

Familial Gastrointestinal Stromal Tumor Syndrome: Phenotypic and Molecular Features in a Kindred

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A B S T R A C T

Purpose

Members of a family with hereditary gastrointestinal stromal tumors (GISTs) and a germline *KIT* oncogene mutation were evaluated for other potential syndrome manifestations. A tumor from the proband was analyzed to compare features with sporadic GISTs.

Patients and Methods

Members of a kindred in which six relatives in four consecutive generations comprised an autosomal dominant pattern of documented GISTs and cutaneous lesions underwent physical examination, imaging studies, and germline *KIT* analysis. A recurrent GIST from the proband was studied using microarray, karyotypic, immunohistochemical, and immunoblotting techniques.

Results

In addition to evidence of multiple GISTs, lentiginos, malignant melanoma, and an angioleiomyoma were identified in relatives. A previously reported gain-of-function missense mutation in *KIT* exon 11 (T → C) that results in a V559A substitution within the juxtamembrane domain was identified in three family members. The proband's recurrent gastric GIST had a 44,XY-14,-22 karyotype and immunohistochemical evidence of strong diffuse cytoplasmic *KIT* expression without expression of actin, desmin, or S-100. Immunoblotting showed strong expression of phosphorylated *KIT* and downstream signaling intermediates (AKT and MAPK) at levels comparable with those reported in sporadic GISTs. cDNA array profiling demonstrated clustering with sporadic GISTs, and expression of GIST markers comparable to sporadic GISTs.

Conclusion

These studies provide the first evidence that gene expression and mechanisms of cytogenetic progression and cell signaling are indistinguishable in familial and sporadic GISTs. Current investigations of molecularly targeted therapies in GIST patients provide opportunities to increase the understanding of features of the hereditary syndrome, and risk factors and molecular pathways of the neoplastic phenotypes.

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INTRODUCTION

Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors of the GI tract, with a wide spectrum of clinical behaviors that span from indolent and curable disorders to highly malignant diseases that metastasize and become lethal.¹⁻³ GISTs have been reported to occur in association with cu-

taneous lesions and other manifestations that may mimic neurofibromatosis and other diseases.⁴ Despite their rarity, GISTs have recently received considerable attention because of the clinical efficacy of molecularly targeted therapy with the selective tyrosine kinase inhibitor, imatinib mesylate (ST1571; Gleevec; Novartis, East Hanover, NJ).^{2,5,6} Acquired mutations in the *KIT* proto-oncogene have

been found to be involved in the pathogenesis of GISTs,⁷ and germline *KIT* mutations have been identified in rare kindreds with multiple occurrences of GIST among blood relatives.^{1,3,8-18} Somatic mutations of *PDGFRA* are an alternative oncogenic event, and such mutations are found in 35% to 40% of GISTs lacking a *KIT* mutation.¹⁹

GISTs were first recognized as a distinct clinicopathologic syndrome in 1983 by Mazur and Clark.²⁰ Until recently, however, GISTs were often diagnosed as smooth muscle tumors, nerve sheath tumors, paragangliomas, fibromatoses, and carcinomas.^{3,21-24} This report describes clinical and laboratory findings in six members in four generations of a family with GISTs and other clinical manifestations among affected relatives. The pattern of inheritance is consistent with autosomal dominance and high penetrance, with diverse manifestations associated with germline *KIT* mutations.

PATIENTS AND METHODS

The proband was evaluated at the Beth Israel Deaconess Medical Center (Boston, MA) after presenting with multiple GI tumors

and subsequent recurrences. A detailed family history indicated an autosomal dominant pattern of diverse cancers and skin lesions in at least six other relatives in the maternal lineage. One family member had meticulously compiled a notebook of medical information on illnesses among the blood relatives, including their clinical records, pathology reports, and death certificates. Four affected (proband, his mother, and his two children) and unaffected family members were evaluated by a team comprising oncologists, dermatologists, cancer geneticists, and other consultants. With informed consent (under a protocol approved by the Institutional Review Board of Dana-Farber/Harvard Cancer Center in accordance with an assurance filed and approved by the Department of Health and Human Services), participating family members released their medical records for review, were interviewed, and had physical examinations, including photographs of informative skin lesions. A detailed pedigree was constructed (Fig 1). A structured questionnaire was completed regarding their personal and family history of neoplasia and other disorders, and blood specimens were collected for genetic analysis.

