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BRIEF REPORT

Gastrointestinal juvenile-like (inflammatory/hyperplastic) mucosal polyps in neurofibromatosis type 1 with no concurrent genetic or clinical evidence of other syndromes

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Abstract

Gastrointestinal "juvenile-like (inflammatory/hyperplastic) mucosal polyps" (JLIHMPs) have been proposed as a neurofibromatosis type 1 (NF1)-specific gastrointestinal manifestation. Juvenile polyposis syndrome (JPS) has also been reported in a NF1 patient, harboring concurrent *NF1* and *SMAD4* germline mutations. Additionally, NF1-like cafe-au-lait spots have been described in biallelic mismatch repair deficiency, another condition featuring gastrointestinal polyps. The *SMAD4* and *BMPR1A* genes that are involved in 50–60% of JPS cases have not been investigated in the ~20 published cases of NF1-associated JLIHMPs with the exception of the abovementioned patient with concomitant JPS and NF1. *NF1* defects have been found in the only two cases exhaustively tested. Therefore, JLIHMP has been questioned as an independent, NF1-specific entity. Incidental associations between NF1 and gastrointestinal polyposes at risk for gastrointestinal carcinoma should not be overlooked, given their implications in terms of clinical surveillance. We describe two patients featuring JLIHMPs in clinically/genetically proven NF1, in the absence of *SMAD4* and *BMPR1A* mutations. In one case, the intervening mucosa was markedly inflamed, unlike JPS. We suggest that JLIHMP probably represents a gastrointestinal lesion specific to NF1.

Keywords Hyperplastic polyp · Inflammatory polyp · Juvenile polyp · Molecular diagnosis · Neurofibromatosis · von Recklinghausen disease

Introduction

Neurofibromatosis type 1 (NF1) is a common hereditary syndrome (affecting ~1:3000 births). ~50% of cases occur de novo due to the high *NF1* mutation rate. NF1 phenotype varies considerably, influencing diagnosis rate. In the gastrointestinal tract, NF1 can present with gastrointestinal stromal tumors (GISTs), Cajal cell hyperplasia (causing peristaltic disorders), and neuroendocrine tumors [1]. Recently, 15 NF1 patients presenting gastrointestinal polyps resembling juvenile

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type have been published, proposing "juvenile-like (inflammatory/hyperplastic) mucosal polyps" (JLIHMPs) as another NF1-specific lesion [2]. Since then, four more cases have been reported [3–6]. One of them featured *NF1* and *SMAD4* germline mutations, revealing the coexistence of juvenile polyposis syndrome (JPS) that is due to *SMAD4* or *BMPR1A* mutations in 50–60% of cases [4]. Furthermore, NF1-like café-au-lait spots have been reported in biallelic mismatch repair deficiency, another condition manifesting gastrointestinal polyps [7]. None of the JLIHMP-bearing NF1 patients, except for the abovementioned JPS/NF1 case, have been tested for *SMAD4* or *BMPR1A* mutations, and *NF1* has been thoroughly investigated in only two cases [2, 4]. Consequently, JLIHMP has been questioned as a NF1-specific entity. The identification of possible incidental associations involving NF1 and polyposis syndromes at risk for carcinoma is clinically relevant. We herein describe two clinically and genetically diagnosed NF1 individuals showing JLIHMPs, wild-type for *SMAD4* and *BMPR1A*, supporting JLIHMP as a NF1-specific manifestation.

Materials and methods

Tissue samples

Tissue samples were retrieved from the archives of the Catholic University and of the Sacro Cuore-Don Calabria Hospital. The cases had been previously characterized by hematoxylin/eosin and immunohistochemistry (CD117 and DOG1 in intramural masses, CD34 in polyps). *PDGFRA* (exons 12/14/18, in intramural masses and polyps) and *KIT* (exons 9/11/13/17, in intramural masses) alterations had been investigated, as had large genomic imbalances and the entire coding region of *NF1* (transcript reference, NM_001042492.2).

