Cancer Genetics 233–234 (2019) 1–6

Cancer Genetics

A familial germline mutation in KIT associated with achalasia, mastocytosis and gastrointestinal stromal tumors shows response to kinase inhibitors

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Abstract

Background: Activating mutations of the tyrosine kinase receptor KIT have been described in both mastocytosis and gastrointestinal stromal tumors (GIST), but are usually found in separate domains and often respond differently to signal transduction inhibitors. We describe here a large family with both GIST, mastocytosis, and achalasia. Affected family members have a unique activating mutation in exon 9 of KIT which show promise to a novel signal transduction inhibitor.

Methods: Clinical data was collected from 15 family members, 7 of whom were variably affected with GIST, achalasia and mastocytosis. DNA was prepared from WBC of 12 subjects (6 affected and 6 unaffected) and exons 9, 11, 13 and 17 of KIT were amplified by PCR and directly sequenced.

Results: A unique activating single base pair mutation in the extracellular domain of KIT was found in all 6 affected subjects resulting in a K>I amino acid change at codon 509.

Conclusions: In the family reported here, a unique mutation in the extracellular domain leads to receptor activation resulting in GIST and mastocytosis as well as achalasia. Initial data suggests that this activation can be suppressed by signal transduction inhibitors and these patients may benefit from such therapy.

Keywords GIST, Mastocytosis, Achalasia, KIT, Imatinib, CD117, Signal transduction.

Introduction

In 1990, Marshall et al. [1] reported a unique family spanning four generations with what appeared to be an autosomal dominant inherited form of achalasia with diffuse esophageal leiomyomatosis, urticaria pigmentosa and mast cell disease. The molecular and genetic basis for the constellation of clinical problems was not evaluated. Subsequently there have been numerous reports of variants of this syndrome associated with activating mutations in the tyrosine kinase receptor KIT [2–12]. Alterations of KIT are of significant interest since patients with some of these mutations and clinical syndromes may respond to small molecular weight signal transduction inhibitor therapy such as imatinib. We report here the characterization of the molecular defect in the family originally described by Marshall as a unique mutation in the extracellular domain of KIT that is responsive to signal transduction inhibitors.

Both germline and somatic mutations have been previously described in the proto-oncogene KIT in gastrointestinal stromal tumors (GIST) and systemic mastocytosis. [2-22]. KIT encodes a transmembrane tyrosine kinase receptor for stem cell factor (SCF) both of which are essential for normal hematopoiesis, melanogenesis and gametogenesis. The overall gene spans more than 70 kb of DNA and includes 21 exons which are alternatively spliced to give several transcripts, the longest of which is 5230 bp [1,13,14,23,24]. Inactivation of KIT or its ligand SCF results in pigmentary, hematopoietic and germ cell defects in mice and pigmentary defects in man, cattle, swine and horses. In addition, mice that

Received August 13, 2018; received in revised form November 16, 2018; accepted February 19, 2019
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https://doi.org/10.1016/j.cancergen.2019.02.001

Table 1 Clinical characteristics of the seven affected family members studied.

Mutation	Number*	Age	Sex	Clinical Problem
Yes	2	51	F	Achalasia, Mastocytosis, GIST resected after GI bleed at Age 42
Yes	3	47	F	GIST, Mastocytosis
Yes	5	39	M	Achalasia, Mastocytosis
Yes	6	35	M	Achalasia, Mastocytosis, GIST
Yes	10	26	M	Achalasia, Mastocytosis
Yes	13	10	F	Achalasia, Mastocytosis
ND	15	1.5	M	Mastocytosis

* See Corresponding Pedigree in Fig. 1.

lack a functional KIT receptor fail to develop the pacemaker cells in the mammalian gut responsible for propagating phasal gut contractions, the interstitial cells of Cajal (ICC) [25]. These cells are found as a neural network embedded in the musculature in the myenteric plexus area of the gastrointestinal tract and strongly express KIT and the stem cell marker CD34 [14]. Both of these are also expressed by GIST and it was based on this similarity that it was postulated that GIST arise from the ICC [23,24]. This suggestion is now widely accepted and strongly supported by the present, and other studies, in which it has been shown that activating mutations of KIT may result in multiple GIST, diffuse hyperplasia of the myenteric plexus and dysphagia.