Cytogenetic analyses were performed on recurrent GIST lesions resected from the proband. Metaphase cells were analyzed by Giemsa-trypsin banding, using standard methods after 5 days of tissue culture.²⁵ Immunoblotting studies for phosphorylated and

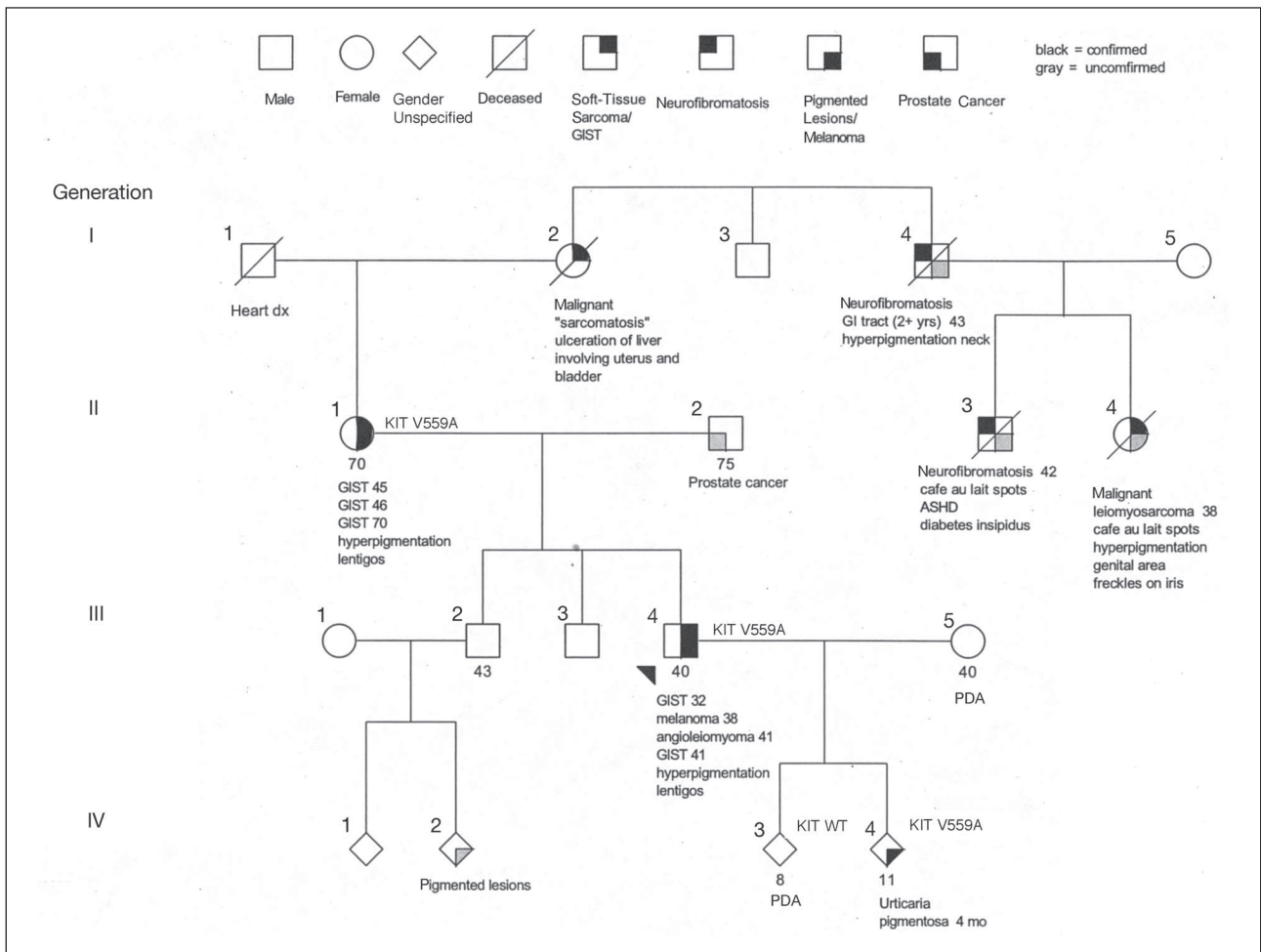


Fig 1. Pedigree of family with multiple gastrointestinal stromal tumors. PDA, patent ductus arteriosus; ASHD, arteriosclerotic heart disease.

total KIT, AKT, MAPK, and STAT5 were performed in total cell lysates from snap-frozen GIST specimens, as described previously.⁷ Equivalency of loading intracellular proteins was demonstrated by immunoblotting the GIST cell lysates for PI3-K p85 (Upstate Biotechnology, Waltham, MA). *KIT* genotyping was performed using a combination of denaturing high-performance liquid chromatography and direct DNA sequencing as previously described.²⁶ cDNA array profiling for the proband's GIST, and comparisons with cDNA array profiles in sporadic GISTs, leiomyosarcomas, synovial sarcomas, and dermatofibrosarcoma protuberans, were performed as described previously.²⁷

