Epithelioid gastric stromal tumours of the antrum in young females with the Carney triad: A report of three new cases with mutational analysis and comparative genomic hybridization

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Abstract. Carney triad is a rare non-hereditary condition affecting young females and characterized by metachronous or synchronous occurrence of epithelioid gastrointestinal stromal tumours (GISTs), pulmonary chondroma and extra-adrenal paraganglioma. The genetic alterations in Carney triad-related GISTs have not been well studied. We evaluated GISTs from three females with incomplete Carney triad for *KIT* and *PDGFRA* mutations and studied the DNA by comparative genomic hybridization (CGH). All GISTs originated in the antrum and had a monotonous epithelioid morphology. Two patients had GISTs and pulmonary chondroma and one had GISTs and paraganglioma. Initial manifestation was GIST (n=1), pulmonary chondroma (n=1) and bladder paraganglioma (n=1). Time to the second component was 2-13 years. Two patients were alive at 108 and 168 months (one with metastases) and one died of the disease 3 years later. All cases were wild-type for *KIT* exons 9, 11, 13, 17 and *PDGFRA* exons 12 and 18. CGH revealed 14 aberrations (mean, 4.7/tumour) including 11 gains (X, 1q, 5p, 8q, 9p, 12p, 13q, 18p, 19q), 2 amplifications (1q, 19p) and one loss (13q). Carney triad-related GISTs do not only lack conventional *KIT* and *PDGFRA* mutations, but they also lack the non-random loss of 14q and 22q characteristic of their sporadic counterparts, suggesting an origin through a distinct pathogenetic pathway.

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Introduction

Gastrointestinal stromal tumours (GISTs) represent the most common primary mesenchymal neoplasms of the GI tract. Histologically, GISTs show spindle cell (70%), epithelioid (20%) or mixed morphology (10%) (1,2). Conventional (sporadic) GISTs comprise ≥95% of the cases. They commonly occur as solitary neoplasms in adults with a peak incidence between 55 and 65 years of age and a slight male predilection (1,2). Approximately 50-60% of sporadic GISTs occur in the stomach, 20-30% in the small intestine and <10% in the colorectum, the oesophagus or elsewhere in the abdominal cavity (1,2). Activating mutations in *KIT* (50-85%) or *PDGFRA* (≤10%) are the most common oncogenic events in GISTs (3-5). Exons 11 and 18 are hot spots for *KIT* and *PDGFRA* mutations in GISTs, respectively (3-5). *KIT* and *PDGFRA* mutations are mutually exclusive. They represent early oncogenic events in GIST tumourigenesis. Recent studies have documented diverse cytogenetic alterations in benign, borderline and malignant GISTs (3,6-10). While some chromosomal anomalies (loss of 14q and 22q) occur frequently and non-randomly in both benign and malignant GISTs suggesting early molecular events, others are associated with biological tumour progression and malignant behavior (6-10). Benign GISTs mainly show chromosomal losses, and malignant/metastatic ones commonly reveal gains and high-level amplifications (7,9,10). In addition, alterations involving diverse tumour suppressor genes have been reported to be predictive of clinical behavior (11).

Less than 5% of GISTs arise in the setting of a syndromic disease (12-15). Such syndromic GISTs lack conventional

KIT and *PDGFRA* mutations (12,16). The genetic events involved in their development are not yet known. Familial GISTs caused by germline mutations in *KIT* or *PDGFRA* differ from sporadic and Carney triad-related GISTs by their multifocal involvement of different parts of the GI tract (mainly the stomach and small bowel) associated with diffuse interstitial cells of Cajal hyperplasia and, rarely, other KIT-related disorders (hyperpigmentation and mast cell proliferation) (17).

Carney triad is a rare non-hereditary condition affecting young females (≤40 years old) and characterized by synchronous or metachronous occurrence of epithelioid gastric GISTs, extra-adrenal paraganglioma and pulmonary chondroma. To date, ~80 cases of the disease have been documented in the literature worldwide (13-15). Diagnosis of the triad requires either all (complete form) or at least two components (incomplete form) of the disease (13-15). The incomplete form is the most common (15). Other facultative manifestations of the disease include non-gastric GISTs and adrenocortical adenoma (15). The intervals between the different components of the disease may exceed 30 years (15). For this reason, the syndrome is probably under-diagnosed. A similar number of apparently sporadic pediatric GISTs have been reported (18-24). Based on the clinicopathologic and molecular similarity, it remains possible that some of these apparently sporadic pediatric GISTs may represent either initial manifestation or a *forme fruste* of Carney triad (20).

