Gastrointestinal Stromal Tumor in Monozygotic Twins Shows Distinct Mutational Status: A Case Report

Marcelo Sobral-Leite  
Jesse Lopes da Silva  
Haynna Kimie Pimenta-Inada  
Andrea Sobral Mendes Boisson  
Priscilla de Almeida Romeiro  
Fernando Mallet Soares Parago  
Marcus Vinicius M. Valadão da Silva

Corresponding Author: Andreia Cristina de Melo, e-mail: andreia.melo@inca.gov.br

Conflict of interest: None declared

Case series
Patients: Female, 62-year-old • Female, 62-year-old
Final Diagnosis: Gastrointestinal stromal tumor (GIST)
Symptoms: Abdominal pain • fatigue
Medication: —
Clinical Procedure: Genetic analysis
Specialty: Oncology

Objective: Unusual clinical course
Background: Gastrointestinal stromal tumors (GISTs) are rare mesenchymal cancers that affect the gastrointestinal tract and are most often located in the stomach and proximal small intestine. The most common molecular genetic abnormalities underlying GIST carcinogenesis are mutations in the tyrosine kinase gene (KIT) and in the platelet-derived growth factor receptor alpha (PDGFRA) gene. To the best of our knowledge, no cases have been reported so far of synchronous diagnosis of GIST in 2 monozygotic twins presenting with clinical and morphological features of sporadic disease.

Case Reports: This report presents the cases of 2 monozygotic twin sisters who were diagnosed with GIST at the same age and who had different KIT exon 11 tumor mutational statuses. In the current report, the screening examination that led to early detection of GIST in one of the sisters was not motivated by any symptom, but by a GIST diagnosis in her twin a few days before. The literature was reviewed for pathological and molecular features associated with prognosis and treatment response. Furthermore, we identified identical genotypes of KIT and PDGFRA polymorphisms in the DNA of both tumors that might be present in the germline DNA. The present case supports the implementation of specific cancer screening in the context of monozygotic twins, regardless of identification of the genetic components involved.

Conclusions: Our report suggests that monozygotic twins with GIST can have different mutational statuses for KIT and PDGFRA. Referral for special screening should be considered for individuals who have a monozygotic twin diagnosed with cancer.

Keywords: Diseases in Twins • Gastrointestinal Stromal Tumors • Proto-Oncogene Proteins c-kit

Full-text PDF: https://www.amjcaserep.com/abstract/index/idArt/929887
Background

Gastrointestinal stromal tumor (GIST) is a cancer with mesenchymal origin in the interstitial cells of Cajal, which belongs to the intestinal autonomic nervous system as a smooth muscle pacemaker [1]. GIST was introduced as a specific disease in 1983, and today it is the mesenchymal tumor most often seen in the gastrointestinal tract [2-4]. Most population-based studies report an incidence of approximately 10 to 15 cases of GISTs per million inhabitants [4].

In 75% to 80% of cases of GIST, the type III receptor tyrosine kinase protein, c-KIT (or CD117), is overexpressed [5,6]. Another receptor tyrosine kinase, the platelet-derived growth factor receptor alpha (PDGF-Rα), is expressed in 5% to 10% of cases of GIST [7]. c-KIT and PDGF-Rα appear to be the main oncogenic drivers involved in GIST carcinogenesis [8]. Thus, immunohistochemistry (IHC) for c-KIT and PDGF-Rα needs to be performed as part of routine diagnosis [6].

Most GISTs are resistant to chemotherapy and the introduction of imatinib mesylate (IM), which targets and inhibits c-KIT, significantly improved overall patient survival [9,10]. Furthermore, the identification of mutations in the c-KIT (KIT) and PDGF-Rα (PDGFRA) genes led to an increased understanding of GIST biology and treatment resistance [11]. Gain-of-function mutations in these 2 proto-oncogenes and their hotspot gene locations have been well described [12]. The mutational statuses of KIT and PDGFRA have emerged as strong biomarkers for GIST prognosis and prediction of response to therapy; the absence of mutations in approximately 10% of cases is associated with reduced effectiveness of IM and shorter survival [13-15].

A number of families with multiple inherited GIST syndromes (MIM #606764) have been described. These syndromes are rare and caused by inherited molecular defects that affect the entire organism [16]. The Carney triad is characterized by gastric GIST, paraganglioma, pulmonary chondroma, and loss of expression of succinate dehydrogenase subunit B but not mutations in KIT or PDGFRA [17]. Carney-Stratakis syndrome is seen in patients who have a germline mutation in any subunit of the succinate dehydrogenase genes and who are young [18]. Several studies have described a number of families with GIST cases that span generations and involve heritable mutations in KIT or PDGFRA. These reports are associated with young age, multiple cases in the family, multiple tumors (more frequently in the stomach and small bowel), and inflammatory fibroid polyps, among other abnormalities [19-27]. Neurofibromatosis type I, one of the most common dominantly inherited genetic disorders, has been described in association with the occurrence of GIST [28]. However, most GISTs are sporadic and risk factors related to sporadic GIST have not been identified [29].