RESULTS

The Proband, Patient III-4

The proband (patient III-4), a male of European origin, underwent resection of multiple tumors of the small intestine at age 32 years. Histologic examination showed multifocal GISTs associated with hyperplasia of the autonomic neural plexus that encompassed the interstitial cells of Cajal (ICC) within the muscularis propria. Physical examination revealed 1- to 3-mm hyperpigmented macules involving the neck, scrotal region, axillae, and buttocks that were suggestive of multiple lentiginos (Fig 2A). An angioliomyoma resected from the skin above his left ankle when he was age 37 was KIT negative by immunohistochemistry. At age 38, a skin biopsy performed from a pigmented skin papule increasing in size, revealing a superficial spreading melanoma extending to 1.1 mm in thickness (Clark level IV). An attempt was made to evaluate the tumor for KIT expression by immunohistochemistry; unfortunately, the remaining specimen was insufficient. Soon thereafter, the proband underwent resection of an enlarging 5.1-cm gastric lesion diagnosed as a recurrent GIST.

Patient II-1

The proband's 70-year-old mother (patient II-1) has a history of vitiligo as well as a pattern of hyperpigmented lentiginos similar to those of her son. She had resection of an unspecified gastric neoplasm at age 45 years, and removal of numerous small intestinal tumors diagnosed as benign proliferative lesions at ages 60 and 64 years. Review of these lesions revealed that they were GISTs. In June 2003, she was observed to have a slowly enlarging soft tissue mass in the lesser curvature of the stomach, consistent with a GIST.

Patient I-2

The death certificate of the proband's maternal grandmother indicated death due to malignant "sarcomatosis," with liver, uterus, and bladder involvement at age 39 years.

Patient I-4

A brother of the proband's maternal grandmother (patient I-4) died at age 43 years. His death certificate indicated "neurofibromatosis, GI tract." He was reported to have had hyperpigmentation of the neck, and had two surgical procedures to remove "buckets of tumors" from his intestines.

Patient II-3

The death certificate of the son of patient I-4 indicated that he died of "neurofibromatosis" at age 53 years. Diabetes insipidus and coronary artery disease were also recorded on his death certificate, but no details were available.

Patient II-4

The daughter of patient I-4, a sister of patient II-3, was reported to have café-au-lait macules and hyperpigmentation of the pelvic area, and was thought to have "neurofibromatosis." She also developed multiple "leiomyomas" of unknown sites, and her death certificate indicated death due to "leiomyosarcoma" at age 38 years.

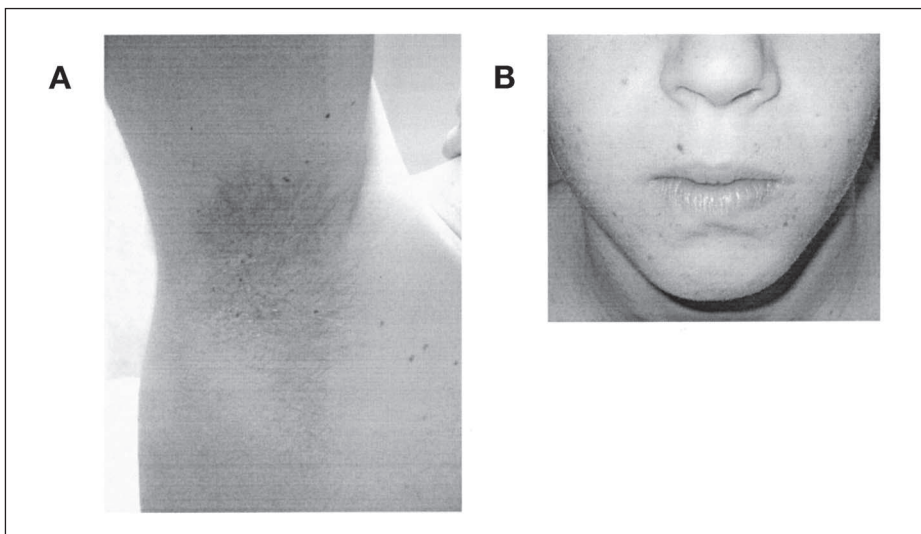


Fig 2. (A) Melanocytic nevi and lentiginos involving the axillary region of patient III-4, and (B) melanocytic nevi and lentiginos involving the face of patient IV-4.

Patient IV-4

The 11-year-old offspring of the proband was evaluated for neurofibromatosis at Children's Hospital of Boston at 8 months of age. At age 2 years, a consulting dermatologist diagnosed classical lesions of urticaria pigmentosa, with several urticarial papules diffusely distributed over his skin (Fig 2B). Subsequently, unusual perioral, inguinal, and genital lentiginos/nevi developed. An abdominal computed tomography scan did not reveal any abnormalities.