Additional immunohistochemistry and genetic analysis

Polyps were immunostained for CD117 and S100 (DAKO, Glostrup, Denmark, rabbit polyclonal), using the DAKO visualization reagent (horseradish peroxidase-conjugated dextran polymer and goat anti-rabbit immunoglobulins), 3,3′-diaminobenzidine, and hematoxylin.

DNA was extracted using the RecoverAll™ Total Nucleic Acid Isolation Kit for formalin-fixed, paraffin-embedded specimens (Thermo Fisher Scientific, Waltham, MA, USA) from areas containing ~100% polyps macro-dissected on slides, following the manufacturer's instructions. *SMAD4* and *BMPR1A* were sequenced as described [8]. Large genomic imbalances of *SMAD4*, *BMPR1A*, and *PTEN* were searched by Multiplex Ligation-dependent Probe Amplification analysis using SALSA MLPA P081, P082, and P158 probemixes (MRC Holland, Amsterdam, Netherlands).

Results

Patients

Two patients with NF1 diagnosis based on clinical manifestation, family history, and genotype were investigated.

Patient 1 (64-year-old woman) presented with melena. Physical examination showed skin café-au-lait spots and facial neurofibromas. Routine laboratory tests were unremarkable. Esophagogastroduodenoscopy/video-capsule endoscopy/contrast-enhanced CT scan showed multiple enhanced duodenal/jejunal/ileal intramural tumors, sometimes bulging on the outer bowel surface, and multiple ileal mucosal polyps. Duodenum/jejunum and ileocolic resections were performed (Fig. 1a). Six-month follow-up is uneventful.

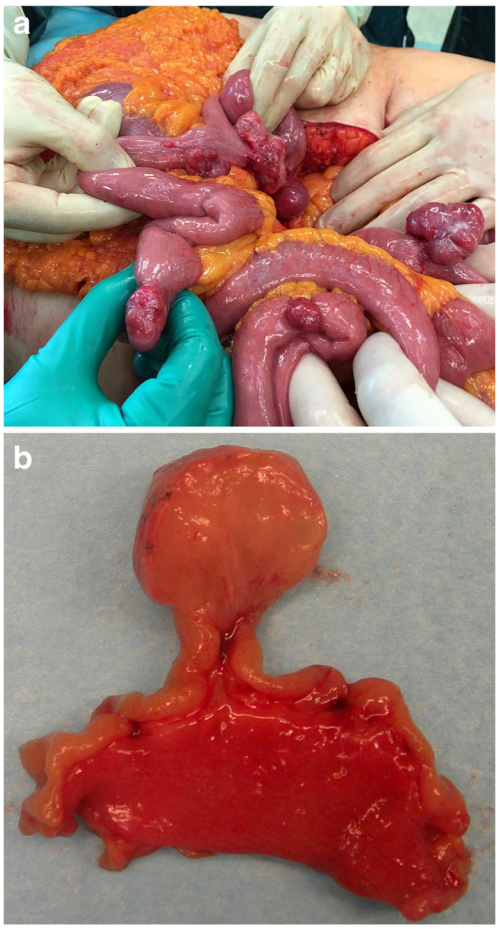

Fig. 1 **a** Appearance of the small bowel of case 1 at surgery. Multiple intramural tumors were observable, often bulging on the visceral outer surface. **b** Appearance of the ileal polyp resected in case 2

Patient 2 (63-year-old man) presented with abdominal discomfort. Physical examination showed skin café-au-lait spots and two ~2-cm leg subcutaneous nodules. Routine laboratory tests were unremarkable. Contrast-enhanced CT scan showed a jejunal intramural tumor and an ileal polyp, both measuring ~2 cm. Jejunum and ileum resections were performed; at surgery, two more intramural nodules were resected (0.5-cm jejunal, 0.4-cm ileal). Figure 1b shows the ileal polyp. The subcutaneous nodules were also removed. Twenty-month follow-up is uneventful.