A peculiar feature of Carney-GISTs is their unusually indolent clinical course that is compatible with long survival, even after occurrence of metastasis. In a review of all published cases (15), only six patients (8%) with the triad died of their metastatic GIST after a follow-up of 1-49 years (mean, 20.6). Specific life-long follow-up guidelines have been proposed for patients with the complete or incomplete form of the triad, as well as for those manifesting one typical component (25).

The cytogenetic features of Carney-GISTs have not been previously evaluated. In this study we examined for the first time the clinicopathologic and molecular genetic profile of Carney-GISTs in three patients with the incomplete triad, with the goal of identifying potential genetic markers that may help delineate Carney triad-related GISTs from other pediatric GISTs.

Case histories

Case 1. A female patient underwent surgical resection of two and three nodules from the right and left lung at age 17 and 22 years, respectively. Radiological diagnosis was also made of an unspecified congenital malformation of the right ninth rib. An emergency 2/3-gastric resection was performed at age 30 because of profuse upper GI bleeding from an ulcerated antrum tumour during pregnancy. At age 42, she underwent a segmental resection of the transverse colon together with multiple tumours in the mesocolon. At that time, ultrasonography showed multiple liver metastases. Radiology revealed extensive involvement of both the lungs by multiple centrally calcified chondromas. Three years later the patient was started on imatinib, but no further follow-up is available. Her family history was negative for similar disease.

Case 2. A 20-year-old female was diagnosed with a paraganglioma of the urinary bladder, which was surgically resected. Three years later, she presented with fatigue and an abdominal mass and was found to have a large gastric tumour with peritoneal and hepatic metastases. She experienced stabilization under chemotherapy, but suffered from significant bone marrow toxicity. The liver lesions were subsequently treated twice by chemo-embolization. She received thalidomide for 10 months and was then switched to imatinib therapy due to the disease progression. She was treated with imatinib on a compassionate use protocol at a dose of 400 mg/day and initially experienced symptomatic relief. However, her GIST lesions continued to progress, and she died 3 months later. The patient's paternal grandmother had angiosarcoma; there was no other significant family history.

Case 3. A 28-year-old female patient presented with GI bleeding and was discovered to have a gastric mass. She underwent surgical resection of the gastric antrum, which contained a 6-cm tumour described as forming 'multiple submucosal gastric lesions'. At the age of 30, she had two benign pulmonary nodules removed and a diagnosis of Carney triad was made. The patient did well until the age of 37, at which time she was found to have a mass in the liver which was positive on a PET scan. The lesion was surgically removed, but a repeat PET scan performed a few months later showed a residual positive signal, so the patient was started on 400 mg imatinib per day. She had some trouble with side effects (leg cramping) and temporarily stopped the drug, but as of her last follow-up was again on 400 mg/day.

Materials and methods

Immunohistochemistry. The present study was approved by The Institutional Review Board. Tissue samples were fixed overnight in buffered formalin and processed routinely for H&E microscopy. Immunohistochemical stains were performed using the ABC method and the following antibodies: vimentin (Vim 3B4, 1:50; Linaris, Wertheim-Bettingen, Germany), smooth muscle actin (1A4, 1:200; Dako, Hamburg, Germany), desmin (D33, 1:250; Dako), h-caldesmon (h-CD; 1:75, Dako), S-100 (polyclonal antibody, 1:2500; Dako), CD117 (anti-Human c-kit proto-oncogene product, polyclonal antibody, 1:50; Dako), CD34 (BI-3C5, 1:200; Zytomed, Germany), and Ki-67 (MiB-1, 1:150; Dako).

Mutation analysis of KIT and PDGFRA. Formalin-fixed, paraffin-embedded tumour tissue was manually micro-dissected under an inverted microscope. For case 1 genomic tumour DNA was isolated using the MagNA Pure system and LC DNA Isolation kit II (Roche Diagnostics, Mannheim, Germany) according to the supplier's instructions. Polymerase chain reaction (PCR) and sequencing of *KIT* and *PDGFRA* were carried out as previously described by Iesalnieks *et al* (26) and Agaimy *et al* (27). For cases 2 and 3, genomic DNA was extracted, purified and subjected to PCR exactly as previously described by Heinrich *et al* (4). The resulting amplicons were screened for mutations by denaturing HPLC, as described.

