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Multiple Gastrointestinal Stromal and Other Tumors Caused by Platelet-Derived Growth Factor Receptor α Gene Mutations: A Case Associated with a Germline V561D Defect

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Context: Gastrointestinal stromal tumors (GISTs) may be caused by somatic or germline mutations of the *KIT* and *PDGFRA* genes, but most GISTs associated with neuroendocrine tumors (NETs) are not, suggesting that other molecular pathways are implicated in their pathogenesis.

Objective: In the course of investigating NETs and GIST genetics, we encountered a patient who had a unique combination of multiple fibrous polyps and lipomas of the small intestine and several gastric GISTs.

Design: The study included the clinical description of a unique patient, DNA sequencing of germline and tumor DNA, and comparative genomic hybridization (CGH) and allelic marker analysis of tumor DNA.

Results: The patient was found to carry a germline *PDGFRA* mutation (V561D) in the heterozygote state; it has only been seen rarely before and only in the somatic state in sporadic GISTs. CGH identified losses of chromosomal regions 1p33–36, 9q12–24, 11q13, and 16q; loss of the 14q region that is commonly lost in NETs and GISTs was shown by DNA marker analysis. These changes are likely to point to secondary and tertiary genetic hits involved in the formation of these rare tumors.

Conclusions: Multiple GISTs and other tumors may be caused by germline *PDGFRA* gene mutations; the V561D mutation can occur in the germline state and lead to a syndrome that should not be confused with other genetic conditions associated with a predisposition to NETs and other tumors. A number of chromosomal loci are likely to be involved in the *PDGFRA* V561D-dependent tumorigenesis, as shown by CGH and other DNA analyses. **(*J Clin Endocrinol Metab* 92: 3728–3732, 2007)**

GASTROINTESTINAL STROMAL tumors (GISTs) may be sporadic or inherited in an autosomal dominant manner either alone or as a component of a multiple tumor syndrome, such as neurofibromatosis type 1 (1–4). There are at least two more genetic conditions, both multiple neoplasia syndromes, with a predisposition to paragangliomas (PGL) and other endocrine or nonendocrine tumors that are associated with GISTs. The first condition that was initially described as the "triad of gastric leiomyosarcoma, functioning extraadrenal paraganglioma, and pulmonary chondroma" (4) is now known as the "Carney triad" (CT) (5–8) and is listed in Online Mendelian Inheritance in Man (OMIM) under catalog no. 604287. The second condition was recently described in patients with PGL and GIST (8). This disease has been referred to as the dyad of "PGL and gastric stromal sarcoma" or the "Carney-Stratakis syndrome" (CSS) (OMIM no. 606864) (9) and has been described in a number of kindreds (10). Mutations of the genes coding for the succinate dehydrogenase (SDH) subunits B, C, and D (*SDHB*, *SDHC*, and *SDHD*) that are associated with familial PGLs (11) appear to be the most likely cause of CSS (12, 13). Mutations (somatic or germline) of the platelet-derived growth factor receptor-α (*PDGFRA*) or *KIT* (the gene coding for c-KIT, the human homolog of the feline sarcoma virus HZ4-FeSV) have been associated with GISTs (14–17). However, these genes are not mutated in CT or CSS patients (12, 13, 18).

In this report, we describe the identification of a patient with the unique combination of multiple GISTs and small intestinal polyps, fibroid tumors, and lipomas, who had no mutations in the *KIT*, *SDHA*, *SDHB*, *SDHC*, and *SDHD* genes, but had a mutation in the *PDGFRA* gene that is for the first time identified in the germline. This patient is different from those described with other *PDGFRA* germline mutations (16, 17) and intestinal neurofibromatosis (INF) because of her unusual tumors. Comparative genomic hybridization (CGH) has not been applied before in INF or any related conditions; CGH in our patient's tumor identified several

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Abbreviations: CGH, Comparative genomic hybridization; CSS, Carney-Stratakis syndrome; CT, Carney triad; GIST, gastrointestinal stromal tumor; HIF, hypoxia-inducible factor; INF, intestinal neurofibromatosis; LOH, loss of heterozygosity; *PDGFRA*, platelet-derived growth factor receptor-α; PGL, paragangliomas; SDH, succinate dehydrogenase.

