Pleomorphic Characteristics of a Germ-Line KIT Mutation in a Large Kindred with Gastrointestinal Stromal Tumors, Hyperpigmentation, and Dysphagia

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ABSTRACT

Purpose: Somatic mutations that result in the activation of the growth factor receptor KIT are commonly found in gastrointestinal stromal tumors (GISTs). Six families have been reported in which a germ-line mutation in KIT is associated with an autosomal dominant predisposition to the development of GISTs. Hyperpigmentation, urticaria pigmentosa, and dysphagia have been described in some, but not all, families. Preliminary correlations between the site of mutation and the clinical phenotype have been proposed, but the strength of these associations is not defined.

Design: A large kindred with multiple GISTs, hyperpigmentation, and dysphagia was identified after the index case presented with multiple GISTs. A germ-line mutation in KIT (W557R) was identified in an affected cousin, after which a large family meeting was held and testing offered. Clinical data were obtained by interview and, whenever possible, medical record documentation.

Results: To date, 19 individuals have been tested, and the mutation has been shown to cosegregate with the syndrome. The phenotypic expression, however, is variable. GISTs, often presenting as upper gastrointestinal bleeding, and hyperpigmentation are common, but not diagnosed in all documented or obligate carriers. Dysphagia is a less prevalent complaint. The diagnosis of GISTs appears to be made at a younger age in more recent generations. Metastatic disease is uncommon.

Conclusions: A germ-line mutation in KIT resulting in an amino acid substitution in the juxtamembrane region is associated with a syndrome of GIST, hyperpigmentation, and dysphagia, although the prominence of each component varies.

INTRODUCTION

Gastrointestinal stromal tumors (GISTs) are commonly described as the most common mesenchymal tumors of the human gastrointestinal tract (1, 2). Interest in these tumors has been spurred by the observation that the type III receptor tyrosine kinase KIT is activated by somatic mutation in the majority of GISTs and that inhibition of KIT activity by the specific inhibitor imatinib often results in dramatic clinical responses (3–6). GISTs are the first solid tumors to respond to targeted small molecule therapy, and it is hoped that the insights gained from the treatment of these rare tumors will be useful in approaching more common malignancies.

KIT activation appears to be a central tumorigenic event in the development of GISTs (7). KIT expression, as measured by CD117 immunohistochemistry, is nearly a diagnostic criterion for GISTs, and KIT gene mutations are identified in 60–90% of cases (1, 7–12). Heterozygous mice with an activating KitV558Δ mutation “knocked in” develop patchy hyperplasia of the myenteric plexus and neoplastic cecal lesions indistinguishable from human GISTs (13). Six families have been reported in which germ-line mutations in KIT result in constitutive activation of the KIT receptor and are associated with a variable clinical syndrome of GISTs, hyperpigmentation, urticaria pigmentosa, and dysphagia (2, 14–20). Another family with a similar clinical syndrome was reported in 1990, but germ-line KIT analysis was not performed (21). Fewer than 20 individuals with germ-line KIT mutations have been described worldwide. We report here a large family of Italian ancestry in which 22 individuals are either documented or presumed carriers of a germ-line KIT alteration. Description of this kindred elucidates the variable clinical presentation of this germ-line KIT mutation, which must be taken into account when assessing possible genotype-phenotype correlations, and allows a preliminary estimate of age-specific penetrance of this specific mutation.

MATERIALS AND METHODS

Patients

Case 1. The index case came to the attention of the Clinical Genetics Service at Memorial Sloan-Kettering in August of 1996. He was a man of Italian descent with no significant past medical history who presented at age 48 with fatigue. In the course of an echocardiographic examination he was noted to have an abdominal mass, prompting a computed tomography scan of the chest and abdomen. This study demonstrated esophageal thickening, a mes-
enteric mass, nodularity of the small bowel wall, multiple pelvic masses, retroperitoneal adenopathy, and a 2-cm right lower lobe lung nodule. A core biopsy of the retroperitoneal mass demonstrated a spindle cell neoplasm with occasional mitotic figures, with immunohistochemistry reportedly positive for vimentin and CD34, but negative for desmin, cytokeratin, epithelial membrane antigen, smooth muscle actin, and S100. He was followed at an outside institution and died ~2 months after presentation with acute renal failure. Limited autopsy demonstrated multiple intestinal nodules consistent with smooth muscle tumor. Metastatic GIST was not documented in the autopsy report.