Pathology and genetic analyses

In patient 1, the resected specimens revealed the presence of 15 jejunal/ileal intramural tumors, (sized 2–54 mm), seven ileal mucosal polyps (10–70 mm), and a sessile 25-mm appendiceal lesion. In patient 2, three intramural jejunal/ileal tumors (4–20 mm), a 15-mm ileal mucosal polyp, and two 20-mm subcutaneous nodules were detected.

Histologically, the small bowel mucosal polyps consisted of epithelial crypts, often dilated/distorted, separated by abundant, markedly inflamed stroma (including mononuclear leukocytes, mast cells, and eosinophils) prevailing over the epithelial component; granulation tissue and superficial ulceration were present; blood vessels often showed fibromyxoid intimal thickening (Figs. 2a–c and 3a–c); *PDGFRA* was wild-type. In case 1, the intervening mucosa was diffusely and heavily inflamed (Fig. 2d), as was the mucosa at the base of the polyp in case 2. Mast cells were numerous in polyps and in the inflamed mucosa (Figs. 2e and 3d). S100 staining did not show Schwann cells/neural alterations. Stromal cells were CD34−. The polyp characteristics were consistent with JLIHMP.

The intestinal intramural tumors were composed of spindled (sometimes epithelioid) cells, CD117+/DOG1+, with skeinoid fibers, and a 3 mitoses/5 mm^2 peak (in the largest tumor), *KIT/PDGFRA*-wild-type, consistent with NF1-associated GIST [9].

The sessile appendiceal lesion showed a serrated architecture involving the whole mucosal thickness, without conventional dysplasia, consistent with a sessile serrated lesion.

