (7 of 78) of primary tumors in 1 study.[59] Also, coexistence of missense and silent KIT exon 11 mutations and missense and nonsense KIT exon 11 mutations was reported twice in the primary tumors.[60,61] Although similar coexistence of different KIT mutations in KIT-TK2 domain was reported in primary mediastinal seminoma,[50] these seem to be extremely rare events in GISTs, since we have not seen double KIT exon 11 mutations in 800 KIT exon 11-mutant GISTs diagnosed at the Armed Forces Institute of Pathology (AFIP). However, apparent nucleotide substitutions were seen in a few cases, but were not reproducible on the same DNA template. Some of such findings may represent PCR artifacts mimicking point mutations, and they have been reported in PCR-based mutation analysis of DNA from formalin-fixed paraffin-embaded (FFPE) tissues.[62]

Although KIT and PDGFRA mutations are believed to represent gain-of-function mutations, three human GISTs

KIT		Mutation type		Human				Canine	
EC	Exon 2	pm	AML						
	Exon 8	delins	AML						
		del	AML						
		dup	AML						
	Exon 9	dup	AML						
JM	Exon 11	dup	AML						Mastocytoma
		del						GIST	Mastocytoma
		pm		Mastocytosis	NK/T-cell Lyphoma	Seminoma	GIST		Mastocytoma
KI	Exon 16	pm	AML						
TK2	Exon 17	pm	AML	Mastocytosis	NK/T-cell Lyphoma	Seminoma			

Figure 4 Distribution and types of KIT mutations identified in different human and canine tumors.

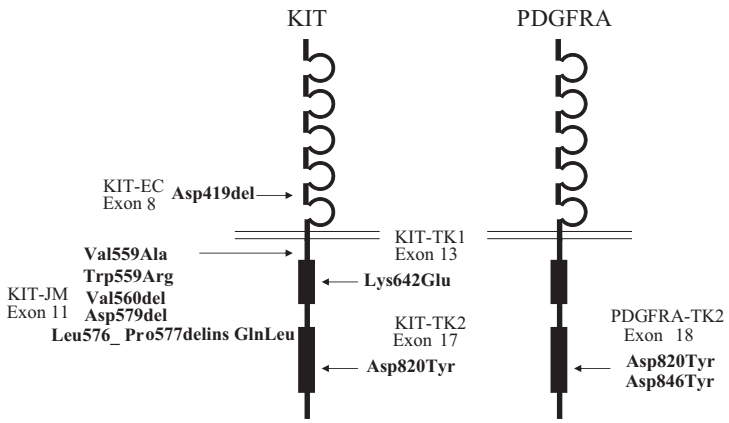

Figure 5 KIT and PDGFRA mutations identified in familial GISTs.

with point mutations causing STOP codon in KIT exon 11 and KIT exon 13,[61,63] and one canine mastocytoma with KIT exon 11 duplication causing a frame shift and STOP codon have been reported.[19] These rare nonsense mutations might, in some cases, reflect secondary changes occurring during tumor progression.

The majority of KIT and PDGFRA mutations are heterozygous. However, in some cases, only mutant allele could be identified by direct sequencing of PCR products.[38,64-66] This may represent true homozygous mutations,[38] or hemizygous mutations created by loss of a second allele.[66,67] Preferential amplification of the allele can mimic homo- or hemi- zygousity as well. Homozygous/hemizygous mutations have been identified in 2 of 13 (15%) incidental GISTs smaller than 1 cm[64]; identical frequency of these mutations has been reported in a study of 56 primary gastric GISTs.[65] However, little is known about their biological potential. Also, shift from KIT/PDGFRA heterozygosity to hemizygosity has been reported in progressive lesions during imatinib treatment.[66,67] An example of homozygous/hemizygous KIT exon 11 mutation is shown in Figure 6.