Table I. Clinicopathological features of Carney-GISTs (n=3).

Case no.	1st tumour (age/site)	2nd tumour (age/site)	Follow-up
1	5x chondromas excised at 17 and 22 years from both lungs	Multinodular epithelioid antrum GIST at 30 years	Multiple liver metastases at 108 months; alive with disease at 168 months (on imatinib)
2	Bladder paraganglioma at 20 years	Multinodular epithelioid antrum GIST at 23 years	Liver and peritoneal metastases simultaneously; deceased due to disease (GIST) at 36 months
3	Multinodular epithelioid antrum GIST at 28 years	2 lung chondromas excised at age 30	Liver metastases at 108 months; alive with disease (on imatinib)

All of the cases were evaluated for the presence of mutations in *KIT* exons 9, 11 and 13, and *PDGFRA* exons 12 and 18. Cases 2 and 3 were also evaluated for mutations in *KIT* exon 17.

Comparative genomic hybridization (CGH). DNA from formalin-fixed, paraffin-embedded tissue slices was prepared using the Nucleospin DNA extraction kit according to the instructions of the manufacturer (Macherey and Nagel, Dueren, Germany). Chromosomal CGH (cCGH) was performed using direct fluorochrome-conjugated DNAs in all cases following the modified methods previously described (28,29). The tumour DNA was labelled with Spectrum Green dUTP (Abbott, Wiesbaden, Germany) and reference DNA with Spectrum Orange dUTP (Abbott) by nick translation. In each CGH experiment, a negative control (DNA from healthy tissue) was included.

The hybridizations were analyzed using a Zeiss fluorescence microscope Axioplan imaging (Zeiss, Jena, Germany) and the CytoVision digital image analysis system (Applied Imaging Corporation, Newcastle Upon Tyne, UK) based on an automated CGH analysis software (CGH extended package). Three-color images were acquired from 15-20 metaphases. Only metaphases of good quality with strong uniform hybridization were included in the analysis.

Results

Pathological findings

Gastric tumours. The data are summarized in Table I. All gastric lesions were multinodular or multifocal separated by bundles of residual muscularis propria. Mucosal ulceration was common. The cut surface of the tumours was grey-white or tan-yellow with focal areas of haemorrhage (Fig. 1A). Histologically, the tumours were predominantly epithelioid consisting of mildly to moderately atypical medium-sized polygonal cells with pale-eosinophilic to clear vacuolated cytoplasm arranged in a nested paraganglioma-like or a non-specific diffuse pattern (Fig. 1B). Case 1 revealed numerous pseudorossettes (Fig. 1C). Mitotic activity ranged from 8-18 per 50 HPFs. Case 1 showed two perigastric lymph node metastases and numerous hyperplastic polyps in the antrum and fundus. The recurrent tumours from case one (0.3- to 6.5-cm nodules) revealed essentially similar features. Immunohistochemistry revealed a strong reactivity for vimentin and CD117 in all cases (Fig. 1D) with variable reactivity for CD34.

Other tumours. Slides of pulmonary nodules from cases 1 and 3 and of bladder paraganglioma from case 2 were not available for review, but detailed pathology reports were available. Excised lung lesions from case 1 and 3 were reportedly osteochondromas and chondromas composed purely of mature cartilage with central areas of calcification.

Mutational status. All cases were wild-type for *KIT* exons 9, 11, 13 and *PDGFRA* exons 12 and 18. Cases 2 and 3 were also wild-type for *KIT* exon 17.

CGH findings. CGH revealed a total of 14 aberrations (mean, 4.7 aberrations/tumour; Table II). There were 11 gains, 2 amplifications and only one loss (gains:losses = 11:1). Case 1 showed gains of the whole chromosome X and the long arm of chromosome 7 (7q35q36), and 19 (19q) and an amplification of 19p (Fig. 2A). In case 2, loss of a part of the long arm of chromosome 13 (13q32q34), gains of a part of the long arm

Table II. CGH results in gastric GISTs of the current patients (n=3).