Patient IV-3

The 9-year-old offspring of the proband had surgery for patent ductus arteriosus in childhood. This child had no manifestations of GIST on computed tomography scan and no cutaneous pigmented lesions, urticaria pigmentosa, or neurofibromata.

Molecular Studies

Blood specimens of the proband (III-4), his mother (II-1), and children (IV-3 and IV-4) were analyzed for *KIT* mutations using previously described methods.⁷ A germline *KIT* exon 11 T → C missense mutation, resulting in a V559A juxtamembrane domain substitution, was found in the proband, his mother, and one child (Fig 3). This gain-of-function mutation has been reported previously in three other families with GIST (two from Japan and one from Italy).^{1,10,11}

The recently excised recurrent GIST lesion from the proband (III-4) was karyotyped and analyzed for *KIT* mutations. A heterozygous V559A mutation was present in the GIST, consistent with the known heterozygous germline V559A mutation status of this individual. No additional

mutations of *KIT* exons 9, 11, 13, or 17 were present. Each of 15 metaphases had a cytogenetic profile similar to those reported in sporadic GISTs, including monosomies of chromosomes 14 and 22, and a small marker chromosome that might have been derived from the deleted chromosome 14 or 22 (Fig 4). Immunostains of the tumor specimen were positive for CD117 (*KIT*) and negative for actin, desmin, and S-100. Immunoblotting demonstrated strong expression of total and tyrosine phosphorylated *KIT* at levels comparable with those in sporadic GISTs bearing exon 11 oncogenic mutations (Fig 5). Immunoblotting showed a profile of phosphorylated signaling intermediates, including phosphorylation of AKT and MAPK, but not STAT5, which is consistent with findings in sporadic GISTs (Fig 5).^{19,28}

Gene Expression Profiling

To define further the molecular characterization of the recurrent GIST tumor, gene expression profiling was performed as previously described.²⁷ The sample ST-3-213-GIST (Fig 6) was compared with 22 other previously analyzed tumors including five dermatofibrosarcoma protuberans, four synovial sarcomas, seven leiomyosarcomas, and five other GISTs.^{27,29} Sample STT1823 was a pulmonary metastasis from the original sample STT94.

Forty-two thousand element printed cDNA gene arrays were used to determine the global gene profile. Sample ST-3-213 clustered within the group of other GISTs based on a gene filtering that left 3,669 genes for clustering. The gene filtering criteria were similar to those used in prior studies.^{27,29} Unsupervised hierarchical clustering shows a

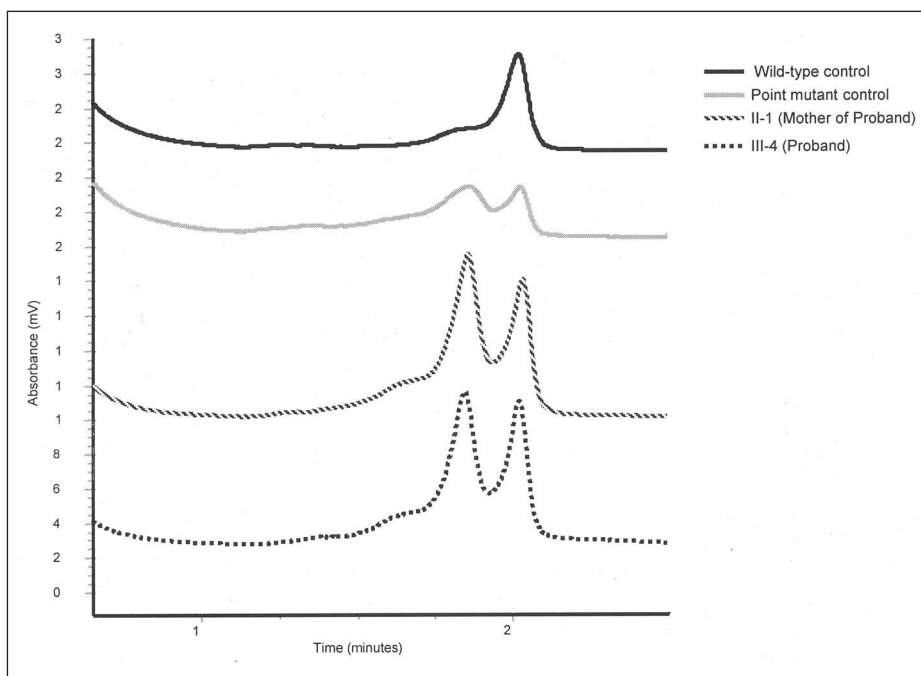


Fig 3. Genotyping of peripheral blood specimens from the proband (III-4) and his mother (II-1). The denaturing high-performance liquid chromatography elution patterns of the subjects indicate heteroduplexes due to a sequence mismatch (complex peak) similar to the heterozygous point-mutant control. In contrast, wild-type *KIT* amplicon elutes as a single peak. Sequencing of subject amplicons revealed a germline V559A *KIT* mutation.