Most of the mutations of KIT described in human disease result in constitutive activation and autophosphorylation of the receptor without ligand binding [13–15,23,24]. Activating mutations have been described in both the extracellular and intracellular domains. In GIST, the most common mutations occur in the inhibitory juxta-membrane alpha helix domain encoded by exon 11 of KIT [13,14,23,24]. Both point mutations and deletions of KIT in this region have been described. The low molecular weight tyrosine kinase inhibitor imatinib blocks activation of KIT by binding the normal ATP binding site and has been shown to be of significant benefit in the treatment of patients with metastatic or locally recurrent GIST [17,26–29]. The highest response rates are in patients with exon 11 mutations (80% response). The response rate in patients with exon 9 mutations is less, but still around 50% while those without KIT mutations have a very low to minimal response to imatinib [28,29]. In mastocytosis, activating mutations of KIT occur primarily in the intracellular enzymatic pocket/activating looped domain encoded by exon 17 and clinical responses to imatinib are rare [9,10]. The reasons behind this discrepancy in response between GIST and mast cell disease remain unclear.

Methods and materials

Several members of the family studied here were originally described by Marshall in 1990 as having familial achalasia [1]. In the present study, clinical information was obtained from 15 family members and peripheral blood, skin, bone marrow and tumor samples were collected from 12 family members after written, informed consent as approved by the Combined Multiple Institutional Review Board of the University of Colorado Denver. DNA was prepared from white blood cells (WBCs), skin samples, bone marrow samples, and tumor sections by standard techniques.

After preparation of DNA, exons 9, 11, 13 and 17 of KIT were amplified by PCR using intronic primers (**Supplemental Table 1)**. Conditions for PCR amplification were a 10 min activation period for AmpliTaq Gold and DNA denaturation at 95 °C. 14 cycles of 95 °C for 30 s, touchdown 65 °C-58 °C for 45 s, and 72 °C for one minute were followed by 25 cycles of 95 °C for 30 s, 58 °C for 45 s and 72 °C for one minute, with a final extension period at 72 °C for ten minutes.

Following amplification, the products were then directly sequenced on an ABI 3100 automated sequencer in the Core Laboratory of the University of Colorado Cancer Center using the same primers used for PCR. All sequencing reactions were performed in both the forward and reverse directions and in duplicate. Alignments and mutation analysis were done using BLAST (National Center for Biotechnology Information) software.

Results

Fig. 1 and Table 1 show the pedigree of the family studied including age, sex, clinical features, and the presence or absence of a mutation in KIT. Of the 15 family members, 7 had one or more clinical manifestations. Three have both achalasia and mastocytosis, 2 have achalasia, mastocytosis and GIST, 1 has GIST and mastocytosis, and 1 has mastocytosis only. In the 12 subjects from whom DNA was obtained there were 6 family members that were variously affected clinically and 6 unaffected family members. Three family members represented by the pedigree in Fig. 1 declined DNA testing (Probands 11, 12, and 15). Two members were clinically unaffected and declined testing (Probands 11 and 12) and one patient was an infant whose parent did not desire to pursue genetic testing at the time (Proband 15).

The family members affected by mastocytosis presented very early in life, as early as one week of age, with blisters and urticaria on the skin of the extremities and trunk and recurrent hives. With advancing age, the urticaria and blistering gradually improved, although most affected members were left with markedly thickened skin, erythema and recurrent bullae. Regarding the 5 family members with achalasia, vomiting and difficulty swallowing usually did not develop until adolescence or early adulthood. Presentation of GIST was variable. Two patients developed GIST in their 20 s, presenting as bowel obstruction and gastrointestinal bleeding, requiring surgical intervention.

