Familial Gastrointestinal Stromal Tumor With Loss of Heterozygosity at *Kit* Exon 11: Genotypic, Histopathologic, Radiographic, and Therapeutic Findings

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Introduction

Gastrointestinal stromal tumors (GIST) represent the most common type of mesenchymal tumor of the GI tract. These tumors are thought to arise from the Interstitial Cells of Cajal. Most of these tumors develop sporadically but rarely they cluster within families. To date, there are sixteen families described in the medical literature with a hereditary GIST syndrome. These families develop GIST at an earlier age than their sporadic counterparts and often occur with associated conditions including urticaria pigmentosa, nevi, hyperpigmentation or other malignancies. Most kindreds with familial GIST have a germline mutation in the *kit* gene on chromosome 4 although there has been a report of a family with a *PDGFRα* gene mutation.

In our study, we describe a kindred with 15 members with GIST. We discovered a mutation in kit exon 11 in germ-line and within tumors from individuals with a high propensity to develop GIST including a case with loss of heterozygosity. We describe the pattern of inheritance, the origin of the tumor from hyperplastic myenteric plexus, the histopathological appearance of these tumors and describe the efficacy of imatinib mesylate (Gleevec, Novartis, Basel, Switzerland) in hereditary GIST.

Pedigree

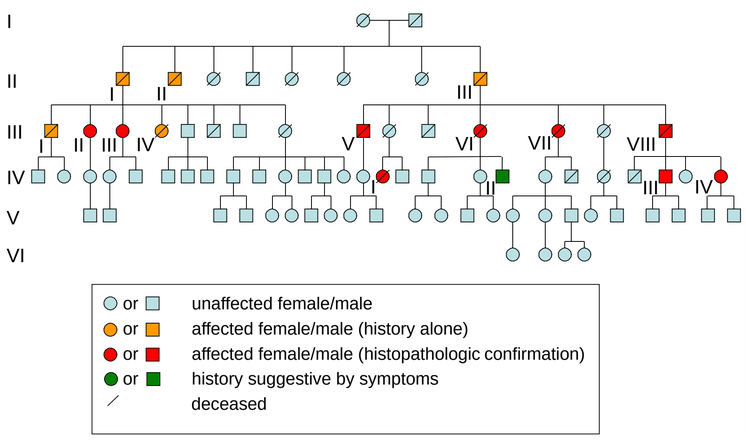

Figure 1. Pedigree of the affected family.

Histopathology

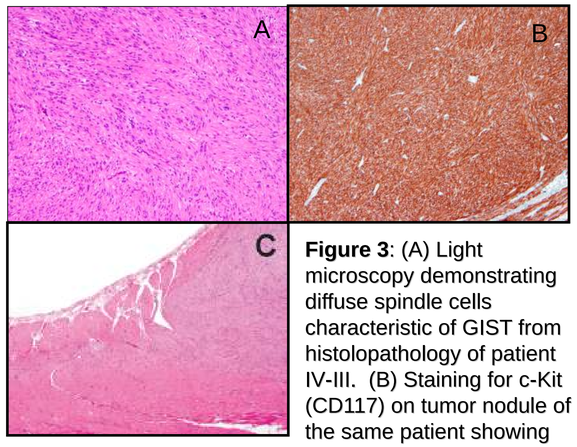

Figure 3: (A) Light microscopy demonstrating diffuse spindle cells characteristic of GIST from histolopathology of patient IV-III. (B) Staining for c-Kit (CD117) on tumor nodule of the same patient showing diffuse positivity. (C) Hypertrophic myenteric plexus with nodularity.

Mutational Analysis

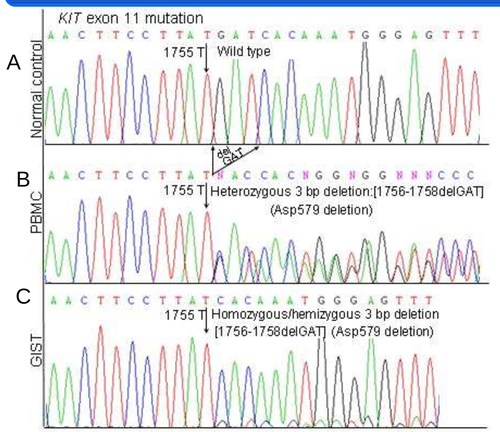

Figure 5. Homozygous/hemizygous deletion at position 1756-1758 (GAT) results in loss of heterozygosity in a GIST from patient IV-III. A) Wild-type *kit* gene from normal control DNA. B) Peripheral blood mononuclear cells from patient IV-III showing germ-line heterozygous deletion of 3 base pairs at position 1756-1758 (GAT) with subsequent 3 base pair frame shift in exon 11. C) Tumor from the same patient shows apparent loss of heterozygosity with deletion of GAT at position 1756-1758 in *kit* exon 11.