The present case report describes 2 monozygotic twin sisters who were diagnosed with GIST at the same age and who had differing tumor mutation statuses in exon 11 of the KIT gene. The role of inherited mutations associated with GIST and genetic susceptibility to cancer is not fully understood. Thus, epidemiological studies and case reports describing diseases in twins have important clinical implications for cancer screening and treatment decisions [30,31].

Case Reports

The study was approved by the Ethics in Human Research Committee of the Brazilian National Cancer Institute, Rio de Janeiro, Brazil, and conducted following Good Clinical Practice guidelines. Written informed consent was provided by each patient.

Tissue for the cases was processed, sectioned, stained, and scored according to the available guidelines [32]. The 2 patients described were diagnosed and treated at different cancer centers in Rio de Janeiro, Brazil. In Case 1, tumor DNA was extracted from frozen tissue collected during palliative surgery. In Case 2, tumor DNA was extracted from a formalin-fixed, paraffin-embedded tissue block (primary tumor) using standard methods. In both cases, primary tumor samples were the source of the DNA. KIT mutational status in exons 9, 11, 13, and 17 and PDGFRA mutations in exons 12, 14, and 18 were analyzed using polymerase chain reaction amplification and subsequent DNA sequencing [33]. Products were analyzed with Sequencher software, version 4.1 (Gene Codes Corporation, Ann Arbor, Michigan, United States). The nomenclature used was in accordance with ENST00000288135.6.

Standard Response Evaluation Criteria in Solid Tumors (RECIST 1.1) were used to assess treatment response [34]. Professionals with specialized certification analyzed and interpreted the clinical, radiological, and pathological data from the patients.

Case 1

The patient in Case 1 was a 62-year-old woman. In 2011, she presented with a urinary tract infection after experiencing a fever intermittently for 1 week. On clinical examination, a pelvic mass was identified. The patient underwent pelvic and abdominal computed tomography (CT), which revealed a homogeneous soft tissue mass in the pelvis that measured 11.0×11.0×11.0 cm and liver lesions suggestive of metastases (Figure 1). An ultrasound-guided biopsy of the pelvis then was performed. Pathological analysis of the material from the patient’s small
intestine revealed morphological features of GIST and IHC staining was positive for c-KIT.

Initially, the patient was prescribed systemic therapy with 400 mg of IM daily. On Day 35 of treatment, she was admitted to the hospital with anemia and abdominal distension, and rapidly developed intestinal obstruction. New CT scans showed a significant increase in the pelvic mass with intestinal obstruction. An emergency ileostomy was performed. Fortunately, at that time, a sample was taken from the abdominal tumor and cryopreserved. Molecular analysis of this GIST sample showed no deletion or missense mutation in KIT or PDGFRA (Table 1).

After the ileostomy, the patient was treated in the Intensive Care Unit for septic shock; mechanical ventilation and vasoactive amines were required. A few days later, her clinical condition deteriorated and she died.

Case 2

The patient in Case 2 was the monozygotic twin sister of the patient in Case 1. In 2001, she was diagnosed with breast cancer, subtype luminal B, and was treated with neoadjuvant chemotherapy, mastectomy, adjuvant radiotherapy, and hormonal therapy. During clinical surveillance in November 2011, she underwent pelvic and abdominal ultrasound as a screening examination; the motivation for it was the GIST diagnosis in her sister. The imaging revealed a pelvic mass measuring 8.5×6.5 cm, which prompted an exploratory laparotomy with optimal resection. The pathological findings from the surgical specimen were a GIST measuring 9×6×6 cm, with 19 mitoses/5 mm². The tumor, which stained positive with IHC for c-KIT, was located in the small intestine. Genomic analysis of the specimen revealed a mutation in exon 11 of the KIT gene: c.1676_1681del [p. (Val559_Val560del)] (Table 1).

Beginning in March 2012, the patient was treated with adjuvant therapy with IM (400 mg daily). She completed 3 years of therapy with mild adverse effects, reporting only recurrent nausea.

Interestingly, in both Case 1 and Case 2, the genotyping of the tumors showed the same polymorphisms in KIT exon 17 (rs55789615), and PDGFRA exons 12 (rs1873778) and 18 (rs3803355). Because the patients described here are monozygotic twins, they carried the same germline DNA sequence, which leads us to suggest that the polymorphisms recorded in the tumor DNA of both sisters are likely to be germline variants.