**Case 2.** The first member of the family to undergo genetic analysis was a 48-year-old woman, a paternal first cousin of case 1, who related a life-long history of hyperpigmentation of the hands, knees, perineum, and circumoral areas. At the age of 40, she noted abdominal bloating and, on pelvic examination, was thought to have a fibroid uterus, for which a hysterectomy was recommended. At the time of surgery, a 15-cm mass was noted adherent to the antimesenteric border of the distal jejunum. Multiple additional nodules, ranging in size from several millimeters to 2 cm, were noted along the antimesenteric border of the entire length of the small bowel, and another lesion was palpated in the lesser curvature of the stomach. Two of the largest small bowel masses were resected, and a diagnosis of multifocal low-grade leiomyosarcoma was made. Since that time, the patient has been followed with serial computed tomography scans. There has been no clinical evidence of progression, and she remains well with a Karnofsky performance status of 100% and no abdominal symptoms or dysphagia.

**Case 3.** The third case is a 57-year-old woman, maternal first cousin of cases 1 and 2, who came to medical attention at age 42 with progressive anemia. Evaluation revealed an ulcerated submucosal mass in the gastric antrum, for which she underwent a partial gastrectomy with Billroth I reconstruction. The original pathological diagnosis was of leiomyoma with epithelioid features, and close clinical follow-up was recommended. On upper endoscopy ~5 years postoperatively, a submucosal mass measuring 2 cm was noted in the gastric remnant. Over the ensuing 10 years, the mass enlarged to >4 cm, and an elective resection was recommended. Before resection, the original specimen was reviewed and a diagnosis of GIST was made, predominantly epithelioid and focally spindle cell, with 1 mitotic figure per 50 high power fields. Neoplastic cells were positive for KIT (CD117). Preoperative physical examination was unremarkable except for hyperpigmentation of the groin. Preoperative computed tomography scan revealed the gastric mass and a 3.4-cm soft-tissue density in the left pelvis. Preoperative positron emission tomography scan was unremarkable except for diffuse curvilinear uptake throughout the abdomen, consistent with bowel wall uptake. A completion gastrectomy was performed, which demonstrated multiple GISTs measuring from 0.1 to 4.0 cm in diameter. Diffuse myenteric plexus thickening was noted in the gastric wall due to hyperplasia of the interstitial cells of Cajal. Also noted at surgery were multiple GIST lesions along the wall of the small bowel, the largest measuring 2.2 cm and associated with small bowel diverticula. Diffuse myenteric plexus hyperplasia was noted in the wall of the small bowel. Immunohistochemistry demonstrated KIT (CD117) and CD34 positivity in the GIST tumors, and KIT expression in the hyperplastic cells of the myenteric plexus.

**Genetic Counseling and Mutation Identification**

The wife of the index case originally sought consultation out of concern for her children. Case 2, after undergoing counseling and providing informed consent, donated a blood sample for KIT testing. To determine whether this individual was a carrier of a KIT gene mutation, genomic DNA was extracted from leukocytes using conventional methods. Exon 11 of the KIT gene was PCR amplified using published primers (22). Direct sequencing demonstrated a single nucleotide substitution at position 1669 (TGG->CGG) in one allele of the KIT gene, resulting in a substitution of arginine for tryptophan at codon 557 (W557R), within the juxta-membrane domain of the KIT receptor. An invitation was then extended to all of the surviving family members to attend a family meeting at Memorial Sloan-Kettering Cancer Center. This meeting was attended by 14 individuals, who provided the family history information used to construct the pedigree represented in Fig. 1. To preserve pri-