Two alternative splicing sites in KIT, which occur at the 3′ end of exon 9 (EC) and 5′ end of exon 15, have been reported.[68,69] Physiological variants of KIT mRNA should not be confused with pathologically altered KIT-mutant mRNA, and the presence of mutation detected at mRNA level should be confirmed in genomic DNA. A recently reported apparent Ser715del in KIT exon 15 encoding KI[57] has been shown to represent a physiological KIT splicing event.[70]

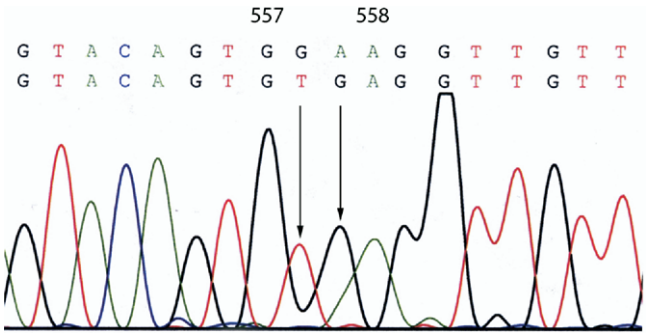

Figure 6 Example of homozygous/hemizygous (1692_1693delinsTG) KIT exon 11 mutation. KIT-WT is shown above KIT mutant sequence.

KIT regulatory domain mutations (exon 9, exon 11)

Nearly all mutations identified in KIT exon 9, a distal part of the KIT-EC domain, represent short, structurally identical duplications of six nucleotides, 1525_1530dupGCCTAT leading to the Ala502_Tyr503dup at the protein level.[61,71-73] However, more recently, another duplication 1537_1545 dupTTTGCATTT leading to the Phe506_Phe508dup at the protein level was reported.[72] Two such duplications have been found among 60 (3.3%) KIT exon 9 mutant GISTs identified at AFIP. An Ala502_Phe506dup affecting the same region of KIT exon 9 has been found in a patient with acute myeloid leukemia.[46] A point mutation leading to Glu490Gly substitution at the protein level[61] and a complex mutation consisting of deletion and inversion of several nucleotides[73] have been recently identified in 2 GISTs as well.

The Ala502_Tyr503dup was first reported in 6 of 8 GISTs that lack KIT exon 11 mutations.[39] A study based on

Table 1 Location of Ala502_Tyr503dup mutant GISTs

Population	No. of cases	Location		
		Gastric	Intestinal	Other non-gastric
Asian	22	7 (31.8%)	13 (59.1%)	2
Western*	106	7 (6.6%)	95 (89.6%)	4
Total	128	14 (10.9%)	108 (84.4%)	6

*Includes 48 GISTs with known location from previously published European, American, and Australian studies and 58 cases from AFIP GIST mutation database.

Table 2 Occurrence of different mutation types among KIT exon 11 mutant GISTs

Study	Type of KIT exon 11 mutation				
	del/delins	pm	dup	Other	Total
Lasota et al.[54]	17 (81%)	4 (19%)	0	0	21
Taniguchi et al.[55]	54 (76.1%)	14 (19.7%)	0	3 (4.2%)	71
Rubin et al.[41]	20 (76.1%)	9 (26.5%)	3 (8.8%)	2 (5.9%)	34
Wardelman et al.[85]	12 (63.2%)	4 (21.1%)	2 (10.5%)	1 (5.3%)	19
Antonescu et al.[74]	51 (63%)	20 (24.7%)	8 (8.9%)	2 (2.5%)	81
Martin et al.[61*]	40 (53.3%)	21 (27.3%)	9 (14.3%)	4 (4%)	74
Andersson et al.[81]	101 (71.6)	23 (16.3%)	17 (12.1)	0	141
Total	295 (66.9%)	95 (21.5%)	39 (8.8%)	12 (2.7%)	441

*Three GISTs with double mutations and one with STOP codon mutation are included in the "other" category.

200 cases has shown the frequency of this type of KIT mutation to be approximately 5% among GISTs from different locations. Also, predilection to intestinal tumors has been suggested.[40] Subsequent studies have revealed strong correlation between intestinal location and presence of Ala502_Tyr503dup.[71,74] However, gastric GISTs with 1525_1530dupGCCTAT have been also reported in the literature.[56,75,76] Based on Western population studies,[64,66,74,76-81] such tumors represent only 6.6% of all KIT exon 9 mutant GISTs. In contrast, combined studies from Asia[56,75,82-84] indicated almost 32% frequency of gastric GISTs among all KIT exon 9 mutants (Table 1). This substantial difference in frequency might be related to ethnical differences between Western and Asian populations.