Six years after the GIST diagnosis, the patient in Case 2 reported abdominal pain. A CT scan demonstrated a solid nodule in her pelvis, attached to the sigmoid, which measured 3.3×2.8 cm.
In July 2018, laparotomy was performed and the lesion was completely resected. The pathology report confirmed recurrence of the GIST. IM was restarted in August 2018 and the patient is continuing to take it and remains free of recurrence.

**Discussion**

This is the first report of GIST diagnosed concomitantly at a similar age in monozygotic twins. Analysis of the tumor molecular pathology, genetics, treatment response, and outcomes in the 2 related patients have resulted in insights about cancer screening and the carcinogenesis of GIST.

To the best of our knowledge, current guidelines for familial GIST screening [35] do not mention screening the other twin if there is no strong family history of the disease. Conclusions about cancer incidence in twins and first-degree relatives of individuals with GIST versus the general population, reported in familial studies, have proven controversial [36-38]. The observations are consistent enough, however, to have led to suspicion about the involvement of a heritable genetic component in the development of the malignancies [31, 39], whereas environmental factors are likely to play an important role in the carcinogenesis of sporadic cancers [40]. Nevertheless, long-term follow-up of a prospective study with Nordic twins (n=80 309 monozygotic twins) estimated an increase in absolute cumulative cancer risk of 14% (95%CI 12-16%) in monozygotic twins diagnosed with cancer as compared with the overall cohort, and 38% of twins were diagnosed with the same type of cancer [41]. In the present report, the screening with abdominal and pelvic ultrasound that led to diagnosis of GIST in Case 2 was motivated by the previous diagnosis in Case 1. Because it facilitated prompt diagnosis, surveillance may be warranted for early detection of asymptomatic disease. It should be noted that although the clinical records for Case 2 included a complete description of the findings from the first CT scans, which identified the primary GIST, the images themselves were unavailable.

Regarding Case 1, the later analysis of the tumor mutation profile showed no pathogenic mutations in KIT exons. That could explain the rapid progression of the advanced disease soon after the patient started treatment with IM. In most cases, patients with wild-type GIST have limited sensitivity to IM [42,43]. In the present case, knowing the patient’s mutational status earlier would not have changed treatment decisions because neoadjuvant therapy is recommended prior to extended resection [44].

A deletion in KIT exon 11 (delVE559-560) was detected in Case 2. Interestingly, this finding suggests that the mutation status

| Clinical features | Case #1 | Case #2 |
|-------------------|---------|---------|
| **Diagnosis**     |         |         |
| Age at diagnosis  | 62      | 62      |
| Sex               | Female  | Female  |
| GIST location     | Small intestine | Small intestine |
| Stage             | IV      | T3NOM0  |
| **Treatment**     |         |         |
| Pre-surgery       | IM 400 mg/day for 3 months | No pre-surgery treatment |
| Surgery           | Palliative ileostomy | Complete resection |
| Adjuvant          | NA      | IM 400 mg/day for 3 years |
| KIT               |         |         |
| Exon 9            | WT      | WT      |
| Exon 11           | WT      | c.1676_1681del [p. (Val559_Val560del)] |
| Exon 13           | WT      | WT      |
| Exon 17           | SNP rs55789615 (2394C>T) | SNP rs55789615 (2394C>T) |
| PDGFRα            |         |         |
| Exon 12           | SNP rs1873778 (1701A>G) | SNP rs1873778 (1701A>G) |
| Exon 14           | WT      | WT      |
| Exon 18           | Intrinsic duplication SNP rs3830355 | Intrinsic duplication SNP rs3830355 |

IM = imatinib mesylate; NA = not applicable.

In July 2018, laparotomy was performed and the lesion was completely resected. The pathology report confirmed recurrence of the GIST. IM was restarted in August 2018 and the patient is continuing to take it and remains free of recurrence.
of GISTs may differ among patients with the same genomic DNA. Mutations in KIT exon 11 are the ones most frequently found in GIST, occurring in about 60% of cases [45-47]. Some studies have reported that patients whose tumors harbor these specific mutations have the best response rates and long-term survival with IM treatment [48-49]. However, deletions in KIT exon 11 involving codons 557-558 are likely to be associated with unfavorable outcomes, but not as an independent risk factor [50]. The variability of mutations found in these oncogenes and the lack of large series with long-term follow-up make treating advanced GIST and establishing criteria based on tumor mutational status challenging [51,52].