**Fig. 1** Pedigree of family transmitting germ-line KIT mutation. Squares, male; circles, female; †, deceased. □, unaffected; □ □, hyperpigmentation; □ □, GIST; □ □, hypopigmentation plus dysphagia; □ □, GIST plus hyperpigmentation; □ □, GIST plus dysphagia; □ □, GIST plus hyperpigmentation plus dysphagia; †, Test W557R (+); †, Test W557R (−).
variable, the genders of certain individuals have been altered, but
the clinical information and family relationships depicted in the
pedigree are otherwise accurate. Whenever possible, medical
records and pathological documentation were sought to confirm
the reported history. Attendees were specifically asked about the
presence or absence of hyperpigmentation, but it was not pos-
tible to perform physical examination of all of the family
members. Several individuals also related a history of dyspha-
gia, but radiographic and esophago-manometric studies were not
performed. All of the individuals at the family meeting, as well
as members later recruited, were offered testing for the family
KIT mutation. For those accepting, after informed consent,
genotyping was performed by denaturing high pressure liquid
chromatography (Transgenomics), and all of the positive results
were confirmed by DNA sequencing.

**Statistical Methods.** The presence or absence of hyper-
pigmentation, dysphagia, and GIST were determined by direct
interview or family reporting, with confirmation through review
of medical records whenever possible. Age at GIST diagnosis
was derived from the patients and medical records. If not pos-
able, the age at diagnosis reported by a first-degree relative was
used. The cumulative incidence of GIST was derived using the
method of Kaplan and Meier, with unaffected individuals cen-
sored at the age of last follow-up or death without GIST. Only
individuals proven to carry the familial mutation, or who were
obligate carriers of the mutation, were used for this calculation.
The risk of developing GIST in different generations was com-
pared using the log-rank test, and mean age at onset in different
generations was compared by ANOVA (SPSS for Windows,
version 9.0; SPSS, Inc., Chicago, IL).

**RESULTS**

**Risk and Outcome of GIST due to W557R Mutation.**

To date, 19 members of the kindred have undergone genetic
testing to date, with 8 carrying the KIT W557R mutation (Fig.
1). The mutation has been identified in all of the affected
members tested and in no unaffected member. There are 22
individuals in the family presumed on either clinical or labora-
tory grounds to be mutation carriers. GISTs have been diag-
nosed in 15 of 22 (68%) presumed carriers, at a median age of
47.5 years (range, 29–77), and in 6 of 8 (75%) documented
carriers. The probability of being clinically diagnosed with
GIST was 91% by age 70 among documented obligate carriers
of the family mutation (Fig. 2). The diagnosis of GIST appears
to have been made at a progressively younger age in successive
generations of this family. The mean age at diagnosis in the
most recent generation has been 34 years, compared with 47
years in the middle generation and 62 years in the oldest (log
rank P = 0.0007; test for trend by ANOVA, P < 0.0001).

The clinical behavior of the tumors in this family appears
relatively indolent. The most common clinical presentation has
been with upper gastrointestinal bleeding, although some family
members have been asymptomatic at the time of diagnosis. A
number of individuals have survived to later ages despite their
diagnosis, and metastatic disease has rarely been a prominent
feature of the clinical course. In at least 2 cases, index lesions
appear to have grown minimally over periods of as long as 10
years. Nonetheless, the predisposition cannot be considered

innocuous. Several individuals have required prolonged hospi-
talizations as a result of gastrointestinal bleeding, and 3 family
members are believed to have died from obstructive complica-
tions of their disease.

**Variability of Manifestations Other Than GIST.** Hyper-
pigmentation was reported to be present in 14 of 22 (64%)
presumed carriers and in 5 of 8 (62.5%) documented mutation
carriers. The hyperpigmentation has typically been on the digits,
elbows, knees, perineum, and face, although the relative prom-
ience of pigmentation in the different areas has varied from
individual to individual. In general, the pigmentation has first
become evident in childhood or puberty, with a tendency to fade
somewhat with aging. In affected areas, the pigmentation has
been more diffuse than speckled.