KIT-JM domain encoded by exon 11 is the most common mutational "hot spot" in GISTs.[38] This helical domain functionally represents an inhibitory element regulating the KIT autophosphorylation in response to growth factor signal by SCF.[5,47,48] Mutations in KIT-JM were the first ones described in GISTs[15] and have been shown to cause constitutive receptor phosphorylation and transforming in murine lymphoblast cell lines in vitro.[15,21] Several types of KIT exon 11 mutations, including deletions, deletion–insertions, point mutations, duplications, insertions, and inversions, have been documented in GISTs.

A great majority of KIT exon 11 mutations are deletion/deletion–insertions (Table 2) leading to the loss of one to several amino acids and occasional insertions of one to two amino acids at the protein level. Typically, such mutations cluster in the 5′KIT exon 11 between 1669_1704 (Lys550_Glu561), but sometimes extend distally involving large portion of exon 11 and eliminating almost two-thirds of KIT-JM.[15,41,54,61,74,81,85] A 1690_1695delTGGAAG (Trp557_Lys558del) is the most common simple deletion identified in GISTs. Deletions in the distal part of exon 11 are seen less frequently. However, their functional significance appears to be similar to the ones seen in 5′KIT exon 11. For example, Asp579del has been shown to cause constitutive phosphorylation of KIT.[21]

More recently, deletions affecting KIT intron 10–exon 11 splice-acceptor sites have been reported. These different size deletions always create a novel intraexonic pre-mRNA 3′ splice acceptor site consistently leading to in-frame Lys550_Lys558del at the protein level.[86,87] According to one study, these mutations were not uncommon and account for 3.9% of KIT exon 11 mutations.[86]

Missense mutations represent the second-most common type of KIT exon 11 mutations in GISTs (Table 2). These mutations cluster in 5′ KIT exon 11 and almost exclusively involve KIT codons 557, 559, and 560[41,55,60,64,66,74,76,78,81,88,90-93] (Figure 7). The Val559Asp, Val560Asp, and Trp557Arg followed by Val559Ala, Val559Gly, and Leu576Pro are the most common missense mutations reported in KIT exon 11 (Table 3). The latter substitution caused by 1748T >C point mutation maps to 3′ KIT exon 11.[41,54,85] Identical Leu576Pro has been found in canine GISTs[94] and canine mastocytoma[20] and more recently in a subset of malignant melanomas.[95,96] This substitution has been shown to cause ligand-independent KIT autophosphorylation.[20]

Duplications, often called internal tandem duplications represent the third-most common type of KIT exon 11 mutations in GISTs (Table 2). These mutations cluster almost exclusively in 3′KIT exon 11 and only 2 of 70 reported[41,61,74,76,77,81,83-85,97-101] affected central and 5′KIT exon 11.[74,84] Size of the duplications varies from 1 to 18 codons and with 1 exception[76] never involved KIT intron 11 and KIT exon 12.[74,61,81,101] Similar KIT exon 11 duplications occasionally involving 3′ KIT exon 11–intron 11–exon 12 have been described in adult patients with acute myeloid leukemia,[45,46] canine mastocytoma,[19] and more recently in pediatric patients with acute myeloid leukemia.[102] Duplications in KIT are associated with constitutive receptor phosphorylation, ligand-independent growth, apoptosis resistance, and altered downstream signaling pathways.[19,20,102] Structurally similar duplications have been found in the juxtamembrane domain of Flt-3, another member of type III receptor tyrosine kinase family in adult and pediatric patients with acute myeloid leukemia.[103,104]

Insertions and inversions are extremely rare KIT exon 11 mutations. A 1694_1695ins TCC leading to Lys558delinsAsnPro at the protein level has been reported in a few cases.[41,55,57,74,85]

Inversions have not been reported in KIT exon 11 in GISTs; however, such mutations, sometimes coexisting