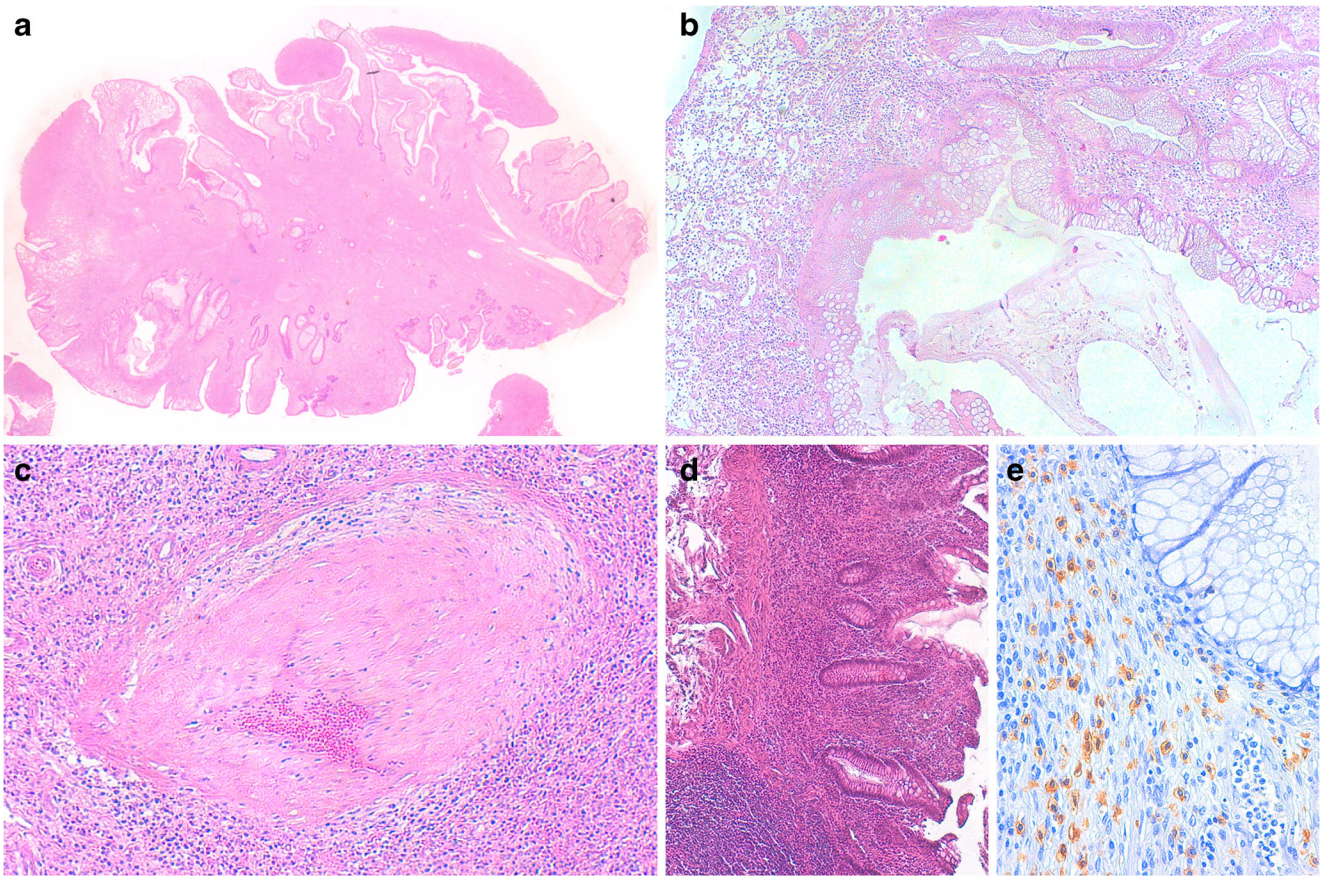

Fig. 2 Representative images of the mucosal lesions found in case 1. At panoramic view, the ileal polyps showed juvenile-like features, with prominent stroma, prevailing over epithelium (**a**). The stroma was inflamed, sometimes rich in granulation tissue; mucosal crypts were distorted (**b**); additionally, vasculopatic changes consisting of fibromyxoid intimal thickening were common (**c**). The intervening ileal mucosa was heavily inflamed (**d**, panoramic view). Numerous mast cells accompanied inflammation, as highlighted by CD117 immunostain in the polyps (**e**) (original magnification: **a** × 5; **b**, **c**, **d** × 50; and **e** × 200)