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genetic changes that may be related to *PDGFRA*-induced tumorigenesis.

Subject and Methods

Clinical studies and tissue samples

The institutional review boards of National Institute of Child Health and Human Development (NICHD), National Institutes of Health (Bethesda, MD) and the Mayo Clinic (Rochester, MN) approved the contact of the patient and her participation in the NICHD protocols 95-CH-0059 and 00C-H-0180 after giving informed consent.

Description of clinical findings

At age 22 yr (in 1977), the patient passed a small amount of blood in the stool. A barium enema was normal. Esophagogastroduodenoscopy revealed a small gastric mass and a duodenal polyp. The latter was excised, and a lipoma was found. At age 32 yr, the patient developed small bowel obstruction. A barium study showed multiple ileal, duodenal, and gastric masses. Laparotomy was performed with excision of the masses; there was a mass in the cecum, but the remainder of the large bowel was normal. Microscopically, the diagnosis was mesenchymal nodules of uncertain origin. The differential diagnosis included multifocal stromal tumor, malignant schwannoma, leiomyosarcoma, and multiple hamartomas. An 8-month course of chemotherapy (cytoxan, adriamycin, dacarbazine, and vincristin) was administered. Two years later, cholecystectomy and excision of a jejunal diverticulum were performed; gastric and small intestinal tumors were noted again. The following year, the patient developed acute small bowel obstruction with abdominal pain and vomiting. At operation, intussusception in the mid small bowel was found. The area of involvement was resected. Upper endoscopy at the age of 47 yr showed partial duodenal obstruction; submucosal prepyloric and duodenal masses were found. Colonoscopy showed moderate to severe diverticulosis and at surgery she was found to have multiple small intestinal tumors that were polypoid, covered by intact mucosa, and protruded into the bowel lumen; several lesions were confluent (Fig. 1A). Upon histopathological examination, these lesions (fibroid tumors) tended to be hypocellular with prominent collagen bundles; tumor cells featured round and spindle-shaped nuclei with very little if any cytoplasm (Fig. 1, B–D). The patient's family was unavailable for studies, but there was no history of similar tumors in any of her relatives.