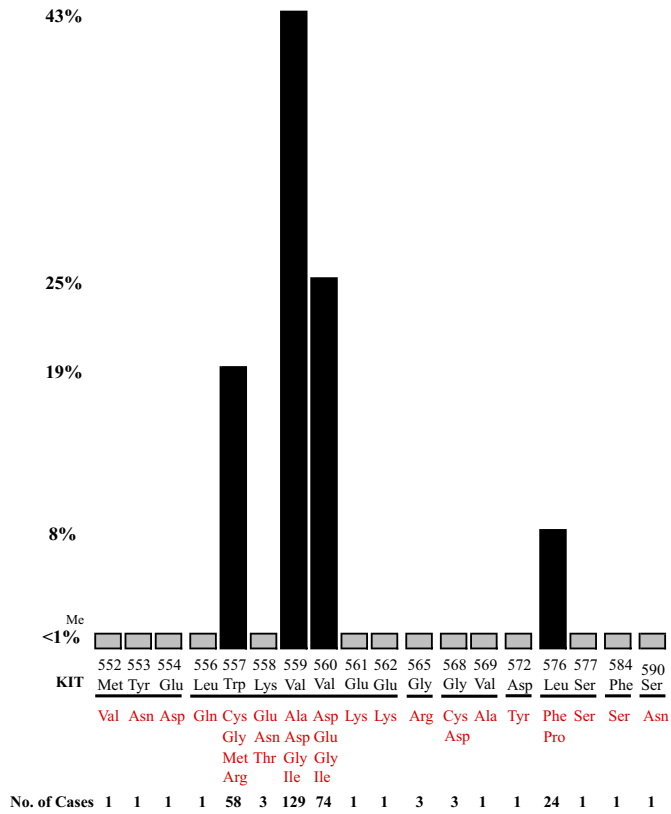

Figure 7 Distribution of 305 KIT exon 11 missense mutations identified in sporadic GISTs. KIT-WT and codon numbers are in black. KIT-mutants are in red.

with deletions (Figure 8), were identified by us in 5′KIT exon 11 (J.L., unpublished observation).

Although KIT exon 11 mutations have been reported in GISTs from different locations from esophagus to anus,[105-112] duplications showed strong predilection to gastric location; 59 of 67 (88%) reported GISTs with KIT exon 11 duplications originated from stomach.[61,74,76,77,83,84,97,101,113]

KIT enzymatic domain mutations (exon 13, exon 14, exon 17)

A 1945A>G point mutation resulting in Lys642Glu substitution at the protein level was initially reported in two GISTs negative for KIT-JM mutation.[39] This mutation affects exon 13 encoding proximal part of the KIT-TK1 (ATP-binding domain) and has been found to lead to constitutive KIT tyrosine phosphorylation.[39] A subsequent study of a relatively large number of GISTs from different locations estimated the frequency of this mutation to be no higher than 2.5%.[40,56]

Recent studies on GISTs, based on Asian population, have reported three tumors with unique missense mutations (Leu641Pro, Val643Ala, and Leu647Pro) affecting KIT exon 13 in the vicinity of Lys642. The biological potential of these mutations is uknown.[114,115]

Table 3 The most common missense mutations reported in KIT exon 11 in GISTs

KIT-mutants	No. of cases	% of mutants in specific KIT codons	% of all KIT exon 11 point mutations
Trp557 mutants			
Trp557Arg	39	67%	13%
Trp557Gly	16	28%	5%
Trp557Cys	2	4%	<1%
Trp557Met	1	2%	<1%
Total: 58			
Val559-mutants			
Val559Asp	82	64%	27%
Val559Ala	23	18%	8%
Val559Gly	23	18%	8%
Val559Ile	1	<1%	<1%
Total: 129			
Val560-mutants			
Val560Asp	58	77%	19%
Val560Gly	10	13%	3%
Val560Glu	5	7%	2%
Val560Ile	1	1%	<1%
Total: 74			
Leu576-mutants			
Leu576Pro	23	96%	8%
Leu576Phe	1	4%	<1%

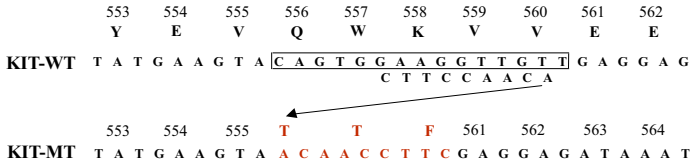

Figure 8 A complex KIT exon 11 mutation consisting of deletion and insertion of inverted complementary sequenced. The boxed nucleotides (KIT-WT) are deleted and inverted complementary sequence (red) is inserted in KIT-mutant (MT).