Dysphagia has been reported by 7 of 22 (32%) presumed
carriers, including 5 of 8 (62.5%) individuals shown by testing
to carry the familial mutation. No individual with dysphagia
was known to have undergone a formal esophagomanometric
evaluation.

**DISCUSSION**

The family described in the current report is the seventh
reported kindred manifesting an autosomal dominant predispo-
sition to GIST (Table 1). Unlike other reported families, clinical
information is available from multiple individuals in this large
kindred, which permits a more complete description of the
syndrome associated with germ-line mutations in KIT. A picture
emerges of substantial clinical variability, with no firm evidence
of genotype-phenotype correlation.

The exon 11 point mutation identified in this family results
in a single amino acid substitution (W557R) in the juxta-
membrane domain of the KIT receptor. This alteration, which
has been reported in one other family (15, 20), is associated with
hyperplasia of the interstitial cells of Cajal in the myenteric
plexus of the bowel wall, demonstrated both in pathological

![Fig. 2 Cumulative incidence of gastrointestinal stromal tumors (GIST) among individuals documented to carry KIT W557R and obligate carriers.](image-url)
material from individuals in this family,\(^7\) and in a previous report of another family with the same mutation (2). The observation of CD117 expression in the cells of the myenteric plexus from case 3 suggests that the constitutive activation of the receptor that results from the W557R mutation is directly responsible for a polyclonal expansion of this subset of cells. It is unclear whether this is also responsible for the observed increased bowel wall uptake noted on the positron emission tomography scan from case 3.

Whereas the W557R mutation appears to be sufficient to result in the expansion of the myenteric plexus, additional somatic genetic alterations are presumably required to generate the neoplastic proliferations recognized as GIST. The exact nature of these additional changes has not yet been described in these tumors, but may include loss of chromosome 14, 1p, 9p, 11p, or 22q, as described in nonhereditary benign or borderline-malignant GIST (7). It is likely that a relatively small number of additional somatic changes are required, as nearly all of the individuals with the mutation eventually developed GIST, 50% of them by the age of 50. The observation of a younger age at diagnosis in more recent generations of the current family suggests that environmental factors may influence the rate of development of GIST. However, this observation can only be considered hypothesis-generating, as a number of factors may produce an appearance of genetic anticipation when, in fact, none exists (23). For example, the availability of computed tomography and its increasingly frequent use in the evaluation of nonspecific abdominal symptoms could lead to a detection bias that would produce an apparent reduction in age at first diagnosis.

In the experience of the family reported here, the clinical behavior of the GIST was generally benign, in that metastatic disease was not a prominent feature of the clinical course in most affected individuals. Despite the absence of metastases in most individuals, the symptoms associated with the development of GIST, particularly gastrointestinal bleeding, resulted in significant morbidity. Imatinib (STI571) has been demonstrated to be remarkably effective in the treatment of nonhereditary GIST (5). Because constitutive receptor activation appears to be central to the pathogenesis of the clinical syndrome associated with germ-line \(^3\)KIT mutation, imatinib may be effective not only in the treatment of hereditary GIST, but also in the prevention of GIST development in these individuals. Balanced against these potential benefits are concerns that dissolution of multifocal tumors distributed throughout the small intestine could result in morbidity and mortality due to bowel perforation or tumoral hemorrhage. To date, no member of the currently described family has received imatinib as an adjuvant for \(\sim 1\) year after resection of a small bowel GIST. She has tolerated the regimen without incident.