Exons 9, 11, 13 and 17 of KIT were all amplified by PCR from all 12 subjects with DNA available and subjected to sequence analysis. No mutations were found in exons 11, 13 or 17 in either affected or unaffected family members. DNA from the peripheral WBCs of all 6 of the affected family members had a single-based pair mutation (A>T) near the 3′end

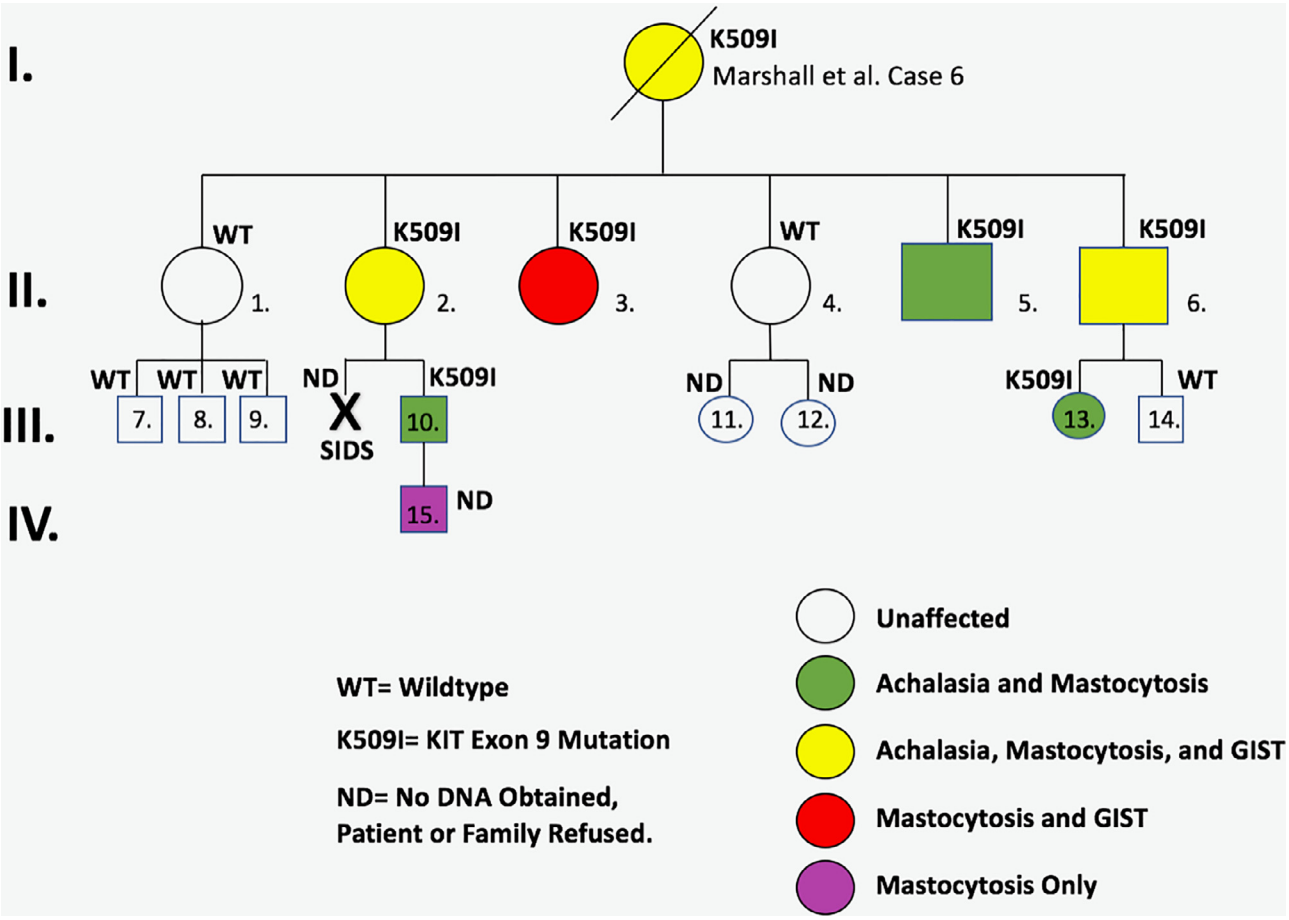

Fig. 1 Pedigree of the family studied. The founder is noted as Case 6 in a prior study by Marshall. Circles are females and squares are males. The numbers in the symbols are the unique patient number and the number over the symbol the age of the subject. The variable clinical conditions in affected family members are depicted by various colors.