A 2131_2136delAAGAAT leading to Lys704_Asn705del in exon 14 at the protein level, encoding a distal part of the KIT-TK1 (ATP-binding domain), was reported in a GIST with KIT exon 11 deletion.[57] However, no such mutation was found in a subsequent study of 31 tumors negative for mutations in other KIT "hot spots," suggesting that this mutation must be rare.[70]

An Asp816Val mutation affecting KIT TK2 domain (exon 17) was the first to be identified in KIT-associated mastocytosis and urticaria pigmentosa and shown to cause ligand-independent autophosphorylation of KIT.[47,48] Although this mutation has never been found in GISTs, a 2487T>A and 2485A>C leading to Asn822Lys and Asn822His at the protein level, respectively, were reported in a few cases.[41,72,77,78,81] Similar missense KIT exon 17 mutations were found in human gonadal germ cell tumors of seminoma/dysgerminoma type, mediastinal seminomas, and sinonasal natural killer/T-cell lymphomas.[49-52] The latter also showed missense mutations affecting KIT juxtamembrane domain.[49]

PDGFRA regulatory domain mutations (exon 12)

Mutations in PDGFRA-JM domain are relatively rare and represent approximately 6% to 9% of all PDGFRA mutations reported in GISTs.[42,43] These mutations consist of point mutations, deletions, deletion–insertions, and insertions.[16,42,43] The most common is 1821T>A leading to Val561Asp substitution at the protein level followed in frequency by deletion/deletion insertions and insertions. In general, these mutations affect the vicinity of codon 561 or a region located immediately 3′ to this codon.

PDGFRA enzymatic domain mutation (exon 14, exon 18)

An Asn659Lys in PDGFRA exon 14 was first reported in KIT-negative gastric GIST.[116] Subsequently, 2 more cases with such missense mutations were reported,[43] and a study based on 200 GISTs negative for KIT exon 9, 11, 13, and 17 and PDGFRA exon 12 and 18 mutations identified 11 PDGFRA exon 14 mutations.[42] A majority of these 11 mutations represented 2125C>A and 2125C>G leading to Asn659Lys at the protein level. However, in 3 cases, variant point mutations, 2123A>T leading to Asn659Tyr, were found instead. PDGFRA exon 14 mutations were linked to gastric location, epithelioid morphology, and low malignant potential/favorable course of disease.[42]

Exon 18 encoding part of TK2 domain harbors almost 90% of PDGFRA mutations and is the most common PDGFRA mutational "hot spot" in GISTs.[42,43] A great majority (70%) of mutations identified in this exon represent missense mutation 2664A>T leading to Asp842Val at the protein level. However, in the vicinity of codon 842, deletion/deletion–insertions have been identified as well. GISTs with PDGFRA exon18 mutations have shown strong predilection to gastric location and epithelioid morphology.[42,76,78] A substitution of tyrosine for the highly conserved aspartic acid at codon 846 (Asp846Tyr) has been reported in both sporadic and familial GISTs. An Asp846Tyr mutation is homologous to KIT exon 17 Asp820Tyr mutation also reported in familial GISTs.[42]

In vitro studies revealed that PDGFRA mutations similarly to KIT mutations cause constitutive receptor phosphorylation and activation of downstream MAPK (mitogen-activated protein kinase) and STAT (signal transducers and activators of transcription) signaling pathways.[16,43,117]

KIT and PDGFRA mutational status in NF1 and pediatric and Carney triad GISTs

Several studies evaluated KIT and PDGFRA mutation status in GISTs from neurofibromatosis type 1 (NF1) patients.[118-124] In general, no mutations in GIST-specific KIT or PDGFRA mutational "hot spots" have been found in multiple tumors from NF1 patients. However, one study identified two KIT (Pro627Leu and Ile653Thr) and two PDGFRA (Pro589Lys and Arg822Ser) missense mutations in two separate lesions from two patients.[120] These mutations might be random genetic events related to the tumor progression. In another study, an identical 1697T>A mutation leading at the protein level to Val559Asp substitution has been identified in three tumors from one patient.[121] Although the patient and first-degree relatives revealed phenotypic features typical for NF1, presence of identical mutations in separated tumors raises the possibility of KIT germline mutation. Unfortunately, the authors were not able to genotype normal tissue and exclude such possibility.