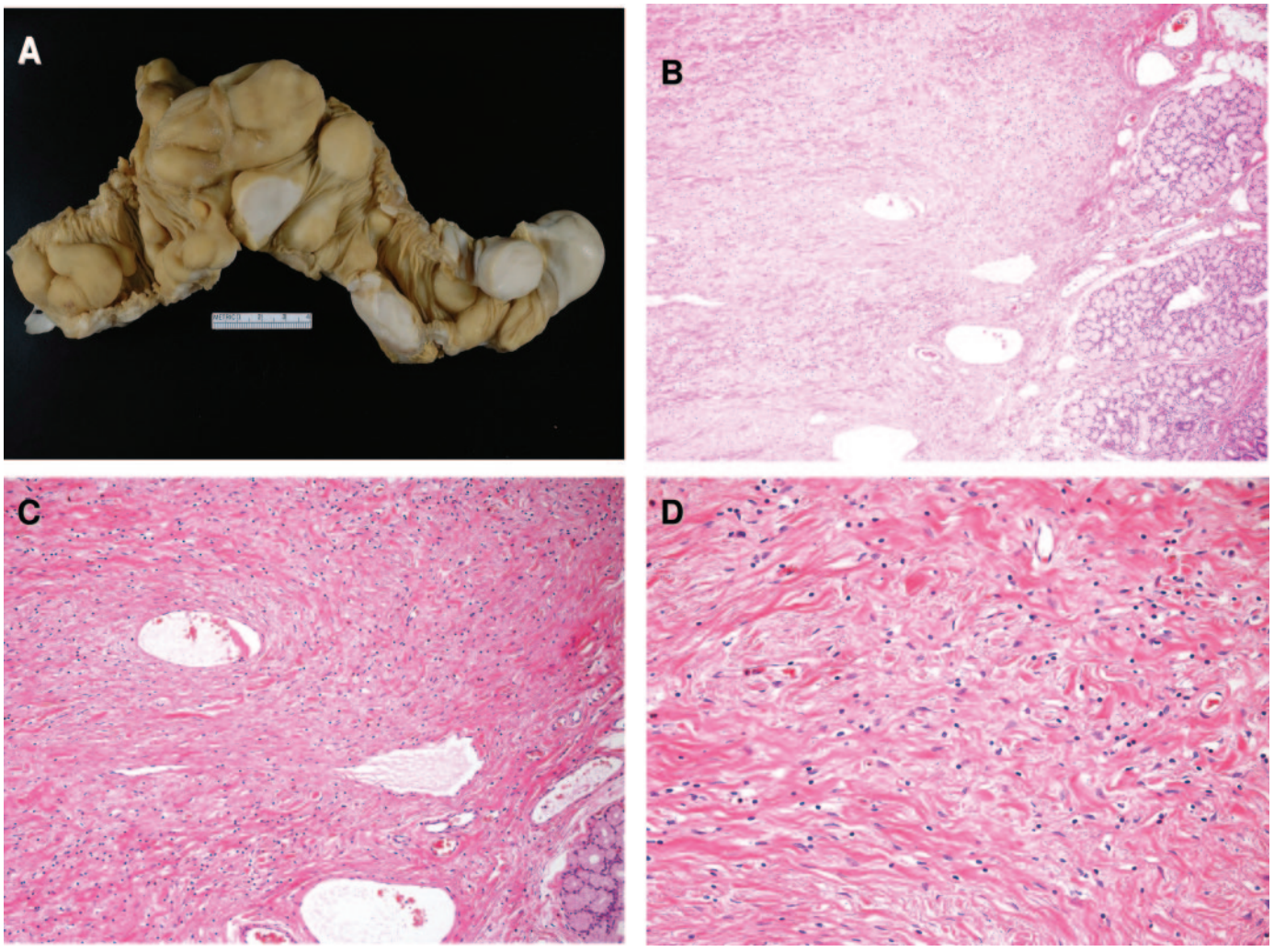

FIG. 1. Multiple small intestinal fibroid tumors. A, Formalin-fixed specimens of small intestine showed multiple polypoid tumors covered by intact mucosa; the tumors were generally hemispherical and protruded into the bowel lumen; some were confluent. The cut surface was white and homogeneous. B, Hypocellular, circumscribed fibrous submucosal tumor with prominent collagen bundles at periphery (*top*) and dilated blood vessels. The tumor did not extend among Brunner's glands (hematoxylin and eosin staining; magnification, ×50). C, Hypocellular fibrous tumor with broad collagen bundles (*upper left*) in submucosa and a few dilated blood vessels; nuclei were round (hematoxylin and eosin staining; magnification, ×100). D, Monotonous hypocellular tumor composed of thick collagen fibers; nuclei were round and spindle-shaped and cytoplasm was not apparent (hematoxylin and eosin stain; magnification, ×400).

Specimen handling

Blood and tissue samples were collected. Tissue was obtained at surgery and processed for routine histopathology and immunohistochemistry after formalin fixation and paraffin embedment; additional fragments were frozen immediately at −70 C for molecular and genetic studies. DNA was extracted using standard methods (18), and all samples were microdissected from surrounding normal tissue.

Sequencing and allelic heterozygosity analysis

Mutation analysis for all exons, exon-intron boundaries, and flanking intronic regions was performed for the *KIT*, *SDHA*, *SDHB*, *SDHC*, *SDHD*, and *PDGFRA* genes as has been published elsewhere (10, 11, 14–18). Frozen tumor material was used for total RNA extraction and cDNA synthesis by reverse-transcriptase PCR and sequencing, using appropriate *PDGFRA* gene primers (15).

Tumor and other DNA samples were also subjected to loss of heterozygosity (LOH) analysis using markers surrounding the *PDGFRA* gene and chromosome 14q (markers *D14S587* and *D14S592*, on 14q13.1 and 14q22.3, respectively). CGH and LOH data analysis was performed as described elsewhere (18).