Case no.	CGH results		
	Loss	Gain	Amplification
1	NR	X, 7q35q36, 19q	19p
2	13q32q34	1q21q42, 12p13, 18p11.31	1q42q44
3	NR	5p14, 8q24.1q24.2, 9p22, 12p13, 13q32q34	NR

NR, no results

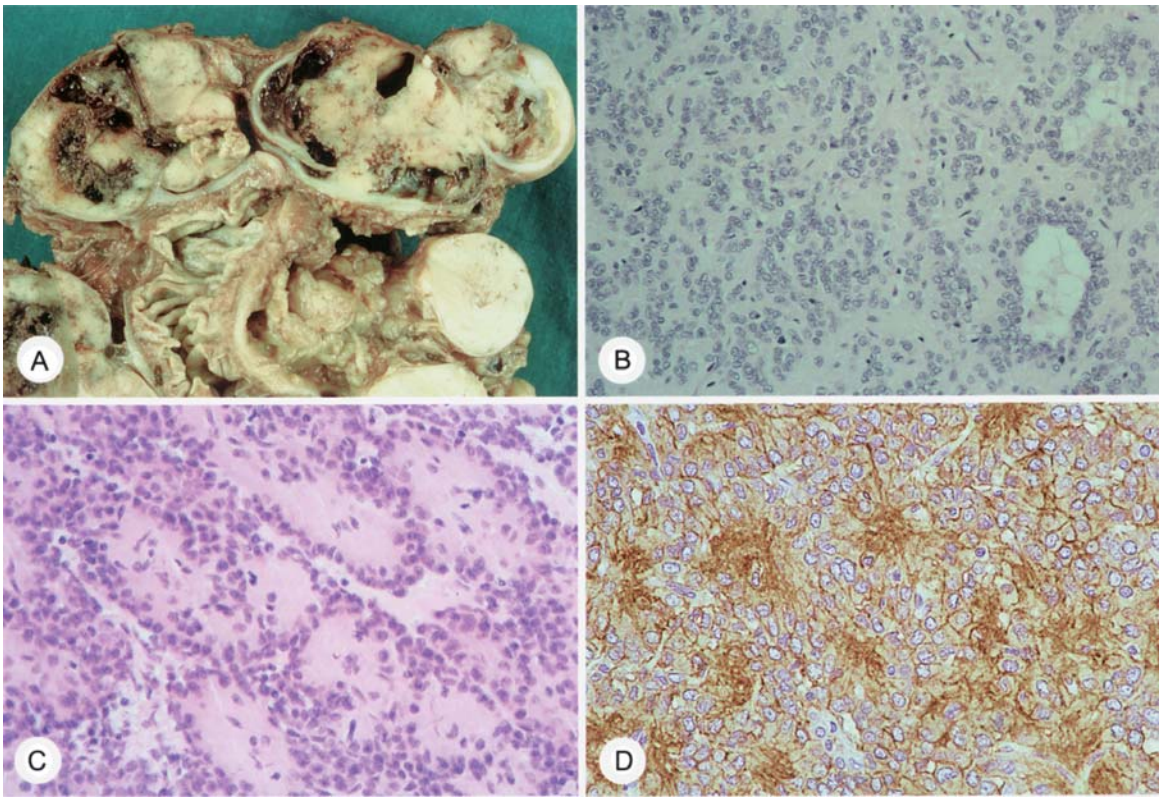

Figure 1. Representative macroscopic, histologic and immunohistochemical findings of Carney-GISTs (case 1). (A) Recurrent GISTs forming multiple tumour nodules in the colon transversum with involvement of the gut wall. (B) Monotonous epithelioid morphology with pseudofollicular structures. (C) Numerous pseudorossettes surrounding neuropil-like cytoplasmic processes that were highlighted by CD117 immunostaining (D).