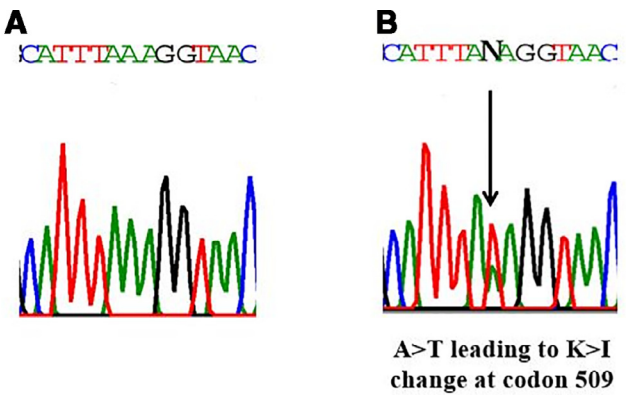

Fig. 2 Partial electropherograms of PCR amplified DNA of KIT exon 9 from an unaffected family member (A) and an affected family member (B). The A>T change in one allele was found in all affected family members leading to a K>I change at codon 509.

of exon 9 of KIT resulting in a lysine to isoleucine (K>I) amino acid substitution at codon 509, while no mutations were identified in unaffected family members (Fig. 2). The same mutation was also found in DNA prepared from the skin and bone marrow from Case 2, and from skin, bone marrow, and resected GIST tumor from Case 3.

Clinical follow up was obtained from available affected family members. Three family members received imatinib for the indications of GIST and mastocytosis and all reported poor tolerance, manifesting as fatigue, fevers, severe nausea and vomiting, and muscle aches. One family member treated with imatinib also experienced a gastrointestinal bleed (GIB) secondary to a bleeding jejunal ulcer several months following initiation of treatment. Review of medical records indicate that the patient did demonstrate radiographic evidence of clinical response before therapy was stopped. He remained off of systemic therapy for seven years until this year until he was enrolled in a clinical trial (#152,187) for mastocytosis to receive a potent, highly-selective oral inhibitor that targets KIT and PDGFRα activation loop mutants. He remains on therapy and has completed one year of therapy to date. After initiation of treatment, the patient underwent interval imaging after approximately 2 months and 4 months on the small molecule inhibitor with abdominal magnetic resonance imaging (MRI). During his 2 month interval examination, a known GIST lesion on the greater curvature of the stomach measured 1.9×1.2 cm, which had previously measured 2.2×1.8 cm 5 months prior, indicating disease response. On his 4 month interval MRI, this lesion was undetectable.