Results

The patient had a germline mutation in the *PDGFRA* gene: V561D (GTC>GAC) in exon 12, in the normal gastric mucosa and the GIST cells; a "control" GIST from a different patient showed only the expected normal sequence (Fig. 2A). Although sequencing showed the presence of both the wild-type and mutant sequences, LOH analysis with 8 chromosome 4q12 markers showed losses around the *PDGFRA* gene (data not shown).

Sequencing of 24 other GISTs from patients with either multiple tumors or family history of GISTs and other neoplasms did not identify any pathogenic *PDGFRA* sequence changes; in addition, these tumors did not demonstrate LOH of the 4q *PDGFRA* region (data not shown). The known *PDGFRA* polymorphisms S478P and IVS17–50delA, and 2472C>T in exons 10 and 18, respectively, were identified in both patients' and normal control samples (data not shown).

CGH showed that the patient's tumor had a number of secondary genetic changes, including losses of chromosomal regions 1p33–36, 9q12–24, 11q13, and 16q. Although 14q losses were not present on CGH, we checked 14q at the tumor with markers *D14S587* and *D14S592*, on 14q13.1 and 14q22.3, respectively, for LOH; both loci showed LOH (Fig. 2B). Interestingly, none of the other 24 non-*KIT*, non-*PDGFRA*-mutant GISTs that we used as controls in the present study showed any 14q LOH (data not shown).

Discussion

GISTs, although rare (5000 new cases-per-year in the United States), are the most common mesenchymal tumors of the gastrointestinal tract. When GISTs occur in association with other tumors, PGLs are the most commonly associated lesions, in the context of genetic conditions such as the neurofibromatosis syndromes (2, 3), CT (4, 5), and CSS (8, 9, 13). GISTs originate from the interstitial cells of Cajal (7), the pacemaker cells that regulate peristalsis in the digestive tract. In common with interstitial cells of Cajal, up to 95% of GISTs express the receptor tyrosine kinase KIT, whose immunohistochemical detection (CD117) together with the marker CD34 (hematopoietic progenitor cell antigen) forms part of the diagnostic tests for these lesions (7). In recent years, it has been established that 75–80% of GISTs harbor somatic, gain-of-function *KIT* gene (7, 14) and approximately 7% in the related *PDGFRA* gene (7, 15). Mutations of both genes generate constitutively activated tyrosine kinase receptors with ligand-independent autophosphorylation and downstream signaling. Most *KIT* somatic mutations involve the intracellular juxtamembrane domain (67% in exon 11) or the upstream extracellular domain (17% in exon 9) with loss of the autoinhibitory role of the spontaneous dimerization domain (7). By contrast, most *PDGFRA* mutations affect the activation loop of the tyrosine kinase domain II (82–89% of the mutations occur in exon 18) and are believed to switch the catalytic domain into its active conformation (7, 15, 19). Mutations in *KIT* and *PDGFRA* genes are mutually exclusive events that result in the activation of common signaling

