Dysphagia, melanosis, gastrointestinal stromal tumors and a germinal mutation of the KIT gene in an Argentine family

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Summary
Gastrointestinal stromal tumors (GIST) are the most common mesenchymatous neoplasms of the human digestive tract. They locate preferentially in stomach, duodenum or small bowel. Usually sporadic, familial cases unrelated to neurofibromatosis may be due to germline mutations in KIT or PDGFRA. We describe the first Argentine family with GIST in which we found, diffuse cutaneous melanosis, lentiginosis, and dysphagia. Dysphagia was not observed in the four families previously described with the same mutation. Histopathology resulted consistent with GIST, and tumor immunohistochemistry was likewise positive for DOG-1, CD117 (KIT) and CD34. The search for germline mutations identified the KIT c.1697T>C (p.559V>A) substitution in exon 11. Treatment with imatinib is furnishing positive results.

Key words. GIST, KIT, dysphagia.

Familia argentina con disfagia, melanosis, tumores del estroma gastrointestinal y mutación germinal del gen KIT

Resumen
Los tumores del estroma gastrointestinal (GIST) son las neoplasias mesenquimáticas más comunes del tubo digestivo humano. Se localizan preferentemente en el estómago, el duodeno o el intestino delgado. Los casos descriptos son por lo general esporádicos pero se describen casos familiares no relacionados a neurofibromatosis debido a mutaciones germinales en el gen KIT o en PDGFRA. Describimos la primera familia argentina con GIST en la que encontramos, melanosis cutánea difusa, lentiginosis y disfagia. La disfagia no se observó en las otras cuatro familias descriptas con la misma mutación. La histopatología y la inmunohistoquímica fueron positivas para DOG-1, CD117 (KIT) y CD34. La búsqueda de mutaciones germlines identificó la mutación c.1697T>C (p.559V>A) en el exón 11 del gen KIT. El tratamiento con imatinib está proporcionando resultados positivos.

Palabras claves. GIST, KIT, disfagia.
20, 2012) or PDGFRA, the gene for the platelet-derived growth factor receptor alpha (OMIM 173490, April 20, 2012); 2) a Carney triad [with lung enchondromas and/or paragangliomas, and absolute female predominition (85%)]; and 3) a very infrequent familial autosomal dominant syndrome of GIST, melanosis and dysphagia, often associated to a germlinal mutation in KIT or PDGFRA, both genes located within human chromosome 4q12.3,4

GIST develop most frequently in stomach (60%), duodenum and small bowel (35%), and less frequently in colon, rectum, esophagus, omentum and mesentery (<5%). Most lesions within the latter two are metastases. Histologically, they include spindle, epithelioid and even pleomorphic cells. These can be studied by immunohistochemistry since they express DOG-1 (100%), KIT (CD117) (95%), heavy caldesmon (80%), CD34 (70%), smooth muscle actin (SMA) (20-30%) (the latter two often reciprocating), protein S100 (10%), and even desmin (less than 5%). Morphological similarities and the common expression of KIT (CD117) and CD34 have allowed authors to postulate that GIST originate from a subpopulation of ICC. An alternative would be pluripotent stem cells from which both ICC and smooth muscle cells expressing SMA arise.3,6

KIT (CD 117) is a transmembrane receptor with tyrosine quinase type III activity. Its ligand is a growth factor dubbed stem cell factor or mast cell growth factor. In addition to ICC, KIT is expressed constitutively in several normal tissues (hematopoietic stem cells, mast cells, melanocytes, basal cells of the epidermis, mammary epithelial cells, germ cells, and cells of certain regions of the central nervous system). KIT is neither expressed in normal stratified epithelium nor found in glandular epithelium of the digestive tract, pancreas, prostate, or endocervix. KIT expression can be detected in GIST; mast cell tumors; seminomas; melanomas; endometrial, ovary, small-cell lung and breast ductal carcinomas; and acute myeloid leukemia.7

The finding of oncogenic KIT mutations in the majority of GIST, and the fact that tumors lacking those mutations show a high degree of KIT phosphorylation, led to the inference that the central tumorigenic event would be the constitutive activation of the KIT receptor, and to propose it as a therapeutic target in GIST.3 Mice with the germline Kit K641E mutation developed KIT activation, ICC hyperplasia and GIST, in a mutant allele dose-dependent, completely penetrant fashion: homozygotes displayed a more extensive hyperplasia, larger tumors and increased mortality from intestinal obstruction than heterozygotes. The predictions have been supported by clinical data in which tyrosine kinase inhibitors have been shown to be effective targeted therapeutic agents for the treatment of sporadic GIST.10,11

We report the first Argentine GIST family with dysphagia, diffuse melanosis, lentiginosis, a c.1697T>C (p.559V>A) in KIT exon 11 (HGMD CM013551) and a favorable response to imatinib. In so doing, we expect to increase the casuistic supporting the current mutant genotype-phenotype correlation, and to widen the latter by including dysphagia.