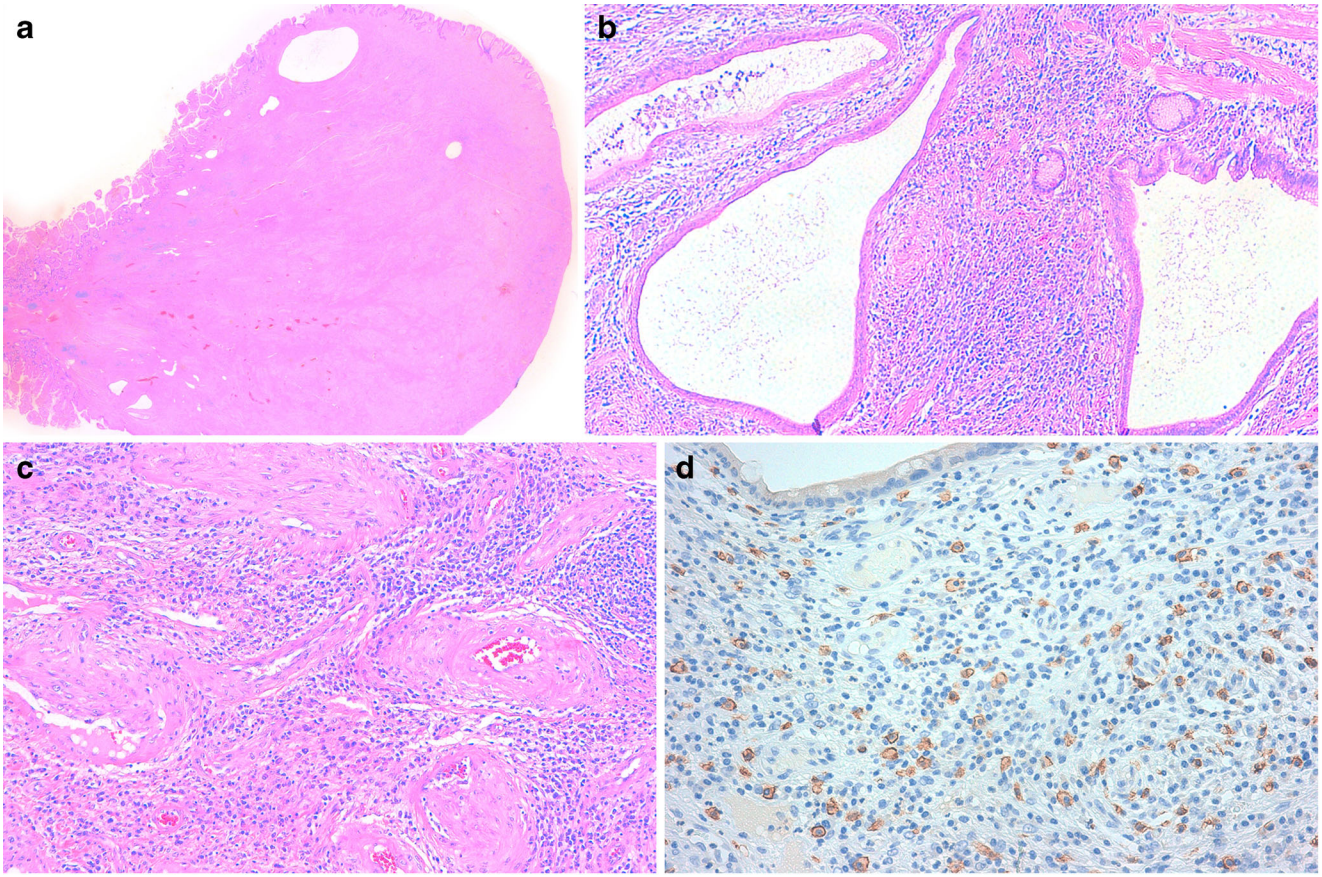

Fig. 3 Representative images of the ileal polyp found in case 2. Similar to case 1, at panoramic view juvenile-like features were evident, with abundant stroma (**a**). Also, in case 2, the stroma was heavily inflamed and granulation tissue-rich, the mucosal crypts were distorted (**b**) and vasculopatic changes were evident (**c**). Numerous mast cells accompanied inflammation (**d**, CD117 immunostain) (original magnification: **a** × 5, **b** × 50, **c** × 100, and **d** × 200)

The two leg subcutaneous nodules revealed a non-encapsulated proliferation of Schwann cells and fibroblasts, with collagen fibrils and mast cells, consistent with neurofibroma.

DNA analysis did not reveal any *SMAD4* or *BMPR1A* alterations nor *PTEN* deletions. Germline *NF1* alterations were detected in both patients: a 1 nucleotide insertion, causing a frameshift (c.3892_3893insA; p.Leu1300fs*14) in case 1, and a 12-to-57 exon deletion in case 2.

Discussion

The recent publication of a case of concurrent NF1 and JPS, genetically confirmed [4] has questioned JLIHMP as an independent entity specific to NF1, as proposed by Agaimy and colleagues [2]. In the other 18 NF1 patients harboring JLIHMP reported so far [2, 3, 5, 6], *SMAD4*/*BMPR1A*, whose germline mutations characterize 50–60% of JPS cases, were not investigated. *NF1* was exhaustively analyzed in 2 of these patients, while large rearrangements were investigated by FISH in a third case [2, 4]. This is relevant since skin café-au-lait spots constitute a trait common to NF1 and biallelic mismatch repair deficiency, a condition featuring gastrointestinal mucosal polyps (usually adenomas), leading to possible misdiagnosis [7].

Therefore, our report of two additional occurrences of JLIHMPs in genetically ascertained NF1 patients, without *SMAD4* or *BMPR1A* defects, supports JLIHMPs as a component manifestation of NF1, with the limit that *SMAD4*/*BMPR1A* variants characterize only 50–60% of JPS cases. Nevertheless, JPS is a very rare condition, with a ~ 1/100,000–1/160,000 annual incidence: therefore, it is very unlikely that most, if not all cases of NF1 with JLIHMPs may harbor JPS defects. Overall, our patients represent ~ 10% of the total cases of NF1 with associated JLIHMPs hitherto identified.