Similarly, studies on pediatric GISTs failed to identify KIT or PDGFRA GIST-specific mutations in a substantial

number of cases.[125-127] However, two separate studies reported KIT exon 9 (Pro456Ser) missense mutation and PDGFRA exon 18 nonsense mutation, respectively.[128,129] These mutations do not correspond to the GIST-specific KIT and PDGFRA mutations and both may represent random molecular events related to ongoing molecular changes in progressing cancer.

Also, no KIT or PDGFRA mutations were identified in GISTs from two cases of Carney triad[122,130] and in a case of rare variant of Carney triad, paraganglioma-gastric stromal sarcoma syndrome.[131]

Frequency of KIT and PDGFRA mutations

Frequency of the KIT and PDGFRA mutations differs between the studies. Several factors contribute to these differences. First, KIT and PDGFRA mutations are unequally distributed among GISTs. For example, studies with a large number of intestinal GISTs will show a higher frequency of KIT exon 9 mutants, whereas studies with a higher number of gastric epithelioid tumors will show a lower number of KIT-mutants and a higher number of PDGFRA-mutants. Moreover, studies based on material from cancer centers and treatment trials might include more KIT-mutants linked to malignant, clinically aggressive GISTs, and fewer PDGFRA-mutants linked to GISTs with indolent course. Thus, the overall frequency of KIT and PDGFRA mutations can be only established based on population studies free on selection bias. However, such studies based on archival material might face technical problems related to the detection of KIT and PDGFRA mutations in FFPE tissues. A mutation detection rate tends to decrease with increasing age of paraffin blocks as reported independently by two different groups.[81,111] Also, large duplications may not be amplifiable from partially degraded DNA.

In our recent population study on GISTs diagnosed in Northern Norway during a 30-year period from 1974 to 2003, frequency of KIT and PDGFRA mutations were 75% and 10%, respectively (J.L., unpublished observation).

Ethnic differences between study populations cannot be completely excluded. For example, none of 172 GISTs, including 122 gastric cases studied in Japan,[55,60] revealed duplications in 3′KIT exon 11. In contrast, the frequency of this type of KIT mutation in Western population varies from 4% to 10% for GISTs from different locations.[74,81,101] Technical problems limiting detection of duplications in FFPE tissues may contribute to this discrepancy, since other studies from Korea and China have been reported KIT exon 11 duplications in GISTs.[83,84,99,100]

Prognostic value of KIT and PDGFRA mutations

The prognostic value of KIT and PDGFRA mutations in primary tumors is controversial. Some of the early studies reported that KIT exon 11 mutations are more common in large and malignant GISTs,[54,55] and adverse prognostic significance of such mutations was suggested.[54,55,90] However, others have also shown these mutations in diminutive, clinically indolent incidental tumors.[64]

More recent studies, based on larger numbers of cases and evaluating both KIT and PDGFRA mutational status, indicated that the type of KIT mutation may correlate with clinical outcome in gastric GISTs. A study based on 421 cases with mutation analysis showed that gastric tumors with KIT exon 11 deletions follow a more malignant course then ones with point mutations.[111] Two other studies pointed that KIT Tyr557_Lys558del represents a statistically significant adverse factor.[61,132] Also, recent studies suggested that PDGFRA-mutant tumors tend to have a low mitotic rate and favorable prognosis.[42,111]

The Ala502_Tyr503dup has been previously associated with clinically malignant tumors and poor outcome.[71,74] However, a recent study based on 145 small intestinal GISTs did not show significant differences in tumor behavior between GISTs with this mutation and tumors with KIT exon 11 mutations. Thus, the previously reported association between Ala502_Tyr503dup and malignancy is most likely a consequence of the high mortality of patients with small intestinal GISTs, as opposed to gastric ones.[112]

A study of 200 GISTs identified two malignant GISTs with 1945A>G (Lys642Glu) and suggested that this mutation might be associated with malignant behavior.[40] Although subsequently two more malignant GISTs with Lys642Glu have been reported,[61,133] other study identified four low- and intermediate-risk tumors with such mutations.[134] Prognostic value of rare KIT exon 13 and KIT exon 17 mutations requires further studies.