Patients and methods

Guided by the family pedigree, the interdisciplinary medical team in charge of the investigation had access to all six affected individuals (proband, mother, four sibs) and two clinically unaffected (a full sister and a half sister from a different father), among adult members of available households, as well as to two symptomatic children from one affected female sib.

After informed consent, all adults were submitted to physical examination, esophageal-gastric-duodenal fibroscopy, computerized tomography (CT) scan of the abdomen, and, with the exception of the unaffected sister, to KIT mutation investigation. Children have only been physically examined to date.

The histopathological diagnosis of tumors and their immunohistochemistry for CD34, KIT (CD117) and CD138 were performed in Argentina. Slides from the proband tumors and organically extracted genomic DNA from seven adults who agreed to the molecular investigation were sent to Boston. In order to confirm the diagnosis and search for mutations, histopathology was repeated, KIT (CD117) and DOG-1 were tested, and the proband DNA was scanned for sequence variations in KIT and PDGFRA. For this, enzymatically amplified exons were submitted to either denaturing HPLC or high resolution melting curve analysis, followed by direct sequencing of variant fragments in all seven adult samples.

Results

The proband (Figure 1, individual III.5), a 29-year-old male with history of dysphagia, entered the Dr Eduardo Castro Rendón State Hospital from Neuquén, Patagonia, Argentina, because of upper digestive tract bleeding. Physical examination found thin skin diffuse melanosis, generalized lentiginosis, and palmar crease hyperpigmentation without compromise of the rest of palm and sole skin. The abdominal CT scan demonstrated multiple exophytic and endophytic lesions disseminated along the digestive tract.
The esophageal-gastric-duodenal fibroscopy revealed a bleeding endophytic lesion in the duodenum and multiple tumors in the upper gastrointestinal tract. Tumors were proven to be GIST by histopathology and immunohistochemistry, and the patient was heterozygous for a KIT exon 11 c.1697T>C (p.559V>A) substitution.

The proband’s eldest brother (Figure 1, individual III.2), a 33-year-old male with history of dysphagia, was found to have intense melanosis and lentiginosis on physical examination. CT scans and endoscopic studies found multiple gastrointestinal lesions that histopathology and immunohistochemistry allowed to identify as GIST. Molecularly, this patient was indeed heterozygous for the KIT p.559V>A mutation.

A 32-year-old sister (Figure 1, individual III.3) with history of dysphagia and 4 years of treatment with corticosteroids for a diagnosis of Addison’s disease later disproven biochemically, had cutaneous hyperpigmentation and multiple gastrointestinal exophytic and endophytic lesions on CT scans. Esophageal-gastric-duodenal fibroscopy made possible to submit some of them to histopathology and immunohistochemistry, confirming the diagnosis of GIST. Heterozygosity for the family KIT mutation was demonstrated in this patient too.

The remaining brother (Figure 1, individual III.6), a 27-year-old male with history of dysphagia, had cutaneous hyperpigmentation, GIST tumors, and the same KIT mutation in the heterozygous state.

The youngest sister (Figure 1, individual III.7), a 26-year-old female with history of dysphagia, had hypermelanosis and was heterozygous for the family KIT mutation. She has not yet been evaluated by endoscopy or CT scan.

Neither the remaining sister (Figure 1, individual III.4), aged 31 years old, nor the half-sister (Figure 1, individual III.1), aged 36 years old, have shown any clinical, endoscopic or tomographic sign of the disease. The KIT genotype of the latter was homozygous for the wild-type allele.

Children of III.3, a 14-year-old female (Figure 1, individual IV.6) and a 9-year-old male (Figure 1, individual IV.7), began with progressive cutaneous melanosis during their fourth year of life, but they have not showed digestive symptoms. The biochemical evaluation ruled out Addison’s disease.

The mother of the proband (Figure 1, individual II.2), a 55-year-old native Argentine female, has suffered since her youth a dysphagia attributed to an esophageal achalasia and treated with repeated esophageal dilations. She displayed intense melanosis of thin and thick skin, lentiginosis, and was heterozygous for the family KIT mutation; however, she had no tumor by upper gastrointestinal endoscopy and CT scan.

The Table 1 summarizes clinical and molecular findings in each individual. The Figure 2 illustrates the cutaneous lesions, the Figure 3 displays a small bowel piece with tumors excised from the proband, and the Figure 4 shows the histopathology of one of those tumors, quite characteristic of a...
GIST of the spindle-cell type, and supporting immunohistochemistry. Tumors were negative for desmin, synaptophysin and S100, and positive for CD34, KIT (CD117), CD138 and DOG-1. Spindle cell areas were positive for smooth muscle actin too. The Figure 5 portrays the homozygous genotype of unaffected individual III.1, and the heterozygous genotype of the affected proband including the KIT exon 11 c.1697T>C (p.559V>A) allele and a normal allele.