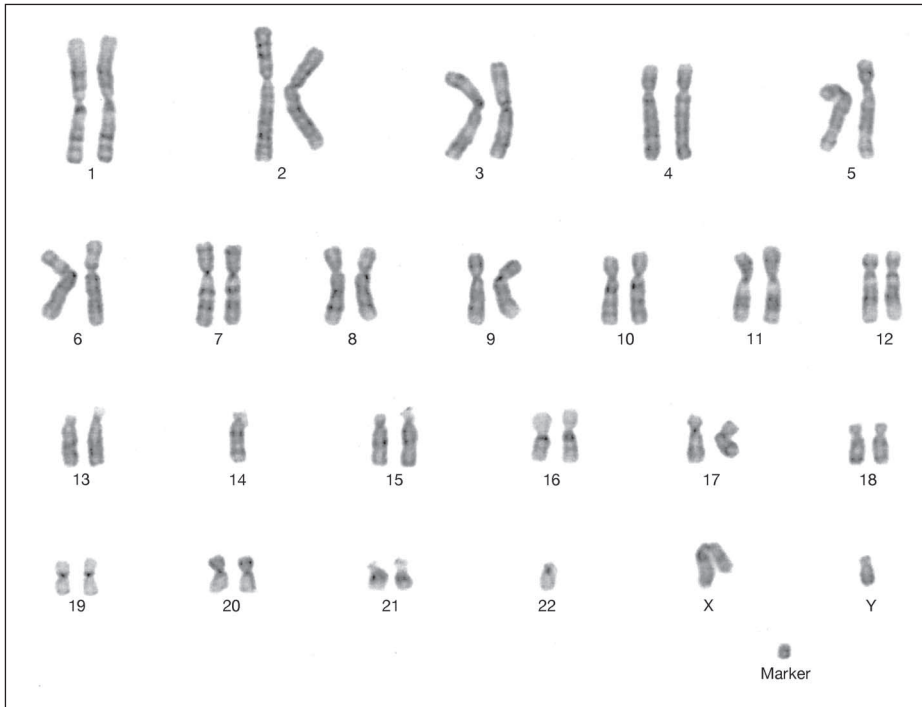


Fig 4. Giemsa-trypsin banded karyotype of proband's gastrointestinal stromal tumors, showing monosomies of chromosomes 14 and 22, and an unidentified marker chromosome.

gene expression profile of sample ST-3-213-GIST that is highly similar to the other GISTs; much more so than to any of the other neoplasms analyzed. The full data set of this cluster is available through the Stanford microarray database (<http://genome-www5.stanford.edu/>).

A small selection of the genes that positively identified and separated the GISTs from the other tumors in the cluster is shown in Figure 6. Note high expression of the sample for *KIT* and *PRKCCQ* (*PKCθ*). The *FLJ10261* mRNA encodes for a novel protein called *DOG1* that was recently found to be expressed by a large number of GISTs.³⁰

DISCUSSION

GISTs, the most common mesenchymal tumors of the GI tract, are derived from transformed neoplastic precursors of the ICC.^{3,21,22,31} GISTs can exhibit a relatively benign and indolent course, or manifest an aggressive behavior with extensive local invasion and distant metastases. Most GISTs arise in the stomach or small intestines, with other lesions appearing in the large intestines, esophagus, omentum, mesentery, and other sites.^{3,21,31} GISTs often express *KIT* (*CD117*) and *CD34*, which can help to distinguish these tumors from other neoplasms, including leiomyosarcoma, other spindle cell tumors, melanoma, and carcinoma of the GI tract.³¹ These molecular findings have improved diagnostic accuracy and facilitate greater accuracy in determining the incidence of GISTs, which are estimated to develop in approximately 2,000 to 5,000 patients annually in the

United States.³¹ The median age at diagnosis of sporadic GISTs in the older literature has been reported to be approximately 60 years.²¹ A recent National Cancer Institute consensus conference³² has noted that the behavior of GISTs cannot be predicted by histopathologic appearance alone, and therefore all GISTs may harbor malignant potential. The overall 5-year survival of patients with GISTs is approximately 40%, with high mitotic index connoting poor prognosis.^{7,13,31}