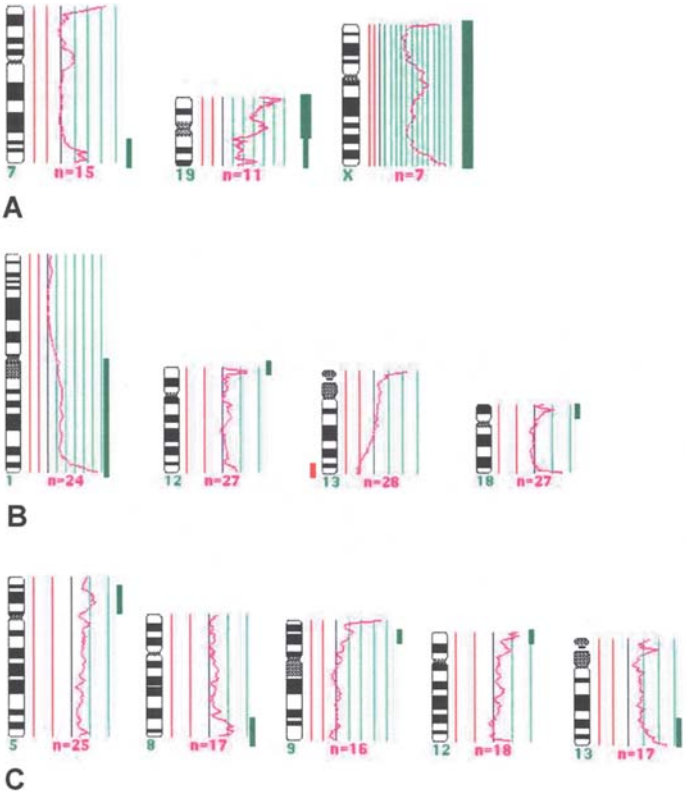

Figure 2. CGH profiles in three patients with Carney-GISTs. (A) Case 1 showed gains of X, 7q35q36, 19q and an amplification of 19p. (B) Case 2 showed loss of 13q32q34, gains of 1q21q42, 12p13, 18p11.31 and an amplification of 1q42q44. (C) Case 3 showed gains of 5p14, 8q24.1q24.2, 9p22, 12p13, and 13q32q34. Red line marked a loss of chromosomal material; green line (thin), a gain of chromosomal material; and green line (thick), an amplification. n, number of analyzed chromosomes.

Table III. Reported *KIT*/*PDGFRA* mutations in pediatric GISTs (n=9).

Case no.	Reference	Age/gender	Site	Mutation	Outcome
1	(21)	13/M	Stomach	*KIT* exon 9	NA
2	(22)	18/F	Stomach	*KIT* exon 9	77 months; alive with disease
3	(22)	20/F	Duodenum	*KIT* exon 11	60 months; no evidence of disease
4	(22)	23/M	Duodenum	*KIT* exon 11	29 months; deceased due to disease
5	(22)	23/M	Jejunum	*KIT* exon 9	4 months; no evidence of disease
6	(22)	24/F	Small bowel	*KIT* exon 9	148 months; alive with disease
7	(22)	27/M	Intra-abdominal	*KIT* exon 11	36 months; deceased due to disease
8	(22)	28/F	Ileum	*KIT* exon 11	48 months; alive with disease
9	(24)	12/F	Stomach	*PDGFRA* 18	25 months; no evidence of disease

NA, not available.

Table IV. Reported congenital malformations in patients with Carney triad (n=4).

Case no.	Reference	Age/gender	Site of GIST	Clinical manifestation	Congenital anomaly
1	(14)	12/F	Antrum	Complete Carney triad	Agenesis of left ear, partial agenesis of right ear
2	(14)	16/F	Antrum	Complete Carney triad	One sibling with major congenital defect of upper limb; died at 40 years of age of pancreatic cancer
3	(22)	14.8/M	Stomach	Complete Carney triad	Microcephaly, global developmental delay
4	Current study	17/F	Antrum	Incomplete Carney triad	Unspecified malformation of the right ninth rib

of chromosome 1 (1q21q42) and of the short arm of chromosomes 12 (12p13) and 18 (18p11.31), and an amplification of 1q42q44 were detected (Fig. 2B). Case 3 revealed gains of parts of chromosomes 5 (5p14), 8 (8q24.1q24.2), 9 (9p22), 12 (12p13), and 13 (13q32q34) (Fig. 2C).