Primary KIT and PDGFRA mutations and imatinib-based treatment

Imatinib mesylate, STI571, commercially known as Gleevec/Glivec™ (http://www.novartis.com) that inhibits KIT, PDGFRA, and ABL tyrosine kinases has been used in the treatment of clinically advanced, unresectable, and metastatic GISTs.[135,136] More recently, sunitinib malate, also known as SU11248 (http://www.pfizer.com), that inhibits KIT and some other tyrosine kinases has also been approved on the same indication.[137,138]

A great majority of patients benefit from imatinib mesylate-based treatment. However, resistance often develops.[139] Type of KIT or PDGFRA mutation may have an impact on imatinib sensitivity.[17] KIT exon 11 mutant GISTs showed better response to imatinib treatment than KIT exon 9 mutant tumors and ones with KIT-WT.[140] A recent study suggested use of higher dose of Gleevec for treatment of KIT exon 9 mutant GISTs.[141] In vitro experiments and preliminary clinical data suggest that GISTs with PDGFRA Asp842Val substitution causes primary resistance to

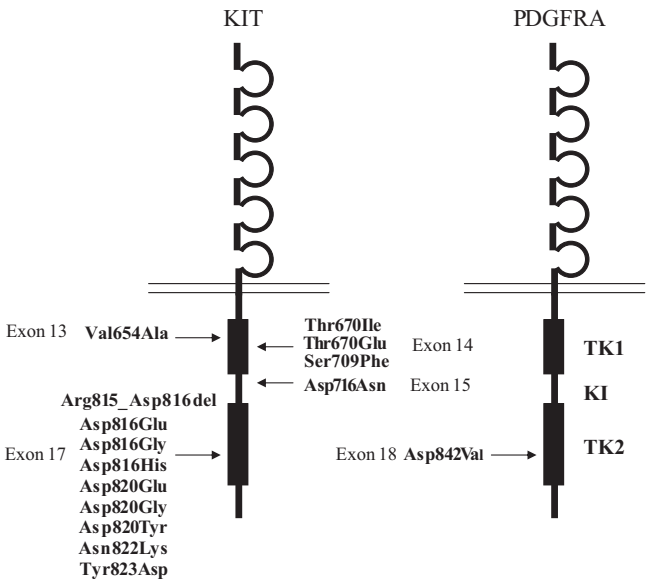

Figure 9 Secondary KIT and PDGFRA mutations acquired during imatinib treatment.

Gleevec.[72] This mutation corresponds to imatinib-resistant KIT Asp816Val mutation in human mastocytosis.[72]

However, an in vitro study showed that other PDGFRA mutants decrease phosphorylation in the presence of imatinib, suggesting that they are imatinib-sensitive.[43]

These findings indicate that KIT and PDGFRA mutational status could be a useful parameter in planning imatinib-based therapy in patients with advanced GISTs.

Secondary KIT and PDGFRA mutations acquired during imanitib-based treatment

An acquired resistance has been reported during imatinib-based treatment and linked to secondary KIT or PDGFRA mutations.[66,142] Initial studies showed that secondary KIT mutations occur in the allele that harbors primary gain-of-function KIT mutation and in a great majority of cases represents missense point mutation affecting the first or second tyrosine kinase domain (Figure 9).[66,89,143-145] Subsequently, polyclonal evolution of multiple secondary KIT mutations has also been reported.[146,147] Clinical significance of monoclonal versus polyclonal evolution is unknown. However, involvement of the first versus second tyrosine kinase domain by secondary KIT mutations may indicate predisposition to more aggressive behavior with earlier metastasis and shorter progression-free survival.[147] Comprehensive, prospective studies are necessary to clarify the significance of KIT and PDGFRA secondary mutations in metastatic lesions and their impact on therapeutic strategies.

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