A number of families with GISTs affecting multiple relatives have been described in recent years (Table 1). Most affected families have been shown to carry a *KIT* germline mutation.^{1,9-11,33-37} One GIST family has recently been identified with a germline *PDGFRA* mutation.³⁸ Akin to most hereditary cancer predisposition syndromes, GIST families tend to have several affected relatives with multiple primary neoplasms, multifocal lesions, and tumors that present at earlier ages than corresponding sporadic GISTs. The kindred in this study demonstrated all of these traits. Whereas other isolated affected kindreds have manifested urticaria pigmentosa or other undefined hyperpigmented skin lesions,^{1,4,8,10,11} the family in our study had a spectrum of cutaneous lesions other than urticaria pigmentosa, including a melanoma, among three carriers of the *KIT* germline mutation and three other relatives with histories suggestive of GISTs (Fig 1). Diverse pigmented skin lesions diagnosed from early childhood included lentigines, benign melanocytic nevi, urticaria pigmentosa, café-au-lait macules, and perioral hyperpigmentation, and the proband

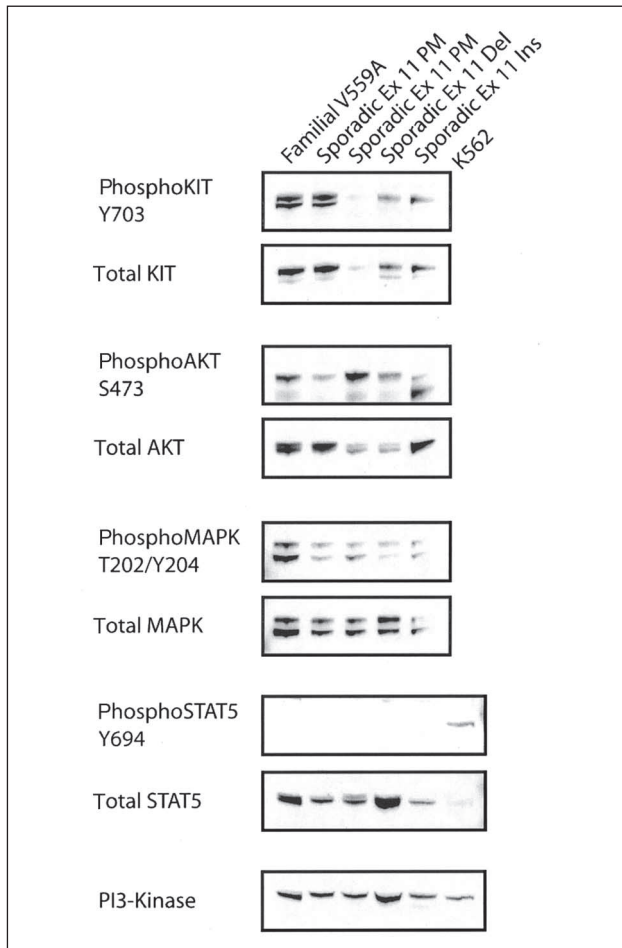


Fig 5. Expression of phosphorylated and total KIT, AKT, MAPK (p42/44), and STAT5 in immunoblots of total cell lysates from primary gastrointestinal stromal tumors (GISTs) in proband (V599A mutation) and sporadic GISTs with *KIT* exon 11 point mutations (PM), in-frame deletion (Del), and in-frame insertion (Ins). K562 leukemia cells provide a positive control for phospho-STAT5. PI3-K stain (bottom) provides a loading validation.

developed a malignant melanoma. A relationship between the *KIT* activation mutation and the development of melanoma is uncertain, given that this mutation is not a typical part of genesis of melanomas, but trials of imatinib and other agents in this condition are ongoing. Death certificates for two of the family members in this study in earlier generations (I-4 and II-3) indicated “neurofibromatosis of the GI tract” as the underlying cause of death. This diagnosis was made at a time when GISTs had not yet been recognized as a distinct clinicopathologic entity. These lesions were likely GISTs, although multicentric GISTs can develop in certain individuals with classic neurofibromatosis.^{39,40}