Discussion

Carney triad-related GISTs differ greatly from sporadic gastric GISTs of adults in terms of demographic, clinicopathologic and molecular features. However, they show similarities to sporadic pediatric GISTs, including presentation in childhood or early adult life, a striking female predilection (>80%), predominantly epithelioid morphology, location in the antrum and lack of conventional *KIT* and *PDGFRA* mutations (16,20-22). To date, ~80 cases of Carney triad and a similar number of apparently sporadic pediatric GISTs have been reported (15,18-24). The clinicopathologic and molecular genetic profiles of these rare neoplasms are heterogeneous. The histogenetic and molecular relationship between them remains unclear. Of the pediatric GISTs reported by Miettinen *et al* (the largest series published to date) including a literature review (n=82), 71% of cases involved the antrum or pylorus and most had an epithelioid morphology (20). This contrasts with gastric GISTs in adults, which occur predominantly in the proximal parts of the stomach (body, fundus and cardia) and most often have a spindle cell morphology (1,2). To date, 34 pediatric GISTs (including two cases with the Carney triad) have been evaluated for mutations in *KIT* and *PDGFRA*. Twenty-five cases (73%) were wild-type for *KIT* exons 9, 11, 13 and 17 and *PDGFRA* exons 12 and 18 (16,19-24). Nine pediatric cases reported had *KIT* or *PDGFRA* mutations (Table III). These mutation-positive pediatric GISTs have a different gender distribution than pediatric GISTs in general (F:M = 1.2:1 vs. F:M = 5.5:1, respectively), with a higher than usual frequency of non-gastric location and *KIT* exon 9 mutations. Young adults with mutation-positive small bowel GISTs tend to be older than those with gastric tumours (23.6 vs. 14.3 years old, respectively), and probably belong to the lower end of the age spectrum for conventional GISTs. Both of the Carney-GISTs included in the previous studies and all three cases in this study were wild-type for *KIT* exons 9, 11, 13 and 17 and *PDGFRA* exons 12 and 18.

To our knowledge, Carney-GISTs have not been investigated by CGH. We found a mean of 4.7 aberrations per tumour, a value that is intermediate between that for benign GISTs (2.6) and those for primary malignant (7.5) and metastatic (9) conventional GISTs (7). In contrast to

conventional GISTs, chromosomal gains/amplifications were strikingly more common in the three Carney triad-related GISTs analyzed herein (gains:losses = 11:1) suggesting that gain of genetic material may be more significant than loss for the development and biological progression of this rare variant of GIST. However, the chromosomal abberations found in our three cases may represent secondary events acquired during biological tumour progression. Recurrent gains in chromosomes 1q, 8 and 12 (as in some of our cases) have been reported in tumours of the Ewing family (30). Our findings suggest a different pathogenetic pathway in tumorigenesis of Carney-GISTs. The recurrent non-random losses of 14q and 22q seen in the majority of conventional GISTs irrespective of site and biological behavior were lacking in the Carney triad-related GISTs analyzed in this study. Chromosomal aberrations in GISTs have been shown to occur independently of *KIT* mutations (8). In our study, one loss involving 13q32q34 was detected in one case (case 2). Alterations in 13q34 (which harbor the tumour suppressor gene ING1) have been frequently detected in squamous cell carcinoma of the head and neck, but also in other neoplasms (31).

The multifocal occurrence of the individual components of Carney triad suggests a genetic predisposition, but there is no evidence that the disorder is inherited (13-15). Carney *et al* suggested an early acquired complex genetic alteration as the basic defect (13-15). Accordingly, an intrauterine hit leading to somatic chromosomal aberrations may be involved in predisposition for Carney triad. The heterogeneity and complexity of the basic genetic alterations and the role of additional genetic, epigenetic or environmental factors involved are reflected by the long intervals between manifestations of the triad in individual patients and the fact that most patients manifest only two components of the disease (15). In keeping with the proposed genetic complexity of the triad, miscellaneous congenital malformative lesions have been described in rare cases (Table IV). Though the association of congenital malformations with an increased risk for childhood cancer is a well-documented phenomenon (32), we encountered no report of GISTs occuring in association with congenital anomalies other than in the setting of Carney triad. Some authors have suggested a histogenetic relationship between the different components of the disease through an embryonic link to the neural crest, suggesting a neurocristopathy as the possible basic defect (33,34). In line with this, a neuroectodermal line of differentiation was suggested in rare Carney-GISTs (35). Neuroectodermal-type pseudorossettes have been also described in rare Carney-GISTs (18) as in the current study (case 1).

In summary, we report for the first time CGH results for Carney-GISTs. Our findings showed a lack of the non-random loss of 14q and 22q seen in most sporadic GISTs irrespective of site and biological grade. This strengthens the notion that Carney triad-related GISTs differ from sporadic or conventional GISTs not only in terms of their clinicopathologic and mutational profiles, but also by virtue of their chromosomal aberrations (lack of losses in 14q and 22q and predominance of gains and high-level amplifications). Alternative oncogenes (other than *KIT* and *PDGFRA*) related to these chromosomal regions and their role in initiation, tumorigenesis and phenotype maintenance of Carney-GISTs remain to be identified. Because of the low case number in our study (related to the extreme rarity of this GIST variant), our findings need to be validated by future studies that might allow segregation of the heterogeneous group of pediatric GISTs into clinically relevant molecular subtypes.

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