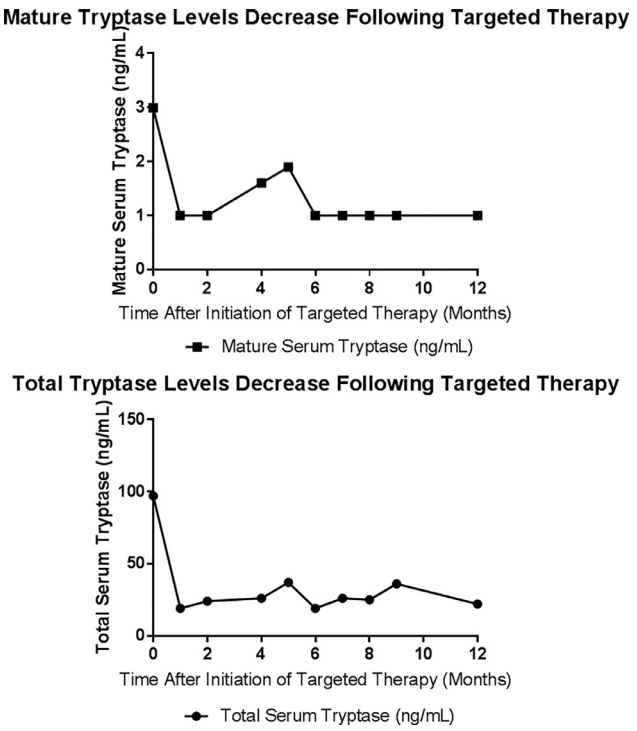

Fig. 3 The patient's serum tryptase levels markedly responded to targeted therapy. These graphs display the patient's pre-therapy serum total and mature tryptase levels (ng/mL concentrations) Tryptase is now established as a biomarker of mast cell activation or as a clinical indicator of the activity of a patient's known mastocytosis, with the mature form of the tryptase enzyme being the most accurate indicator of disease severity. His pre-treatment total tryptase level was elevated at 96 ng/mL (normal values 1.0–11.4 ng/mL) and mature tryptase level was 3.0 (normal values <1.0 ng/mL, note that a negative value or the lowest reported value by our laboratory is <1.0 ng/mL) and decreased to 19 ng/mL and <1.0 ng/mL respectively following one month of targeted therapy. These levels have stayed within the range of 19.0–37.0 ng/mL for total tryptase and <1.0–1.9 ng/mL for mature tryptase since initiation of therapy. Notably the smaller peaks noted within in each lab value occurred when the patient was off therapy for two weeks secondary to a GI bleed.

Furthermore, prior to initiation of therapy, the patient underwent a bone marrow biopsy which demonstrated 15% mast cells with nodular infiltration, consistent with his diagnosis of systemic mastocytosis. After 6 months of therapy, his bone marrow biopsy showed no evidence of mastocytosis, again indicating disease response. Tryptase levels, a biomarker of active mastocytosis [30], have also been followed on this patient, with marked reduction following the initiation of therapy (Fig. 3). Additionally, he reports decreased dysphagia and improvement in his chronic abdominal pain while on therapy. All of these functional tests indicate a robust response to this highly selective alternative KIT inhibitor.

Discussion

There are multiple previous reports of families with germline KIT mutations and a variable clinical picture which may include isolated or combined syndromes including GIST, mastocytosis, and dysphagia [2–12,22]. The variability in the clinical picture between families as well as between individuals within single families is sometimes striking even when the mutation is identical. In most of the families described to date the mutation found was in the juxtamembrane location encoded by Exon 11, similar to that seen in isolated GIST [1–3,5–8]. These include both point mutations and deletions, all of which are in frame and are thought to result in spontaneous ligand-independent tyrosine kinase activation. The KIT juxta-membrane domain is clearly important in signal transduction both through interactions with adapter proteins and phosphatases, and through modulation of KIT catalytic activity. The presence of a mutation in this area portends a poor prognosis in general, but also indicates the high likelihood of a response to treatment with imatinib [13,23,24]. In the family described here we have characterized a germline mutation in the extracellular domain of KIT in exon 9, which is both activating and appears responsive to signal transduction inhibitors.

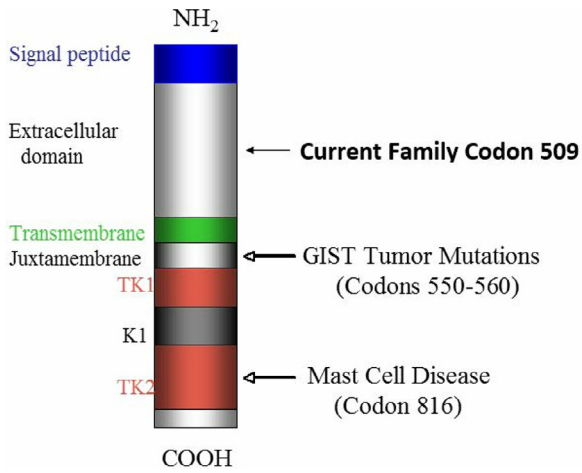

Fig. 4 The structure of KIT and the location of the most common mutations in various clinical diseases. In GIST the most common site of mutations is in the intracellular juxtamembrane domain encoded by exon 11 and encompassing codons 550 to 560. Less common mutations are found in the other areas noted. In contrast most mutations of KIT in mast cell disease are found in the intracellular tyrosine kinase domain encoded by exon 17, particularly codon 816. In the family studied here a unique mutation is described in an extracellular domain at codon 509 encoded by exon 9.