Hereditary cancers tend to occur at earlier ages when compared with their sporadic counterparts. The median age at diagnosis of GIST syndrome is 46 years in the nine GIST syndrome families reported to date, more than a decade earlier than sporadic (nonhereditary) GISTs re-

ported in the literature.¹⁰ Earlier ages at diagnosis of familial GISTs might be due to greater attention to the disease, earlier onset of tumors, or both.⁴ Knudson⁴¹ postulated that at least two hits (mutations) are required to transform a normal cell into a cancer cell. In most hereditary forms of cancer, one mutation has been transmitted through the germline, and additional somatic mutations are needed to initiate the process of neoplastic transformation. Recent data from a transgenic mouse model, as well as histologic findings in our proband, suggest that germline *KIT* activation results in hyperplasia of the ICC cells from which GISTs derive.⁴² Additional mutations, including the typical deletions of chromosomes 14 and 22, might promote the transition from hyperplasia to neoplasia. This inference is supported by studies of clonality in lesions in two different kindreds with familial GIST syndrome (one kindred with an exon 11 point mutation, the other with an exon 17 point mutation described by Chen et al).³⁵ Among patients in the study by Chen et al,³⁵ the diffuse ICC hyperplasia present within the muscularis propria of the GI tract was found to be polyclonal in nature. In contrast, discrete mass lesions diagnosed as GIST syndrome were monoclonal, and different GIST lesions from the same patient were derived from independent clones.

Our report provides the first evidence that genetic mechanisms of neoplastic progression are similar in familial and sporadic GISTs. The familial GIST syndrome karyotyped in this study had an abnormal cytogenetic profile (deletions of chromosomes 14 and 22) indistinguishable from those reported in most sporadic GISTs.¹⁹ Similarly, our preliminary studies of cell signaling intermediates suggest that the biologic ramifications of *KIT* activation are similar in familial and sporadic GISTs. AKT and MAPK (ERK1 and ERK2) were extensively phosphorylated in the proband’s familial and other sporadic GISTs, whereas phosphorylation of STAT5, which is phosphorylated after *KIT* activation in myeloid cells and fibroblasts,^{43,44} was notably absent in both sporadic GISTs²⁸ and in the proband’s GIST studied herein (Fig 5). The biologic similarity between familial and sporadic GISTs was underscored by cDNA array profiling of the proband’s GIST (Fig 6). This familial GIST clustered with sporadic *KIT*-mutant GISTs, and featured strong expression of the specific GIST markers *KIT*, *PRKCQ/* *PKCθ*, and *DOG1/FLJ10261*.²⁷

Little is known about the epidemiology and environmental risk factors for sporadic occurrences of GIST, in part because of their rarity.^{21-24,31,34,45} However, the recent therapeutic clinical trials in GIST patients provide exceptional opportunities to collect risk factor data from study participants to identify other genetic and possible environmental factors contributing to the development of GISTs. With the uncertain role of environmental risk factors, it is