 Syndromic GISTs account for approximately 3% to 4% of all GIST cases and their clinicopathological characteristics are different from the sporadic cases [16]. The peculiar clinical and pathological features of the Carney triad and Carney-Stratakis syndrome – early age at diagnosis, mainly gastric GISTs, multiple neoplasms, and lack of c-KIT expression – do not correspond to the disease descriptions for Case 1 or Case 2, which involved older patients who had non-gastric GISTs and high expression of c-KIT [17,18]. It is important to note that the familial Carney syndrome has been described in 2 monozygotic twins brothers, who were aged 12 and 13 years old, respectively [53].

In recent years, some familial GISTs with germline mutations in KIT have been identified [21,54,55]. These families exhibit a variety of clinical syndromes with tumors in multiple sites, hyperpigmentation, urticaria pigmentosa, and dysphagia [22,24,25], which also do not match the findings in Case 1 or Case 2, or in their family members. Because the mutation in the KIT exon 11 in Case 2 was not present in the tumor in Case 1, it appears to be somatic rather than germline. On the other hand, the concomitant occurrence of variations in KIT exon 17 and PDGFRA exons 12 and 18 are more likely to be germline polymorphisms.

The involvement of PDGFRA germline mutations has been described in a few cases of familial GIST [27,56,57]. The polymorphisms in PDGFRA identified in the tumors from the patients in Cases 1 and 2 are not likely to be associated with oncogenic events. To our knowledge, no association between these polymorphisms and familial GIST has been described. Unfortunately, germline DNA isolation and sequencing were not undertaken in the cases in the present report. We are aware that germline DNA from Case 2 could be the same as that from the deceased patient in Case 1 because the patients were monozygotic twins. It would be insightful to perform whole-exome sequencing of the germline DNA of one of the monozygotic twins. Although no genetic variations have been identified that clearly predispose patients with GIST to higher risk, in the case of monozygotic twins, such as in the present study, the potential for co-development of tumors should be discussed when making recommendations for cancer screening.

 Appropriately designed genomic studies should be considered to search for germline polymorphisms associated with sporadic GISTs and investigate germline variations in KIT, PDGFRA, and other candidate genes, such as NF1, BRAF, SDHB, and SDHD. Until such research is carried out and potential genetic findings are validated, our recommendation to perform cancer screening in monozygotic twins could contribute to early detection of GIST and other types of cancer.

Conclusions

The description of the two cases in the present report raises new questions about screening, diagnosis, and treatment of and prognosis in patients with cancer who are monozygotic twins. Even though different clinical stages could explain the different outcomes in our 2 patients, we have demonstrated that the mutational status of KIT exon 11 may differ in tumors with the same germline DNA. That, in turn, could have an impact on treatment resistance and prognosis, given data from previously published series. Even though the relationship of hereditary to the disease course in the present case remains unclear, the findings suggest that referral for specialized screening should be considered for monozygotic twins who have been diagnosed with cancer.

Acknowledgments

The authors are indebted to all of the patients and their families for their trust and participation in the study and the provision of biological material for research purposes. The authors also thank all the individuals who supported the 2 patients during their treatment and made the case report possible: Rosane Vianna-Jorge, Gustavo Stefanoff, Marjanka K. Schmidt, Jan Schellens, Monica Padoan, and Romeo Rigby.

Ethics Approval

The present study was approved by the Ethics in Human Research Committee of the Brazilian National Cancer Institute, Rio de Janeiro, Brazil, under registration number CAAE 34313020.6.0000.5274 and conducted following Good Clinical Practice guidelines.

Conflicts of Interest

None.
52. Joensuu H, Eriksson M, Hall KS, et al. Survival outcomes associated with 3 years vs 1 year of adjuvant imatinib for patients with high-risk gastrointestinal stromal tumors: An analysis of a randomized clinical trial after 10-year follow-up. JAMA Oncol, 2020;6(8):1241-46

53. Boccon-Gibod L, Boman F, Boudjemaa S, et al. Separate occurrence of extra-adrenal paraganglioma and gastrointestinal stromal tumor in monozygotic twins: Probable familial Carney syndrome. Pediatr Dev Pathol, 2004;7(4):380-84

54. Thalheimer A, Schlemmer M, Bueter M, et al. Familial gastrointestinal stromal tumors caused by the novel KIT exon 17 germline mutation N822Y. Am J Surg Pathol, 2008;32(10):1560-65

55. Li FP, Fletcher JA, Heinrich MC, et al. Familial gastrointestinal stromal tumor syndrome: Phenotypic and molecular features in a kindred. J Clin Oncol, 2005;23(12):2735-43

56. Pasini B, Matyakhina L, Bei T, et al. Multiple gastrointestinal stromal tumors caused by platelet-derived growth factor receptor alpha gene mutations: A case associated with a germline V561D defect. J Clin Endocrinol Metab, 2007;92(9):3728-32

57. Ricci R, Martini M, Cenci T, et al. PDGFRA-mutant syndrome. Mod Pathol, 2015;28(7):954-64