PTEN can be involved in the pathogenesis of juvenile-type polyps in the setting of PTEN hamartoma tumor syndrome [8, 10]. The latter condition typically features associated illnesses such as macrocephaly, thyroid/breast cancer, and skin tumors; the lack of these signs and of *PTEN* deletions argues against its presence in our patients.

In one of our cases, the intervening non-polypoid intestinal mucosa revealed markedly inflamed, supporting a nosological separation between JPS polyps and NF1-associated JLIHMPs, as the intervening mucosa between polyps in the former condition is typically normal [10].

The inflammatory infiltrate of NF1-associated JLIHMPs can include eosinophils and, sometimes, a perivascular onion-skin pattern of stromal cells, mimicking inflammatory fibroid polyp (IFP). The lack of CD34 staining in our and in most of the previously reported cases [2] does not help in the differential diagnosis, since intestinal IFPs are often CD34– [11]. However, in NF1-associated JLIHMPs, eosinophils are never prominent and stromal onion-skin pattern is sporadically present and often only partially outlined [2]; moreover, we found no *PDGFRA* mutations, characterizing most IFPs [12].

The pathogenesis of NF1-associated JLIHMPs is presently unclear. An involvement of chronic inflammation secondary to disordered gut motility, hyperactivation of fibroblasts due to neurofibromin loss, and vascular pathogenesis has been hypothesized [2]. A role of the latter is supported by its involvement in other congenital malformations and functional disabilities found in NF1, and by the vasculopathic changes detected in association with gastrointestinal NF1 manifestations, including JLIHMPs (where these changes probably constitute a diagnostic hint) [2]. Our finding of a heavy inflammation with numerous mast cells suggests a possible pathogenetic role of these leukocytes. Indeed, mast cells are deregulated in NF1 due to *NF1* haploinsufficiency, determining a loss of inhibition of RAS, a key factor downstream of c-kit, their principal molecular effector [13]. Mast cells have been implicated in the pathogenesis of inflammatory bowel diseases [14], conditions featuring inflammatory polyps [10], sometimes signaled in association with NF1 [3, 15]. The *NF1* haploinsufficiency-dependent RAS deregulation, known to alter also fibroblast and endothelial cell functions [13], can contribute to the abundance of stroma and to the fibromyxoid intimal thickening (coherent with NF1-associated vasculopathy) typical of NF1-associated JLIHMPs [2]. Further studies are warranted to test these hypotheses and to clarify the pathogenesis of NF1-associated JLIHMPs and the reason of their presence only in a minority of patients.

In conclusion, we report two cases of JLIHMPs in a NF1 setting (diagnosed clinically and genetically), wild-type for both *SMAD4* and *BMPR1A*, to the best of our knowledge accounting for 2/21 (9.5%) of NF1-associated JLIHMPs hitherto published, and 2/4 (50%), 2/3 (66%), and 1/3 (33%) of those in which a genetic test for *NF1*, *SMAD4*, or *BMPR1A*, respectively, has been reported [2–6]. Therefore, although the exceptional concurrence of gastrointestinal polyposis syndromes and NF1 should be considered in clinical practice, our findings support NF1-associated JLIHMP as a possible specific gastrointestinal NF1 manifestation.

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Contributions RR conceived and designed the study and wrote the manuscript; GR and GS performed DNA Sanger sequencing; MGP and MG performed MLPA analysis; GQ, SeA, and GZ collected clinical data; GQ and SeA performed surgery; GR, MCG, FC, MGP, SaA, MG, GZ, and RR analyzed data. All authors critically reviewed the manuscript and gave final approval for publication.

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Compliance with ethical standards

Informed consent was obtained from all individual participants included in the study. All procedures performed were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Conflict of interest The authors declare that they have no conflict of interest.

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