Materials & Methods

The proband patient was evaluated at the Sarcoma Center at M.D. Anderson Cancer Center (MDACC) after diagnosis with metastatic GIST. The history and physical, radiographic imaging and histopathology was reviewed. The patient provided a detailed family history of multiple relatives with GIST. Through informed consent under a protocol approved by the IRB, willing family members released their medical records for review and were interviewed by phone. Medical records, pathology slides and reports , endoscopy/colonoscopy reports and CAT scan films and reports were obtained for patients with a history of GIST or symptoms consistent with possible GIST. Pathology slides and radiographic images were reviewed by sarcoma pathologists and radiologists, respectively.

Tumor was available from four of the family members with GIST(III-II, III-III, IV-III, IV-IV) and independently reviewed by a sarcoma pathologist at MDACC. Both light microscopy and immunohistochemistry were performed on the tissue specimens. Each specimen was stained for c-Kit, CD34, Smooth Muscle Actin, desmin, pan-cytokeratin and S-100 proteins. Stains were graded as strong, weak or negative and diffusely positive, focally positive or negative. The gross maximal tumor dimension and number of mitoses per ten high powered field were recorded.

DNA was isolated from paraffin-embedded or from peripheral blood mononuclear cells (PBMNC) by using a QIAamp DNA(Qiagen Inc., Valencia, CA) according to the manufacturers instructions. Nucleotide sequencing was analyzed with a 3730 X 1 DNA from Applied Biosystems. The Genomic DNA sequences of *kit* exon 9,11, 13, 15, 17 and pdgfr-a exon 12, 18 were analyzed in all available GIST specimens and PBMNCs

Gross Pathology

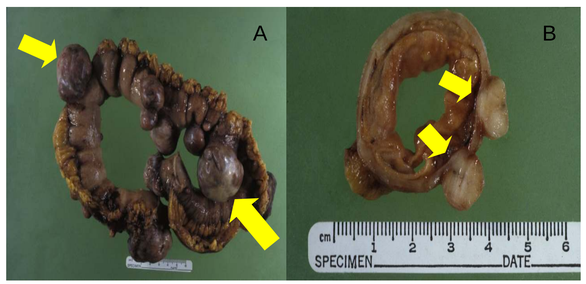

Figure 2. (A) Gross pathological specimen of small bowel demonstrating numerous tumor nodules arising from the submucosal layer of patient IV-III.

(B) Magnified view of same patient with illustration of two tumor nodules arising From a segment of hypertrophied small bowel wall.

Therapeutic Response

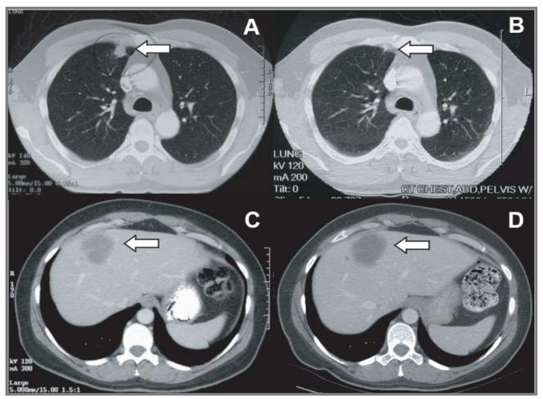

Figure 4: (A&B) Before and after initiation of imatinib (IM) therapy in pt IV-III. (C&D) Before and after initiation of IM therapy in pt IV-IV

Results

Fifteen members were identified in this family with histopathologic diagnosis of GIST or probable GIST. Available surgical findings revealed multiple tumors arising in the submucosa of the small intestine. Histopathology revealed microscopic proliferation of the myenteric plexus with areas of microscopic tumor nodularity (Figure 3C). Four patients in this kindred were treated with imatinib and had radiographic and clinical evidence of therapeutic benefit. An in-frame, three base pair deletion from 1755-1758 (codon 579) in exon 11 of the *kit* gene was identified in tumor and normal tissue of this family. Evaluation of one GIST revealed apparent loss of heterozygosity in *kit* exon 11 suggesting either a homozygous mutation or loss of the wild-type locus while the other tumors were heterozygous.

Conclusions

This study describes a kindred with a high propensity to develop GIST in association with hyperpigmentation and variable melanocytic nevi but not dysphagia. Familial GIST may be associated with apparent loss of heterozygosity at the locus of germ-line *kit* mutation. The germline deletion of codon 579 appears to confer proliferation and nodularity of the myenteric plexus, synchronous tumors at presentation, a metastatic phenotype, and *in vivo* sensitivity to imatinib therapy.

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