In addition to predisposing to GIST, germ-line \(^3\)KIT mutations result in other gastrointestinal pathology and particularly in disrupted bowel motility. Members of the current family, like another reported previously (16), often reported dysphagia. In the family described by Hirota \textit{et al.} (16), dysphagia was associated with low-resting lower esophageal sphincter pressure and abnormal peristalsis, consistent with disruption of the function of the esophageal myenteric plexus. In addition to dysphagia, severe gastroesophageal reflux symptoms were reported by several members of the current kindred, suggesting that abnormalities of both peristalsis and sphincter tone may be clinically relevant in these families.

It is not clear whether different germ-line mutations in \(\textit{KIT}\) lead to different clinical syndromes, particularly regarding those elements other than GIST. The mutation in the family with dysphagia reported by Hirota \textit{et al.} (16) was in the tyrosine kinase II domain of the protein, as opposed to the juxta-membrane substitution in the current family. This indicates that esophageal dysmotility is likely to be a general feature of the syndrome rather than a specific correlate of tyrosine kinase II mutations. Another report suggested that individuals with germ-line mutations in the tyrosine kinase I domain were predisposed to GIST but not to other components of the syndrome such as hyperpigmentation or urticaria pigmentosa (17). The experience reported here suggests that caution should be exercised when assessing possible genotype-phenotype correlations in this disorder. Members of the current family, with a “typical” juxta-membrane domain mutation, reported every combination of GIST, hyperpigmentation, and dysphagia, but few individuals reported all three. Whereas it is possible that there is variable expression of the predisposition, several individuals did not report particular components until they were questioned in detail.

The present family is the largest yet described with a germ-line \(\textit{KIT}\) mutation resulting in a hereditary predisposition to GIST. Whereas the development of GIST was nearly uniform and often resulted in significant morbidity, metastatic disease

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\textbf{Table 1} Reported families transmitting germ-line \textit{KIT} mutations

<table>
<thead>
<tr>
<th>Family</th>
<th>Mutation</th>
<th>Domain</th>
<th>GIST(^a)</th>
<th>Ages</th>
<th>Site</th>
<th>Pigment</th>
<th>Urticaria pigmentosa</th>
<th>Dysphagia</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (19)</td>
<td>559delIV</td>
<td>JM</td>
<td>+</td>
<td>60, NS</td>
<td>ND</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>2 (17)</td>
<td>K642E</td>
<td>TK1</td>
<td>+</td>
<td>67, 40</td>
<td>SB</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>3 (15:20)</td>
<td>W557R</td>
<td>JM</td>
<td>+</td>
<td>69, 52</td>
<td>SB</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>4 (14)</td>
<td>V559A</td>
<td>JM</td>
<td>+</td>
<td>18, others NS</td>
<td>G, SB</td>
<td>+</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>5 (18)</td>
<td>V559A</td>
<td>JM</td>
<td>+</td>
<td>41, 45, others NS</td>
<td>G, SB</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>6 (16)</td>
<td>D820Y</td>
<td>TKII</td>
<td>+</td>
<td>71, 70, 65, 57, 36</td>
<td>G, SB</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Current</td>
<td>W557R</td>
<td>JM</td>
<td>+</td>
<td>See text</td>
<td>G, SB</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

\(^a\)GIST, gastrointestinal stromal tumor; JM, juxta-membrane; TKI, tyrosine kinase I; TKII, tyrosine kinase II; G, gastric; SB, small bowel; NS, not stated; ND, not described.

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\(^7\)C. R. Antonescu, manuscript in preparation.
was rare. The age of diagnosis of GIST varied widely, and family members were inconsistent in reporting the presence of other components of the syndrome. This variability must be taken into account when assessing other families for the possible existence of this disorder. Whereas careful monitoring for the development of GIST is reasonable, the indolent clinical course and multifocality of the disease suggests that surgical intervention should be deferred in the absence of complications. The role of imatinib as a therapeutic or preventive intervention in these rare individuals remains to be defined.

ACKNOWLEDGMENTS
We acknowledge the members of this family who gave their time so generously and enthusiastically. Drs. John Robinson and Rini Pawlingal, and Alice Schluger for technical assistance.

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