This discovery of an exon 9 K509I mutation in the extracellular domain of KIT in this family represents the fifth time this mutation has been described in the literature (Table 2, Fig. 4) [10,12,20,21]. Three of the families described in prior reports demonstrated mastocytosis alone and showed good response to imatinib and other small molecule inhibitors [10,20,21]. The fourth reported instance of an exon 9 K509I mutation was a germline mutation found in an individual with GIST and systemic mastocytosis, and other affected family members were not described in association with this case presentation, likely signifying a sporadic germline mutation [12]. The patient described in the isolated case report had no hyperpigmentation or dysphagia and unfortunately did not show response to imatinib [12]. Thus, the family described in

Table 2 Literature reports of exon 9 K509I mutations in the extracellular domain of kit.

	# of Affected Individuals	Mastocytosis	GIST	Esophageal Dysmotility	Imatinib Sensitivity for Mastocytosis Symptoms
Zhang et al. [10]	2	Yes (2)	No	Yes (1)	Sensitive (*in vitro* and *clinically*)
Speight et al. [12]	1	Yes (1)	Yes (1)	No	Resistant (no clinical response of GIST)
de Melo Campos et al. [20]	2	Yes (2)	No	No	Sensitive (*in vitro* and *clinically*)
Chan et al. [21]	1	Yes (1)	No	No	Sensitive (clinically, but not tolerated)
Current Study	7	Yes (7)	Yes (3)	Yes (5)	Sensitive (clinically, but not tolerated)

this study represents the first described family with hereditary GIST, mastocytosis, and achalasia from the exon 9 K509I mutation. Notably, this mutation is absent from the Genome Aggregation Database (gnomAD), indicating its rarity in large normal populations [31]. Per the gnomAD dataset, the missense *z*-score constraint metric of missense mutations, a statistical model of predicting de novo mutation from large exome sequencing datasets, for KIT overall is $Z = 2.77$ [31,32]. This is a high *Z*-score demonstrating intolerance to missense variation [31]. The gene is particularly constrained within exon 9, as demonstrated by only single digit levels of mutation within that exon in the gnomAD dataset [31]. In essence, this mutation at this specific locus within KIT is a rarity, and the number of affected subjects within this family makes a substantial contribution to what is currently published regarding this mutation.

Of particular note in this report is the robust response demonstrated by the individual treated with an alternative KIT inhibitor. This individual, a known carrier of the K509I mutation, demonstrates the full phenotype of the syndrome we describe including achalasia, GIST, and mastocytosis and has enjoyed a superb response to therapy in all facets of his disease. He has no clinical or pathological signs or symptoms of mastocytosis, had a marked radiographic improvement in his known GISTs, and actually reports improvement in his dysphagia. Given his strong improvement, we have suggested that other affected family members enroll in the clinical trial to receive this alternative KIT inhibitor, and this therapy may likely provide benefit to others found to harbor this mutation.

Familial activating mutations of KIT give rise to GIST in combination with other clinical features including mastocytosis, skin hyperpigmentation and dysphagia. Exactly why different activating mutations appear to result in different but similar systemic illnesses is not entirely clear. Of the 37 families or individuals reported with germline KIT mutations, including the family reported here, there is almost always some combination of GIST, a skin disorder regarding systemic mastocytosis or pigmentation issues, and gut dysmotility disorders, even though the mutations are found in different domains of the receptor. The biggest questions remaining are whether all manifestations of the clinical syndrome(s) and corresponding genotypes will respond to imatinib or other signal transduction inhibitors. Regarding disease responsiveness in this family with an exon 9 K509I mutation, results are highly promising with a novel signal transduction inhibitor.

Funding

This work had no specific funding.

Disclosure/Conflict of Interest

There are no conflicts of interest to declare for any authors.

Author Contributions

Alison Halpern: Conceptualization, data curation, methodology, writing - original draft, writing - review and editing. Robert Torphy: Conceptualization, data curation, methodology, writing - original draft, and writing - review and editing. Martin D. McCarter: Conceptualization, writing - review and editing. Cosimo G. Sciotto MD: Conceptualization, Investigation, data curation, writing - review and editing. L. Michael Glode MD: Conceptualization, writing - original draft, writing - review and editing. William A. Robinson MD Ph.D.: Conceptualization, Investigation, Supervision, Methodology, Formal analysis, Project Administration, writing - original draft, and writing - review and editing.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cancergen.2019.02.001.

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