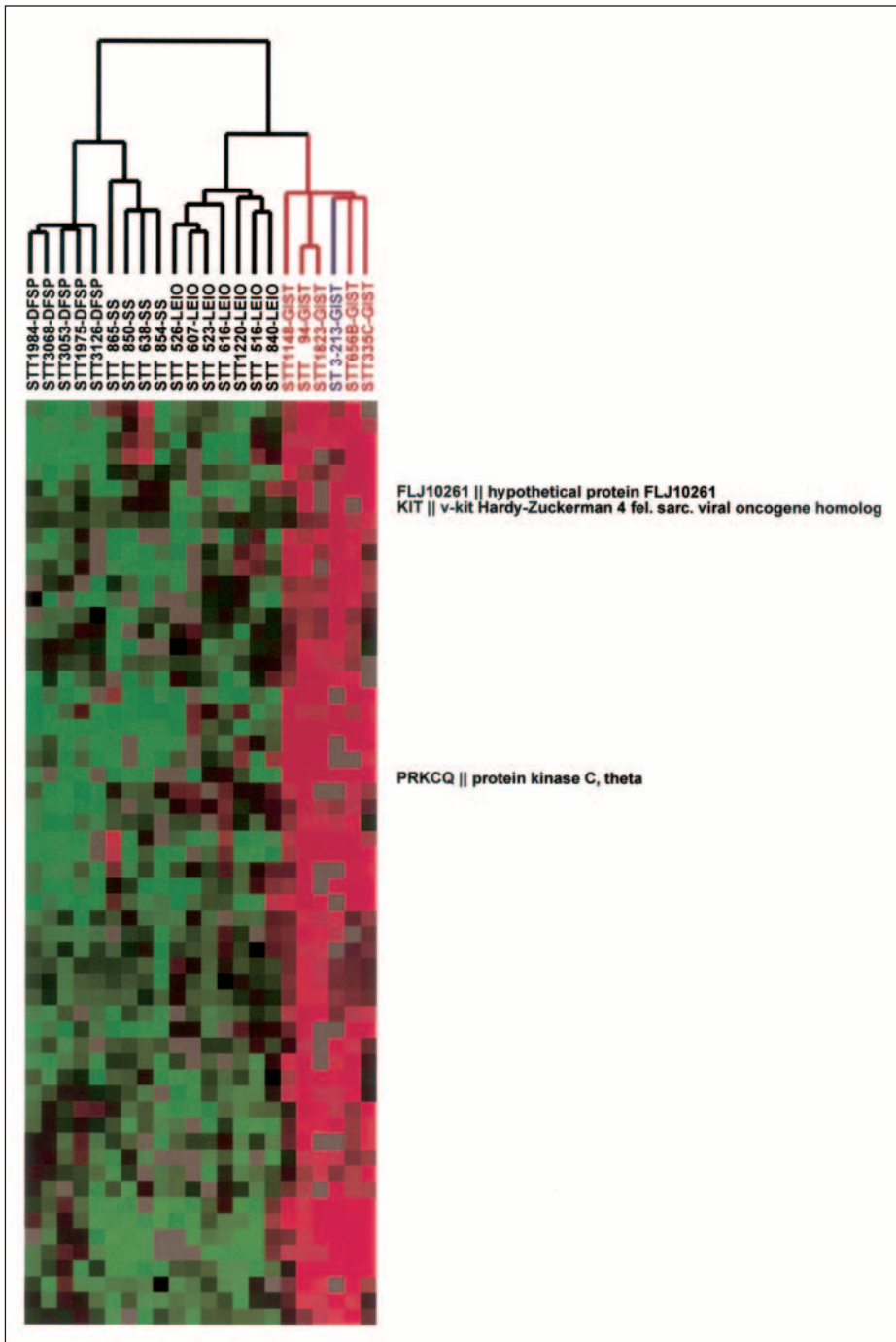


Fig 6. Gene expression profiling of case ST-3-213-GIST (proband) compared with 22 other soft tissue tumors. A portion of the 3,669 genes used for the procedure is shown (see text for details). Unsupervised hierarchical clustering shows that the case gene expression profile is highly similar to other gastrointestinal stromal tumors (GISTs), much more than to any other neoplasm.

possible that germline mutations in genes other than *KIT* and *PDGFRA* may also predispose to hereditary GISTs.^{21-24,31,34,45} Studies are needed to identify the other signaling pathways that trigger the fully malignant phenotype in GIST cells with *KIT* mutations.

The finding of kindreds with germline *KIT* mutations raises the possibility of predisposition testing in unaffected relatives to distinguish individuals at high risk of developing

GIST from those at virtually no risk. The diverse skin lesions reported in this and other publications may be associated with specific *KIT* mutations, raising the possibility of genotype-phenotype relationships. Together with detailed case reports, epidemiologic studies can provide concerned families with reliable information about the penetrance of *KIT* mutations, the spectrum of associated lesions, recommendations for surveillance for GISTs, and surgical or

Table 1. Reports of Other Physical Findings in Familial GIST Kindreds in the Literature

Mutation	Nationality of Proband	No. of Affected Relatives†	Skin Lesion Features*			
			Hyperpigmentation	Urticaria Pigmentosa	Nevi	Achalasia
<i>KIT</i> exon 11 (Val ⁵⁵⁹ → Ala ⁵⁵⁹)						
Li (present report)	United States	7	✓	✓	✓	—
Maeyama et al ¹	Japan	14	✓	—	✓	—
Nishida et al ¹⁰	Japan	7	✓	—	—	—
Beghini et al ¹¹	Italy	4	✓	✓	—	—
<i>KIT</i> exon 11 (Trp ⁵⁵⁷ → Arg)						
O'Brien et al, ³⁴ Hirota et al, ³⁶ Chen et al ³⁵	Canada	2	—	—	—	—
Robson et al ⁴	United States	22	✓	—	—	✓
<i>KIT</i> kinase domain 1 (Lys → Glu ⁶⁴²)						
Isozaki et al, ³³ Handra-Luca et al ³⁷	France	2	—	—	—	—
<i>KIT</i> exon 17 (Asp ⁸²⁰ → Tyr)						
Hirota et al ⁹	Japan	6	—	—	—	✓
<i>PDGFRA</i> exon 18 (Asp ⁸⁴⁶ → Tyr)						
Chompret et al ³⁸	France	5	—	—	—	—
Not Tested						
Marshall et al ⁸	United States	14	—	✓	—	✓

Abbreviation: GIST, gastrointestinal stromal tumor syndrome.
 *Dashes indicate absence of GIST-associated lesions in reported families.
 †Confirmed or likely GIST by examination, medical record, or death certificate.

potentially targeted interventions such as imatinib that could reduce the risk of these tumors.

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Authors' Disclosures of Potential Conflicts of Interest

The following authors or their immediate family members have indicated a financial interest. No conflict exists for drugs or devices used in a study if they are not

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