The treatment of gastrointestinal tumor-affected family members with imatinib, a tyrosine kinase inhibitor, achieved the progressive reduction of melanosis and tumors, according to the follow-up by CT scan and endoscopy.

**Discussion**

The analysis of the pedigree reported herein (Figure 1) is consistent with autosomal dominant inheritance, even though no male-to-male transmission event has been registered yet. All symptomatic individuals molecularly investigated were heterozygous for the family mutation. We were not able to find evidence of reduced penetrance of the disease as such, since the only unaffected individual available for molecular diagnosis (Figure 1, individual III.1) was homozygous for the wild-type allele. However, not every affected individual manifested dysphagia and tumors, and the penetrance of each abnormal feature increased differentially with age.

**Figure 2. A) Diffuse skin hyperpigmentation with lentiginosis, B) lentigines on soles.**

<table>
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<tr>
<th>Pedigree ID</th>
<th>Age at diagnosis and evaluation (years old)</th>
<th>Hyperpigmentation*</th>
<th>Dysphagia**</th>
<th>Gastrointestinal tumors***</th>
<th>Melanoma</th>
<th>Other malignancy</th>
<th>KIT exon 11 c.1697 (p.559) genotype</th>
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* Hyperpigmentation started at 4 years in individuals IV.6 and IV.7, and would have started before age 10 in all other affected ones. It has been progressive, with increasing lentigine numbers.

** Dysphagia would have appeared within the second decade in all affected individuals at 20 years old or more.

*** Gastrointestinal tumors excised from all four patients labelled as + have been diagnosed as GIST based on their histopathology and immunohistochemical positivity for at least CD117 and CD54.
The most penetrant and earliest feature was the hyperpigmentation (Figure 2 A), which was found in all heterozygotes and appeared during the first decade in two and perhaps in most if not in all cases. We observed intense and diffuse melanosis as in all published families with the same mutation, and lentiginosis (Figure 2 B) as in two of them; but in this family the melanosis was not restricted to axillary and inguinal regions, no family member had had urticaria pigmentosa, and no melanoma had been diagnosed.\textsuperscript{4,12,13} In this family, only the proband had gastrointestinal bleeding. Otherwise, the predominant symptom and reason for seeking medical help was the dysphagia. This would have been present since the second decade among all affected individuals older than 25 years, and was especially relevant in the individual II.2. None of the families previously reported with the same mutation displayed this symptom, even though it did associate to substitutions in \textit{KIT} exon 11 W557R and exon 17 D820Y.\textsuperscript{4,14,15} Actually, GIST-associated dysphagia would not be due to esophageal achalasia but to a peculiar esophageal abnormality. This consists of mild esophageal dilation, abnormal, non-propulsive esophageal contractions with low cardiac resting pressure, and hyperechogenic thickening of the parietal layer located between the longitudinal and circumferential muscle fiber layers, and corresponding to the myenteric plexus. Oppositely, the typical esophageal achalasia associates to a poorly developed or absent myenteric plexus.\textsuperscript{15} Gastrointestinal tumors were not found before the third decade, and the individual II.2 has not shown them yet, even though she is already half way in her sixties.

\textbf{Figure 3.} Excised small bowel piece with tumors.

\textbf{Figure 4.} Tumor sections showing: A) the typical aspect of a GIST of the spindle-cell type (hematoxyline-eosine staining), B) its hypercellularity and low mitotic index, C) its immunohistochemical positivity for CD117.
which could be an example of reduced feature penetrance. Regardless, the average age for tumor diagnosis in this family was significantly lower than expected for sporadic lesions. In fact, among all nine families reviewed by Li et al., GIST appeared at an average age of 46 years old, more than a decade earlier than for non-familial cases.4

The histopathology indicated that tumors were spindle cell-type GIST. Several tumors obtained from the duodenum and jejunum revealed a low mitotic index. In spite of this finding, their size larger than 1 cm (Figure 3), as well as the hypercellularity and the mucosal infiltration detected in some of them, allowed us to recognize the high malignant potential of the affection.

The presumptive diagnosis was supported by positive CD34 and KIT (CD117) immunoreactivity (Figure 4 C). DOG-1 positivity made the diagnosis certain.5 The investigation of germline mutations identified the KIT c.1697T>C (p.559V>A) substitution in exon 11. This locates to the juxtamembrane domain of the receptor and allows to predict its constitutive, gain-of-function activation.6

We have treated all gastrointestinal tumor-affected family members with imatinib, a tyrosine kinase inhibitor, as it was previously suggested.10,11 These are the first patients with this mutation to undergo this treatment, which has caused a progressive reduction of the melanosis and of the tumor size as followed by endoscopy and CT scan.

Acknowledgements

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Databases


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References