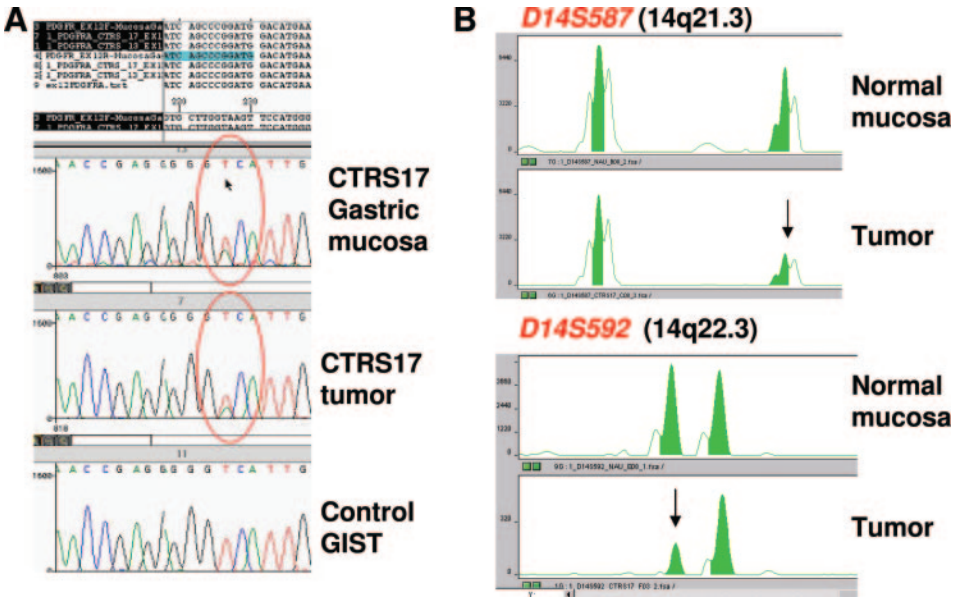

FIG. 2. A, The patient had a germline mutation in the *PDGFRA* gene; the mutation is V561D (GTC>GAC) in exon 12. Both the normal gastric mucosa (*upper sequence panel*) and the tumor cells had this mutation (*middle panel*); a GIST from an unrelated patient showed only the normal sequence (*lower panel*). B, Losses of markers from the commonly lost in GISTs 14q region in the patient's tumor; both *D14S587* and *D14S592* showed losses (*arrows*) of genetic material in one allele at the tumor specimen.

pathways including the PI3K/AKT antiapoptotic, JAK/STAT3 transcriptional, and Ras/MAPK mitogenic pathways that respond to STI571 and its analogs (7, 15, 19, 20).

Germline *PDGFRA* mutations have also been described in two kindreds that presented with multiple gastric GISTs or the condition known as INF (16, 17) and without any other associated lesions. Although these patients had multiple GISTs, our patient did not have INF by histology, but had a unique combination of GISTs, polyps, fibroids, and lipomas that has never been described before. The V561D germline mutation that was identified in exon 12 of the *PDGFRA* gene in our patient lies in a "hot spot" for genetic alterations of the juxtamembrane domain of the gene; it has already been identified in more than 18 sporadic GISTs, and the homologous mutation in the *KIT* gene, V559D, has also been identified in sporadic and familial GISTs and is associated with phosphorylation of the receptor and ligand-independent activation (7, 14, 19). The substitution of the small hydrophobic valine with a larger and negatively charged aspartate is believed to change the "close" autoinhibitory conformation (hairpin loop that docks in the interface between the kinase N and C lobes, maintained by hydrophobic interactions with site chains of V643, Y646 (*KIT* C-helix), C788, and I789 (*KIT* C-lobe), to an "open" activated conformation by hiding a charged position away from solvents in a hydrophobic site (19).

The tumor in our patient was also examined by CGH. Sporadic, familial, and neurofibromatosis type 1-associated GISTs have been extensively analyzed by CGH; among the most frequent findings are chromosome 14q and 22q monosomy followed by 1p, 11p, 9q deletions and 8p and 17q gains (7, 18). These regions overlap with only a few of the changes identified in a GIST from our patient. The common 14q loss (26) was not present in our tumor by CGH but was confirmed by LOH studies (Fig. 2B). 14q deletion is a common occurrence in sporadic GISTs, regions 14q11-q12 and 14q23-q24, in particular; on 14q13.1 lies the hypoxia-inducible factor (HIF)-proly-hydroxylase 3 gene and on 14q23.2 lies the HIF-1 α subunit (*HIF1A*) gene. The HIF pathway is involved in the pathogenesis of GISTs and many other mesenchymal tumors, as well as neuroendocrine tumors. 1p and 9q also demonstrated losses in agreement with findings in sporadic GISTs and other tumors of related conditions (18). Other abnormal regions such as 11q13 and 16q may represent genetic changes specific to GIST of our patient. It would be interesting to see whether genes participating in neurofibromatosis type 1- or *PDGFRA*-linked oncogenetic pathways (20) are important in the pathogenesis of the condition identified in our patient and located in the genomic regions identified in this study.

In conclusion, we identified a unique patient with multiple GISTs and other tumors linked to a germline *PDGFRA* gene V561D mutation; this combination of tumors has not been seen before in any syndromic condition associated with GISTs, neuroendocrine tumors, and related tumors. We also found a number of chromosomal loci that are likely to be involved in the *PDGFRA* V561D-dependent tumorigenesis in this patient.

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