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# Histological variations in juvenile polyp phenotype correlate with genetic defect underlying juvenile polyposis

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## Abstract

**Background—**Juvenile polyps are distinct hamartomatous malformations of the gastrointestinal tract that may occur in the heritable juvenile polyposis syndrome (JPS) or sporadically. Histologically, juvenile polyps are characterised by a marked increase of the stromal cell compartment but, an epithelial phenotype has also been reported. JPS has an increased risk of colorectal cancer but sporadic juvenile polyps do not. In 50–60% of JPS patients a germline mutation of the TGF-β/BMP pathway genes *SMAD4* or *BMPR1A* is found. This study compares the histological phenotype of juvenile polyps with a *SMAD4* or *BMPR1A* germline mutation and sporadic juvenile polyps.

**Methods—**H&E slides of 65 JPS polyps and 25 sporadic juvenile polyps were reviewed for histological features and dysplasia. Systematic random crypt and stroma counts were obtained by count stereology and a crypt-stroma ratio was determined. All polyps were subsequently categorised as type A (crypt-stroma ratio <1.00) or type B (crypt-stroma ratio ≥1.00), the latter referring to the epithelial phenotype. Cell cycle activity was assessed using immunohistochemistry of the proliferation marker Ki67, and mutation analysis was conducted for *KRAS* and *APC* to determine the involvement of the adenoma-carcinoma sequence.

**Results—**Juvenile polyps with a *SMAD4* germline mutation were predominantly type B, whereas, type A was more common among juvenile polyps with a *BMPR1A* germline mutation, but this distinction could not be ascribed to differences in cell cycle activity. Dysplasia was equally common in JPS polyps with either a *SMAD4* or *BMPR1A* germline mutation, where the involvement of the adenoma-carcinoma sequence does not seem to play a distinct role.

**Conclusion—**juvenile polyps in the setting of JPS exhibit distinct phenotypes correlating with the underlying genetic defect.

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### Keywords

Juvenile Polyposis; juvenile polyp; phenotype; genotype; colorectal cancer; SMAD4; BMPR1A

## INTRODUCTION

Juvenile polyposis syndrome (JPS) is a rare autosomal dominant disorder characterised by the presence of multiple juvenile polyps in the gastrointestinal tract. Patients present during the first or second decade of life and have a markedly increased risk of colorectal cancer at later age.5,7,9. Clinical diagnosis is made when any one of the following criteria is met: (1) more than 3–5 juvenile polyps in the colorectum; (2) juvenile polyps throughout the intestinal tract; or (3) any number of juvenile polyps in combination with a positive family history of JPS.9,19,25. Sporadic juvenile polyps are a more common finding, occurring in up to 2% of the paediatric population, and are considered benign solitary lesions of the colorectum 10,[19].

Juvenile polyps most often have a spherical appearance with extensive surface erosion; a marked increase of the stromal cell compartment; inflammatory and reactive changes of the epithelium; and distorted and dilated crypts. Several reports have noticed a more lobulated and epithelial phenotype in a subset of juvenile polyps [19].

In 50–60% of JPS patients a germline defect in *SMAD4* or *BMPR1A* of the TGF-β/BMP signalling pathway, is found [16,17,28]. Inactivation of these genes in mice leads to a JPS-like phenotype. *Smad4* mutant mice develop gastrointestinal polyps characterised by elongated and dilated tubular structures lined by hyperplastic or serrated epithelium and a moderate expansion of the stromal cell compartment. Although mostly hyperplastic, the epithelium may show some atypia with occasional foci of dysplasia [15,27]. In *Bmpr1a* mutant mice, polyps had cystically dilated and distorted glands filled with mucin and inflammatory cells surrounded by fibrous stroma [14]. Moreover, inhibition of BMP signalling in mice (by transgenic expression of noggin, a BMP inhibitor, under control of a villin promoter) leads to branching and budding of the intestinal epithelium, crypt dilatation and reactive inflammatory changes. At later stages these mice develop foci of dysplastic epithelium and adenomatous change[13].

These mouse models all display features reminiscent of JPS but, differences may exist in the phenotype depending on the genetic background. In this study, we compared the histological phenotype of human juvenile polyps with a *SMAD4* or *BMPR1A* germline mutation and sporadic juvenile polyps. In addition, the possible role of the conventional adenoma-carcinoma sequence in the neoplastic progression in JPS was evaluated.

## METHODS

### Patients and tissue

Archival material from patients with one or more juvenile polyps was collected from The Johns Hopkins Polyposis Registry and clinic (Baltimore, MD, USA) and two academic hospitals in the Netherlands (Academic Medical Centre, Amsterdam, and University Medical Centre, Utrecht). The study was carried out according to the guidelines and with approval of the ethical committee of these institutions. Clinical and family history data were examined and polyps were carefully reviewed by an experienced GI pathologist (GJAO) to confirm the diagnosis of JPS or sporadic juvenile polyps. All JPS patients were analysed for germline defects of *SMAD4*, *BMPR1A*, *PTEN*, *ENG* and *TGFBRII* through direct sequencing and multiplex ligation-dependent probe amplification (MLPA).6,28 Thirty-nine

patients (90 polyps) were included in this study, including 8 patients (21 polyps) with a *SMAD4* germline defect, 6 patients (44 polyps) with a *BMPR1A* germline defect and 25 sporadic juvenile polyps.

## Histological characterization

H&E stained slides of all juvenile polyps were systematically scored for individual histological features possibly associated with juvenile polyps (i.e. crypt distortion and dilatation, crypt density, stromal expansion, surface erosion, inflammatory and reactive change of the epithelium, vascular proliferation, Paneth cell metaplasia and thickening of the basal membrane, eosinophilia). Evaluation revealed 2 general phenotypes, namely that of a *classic* juvenile polyp comprising a prominent stromal compartment, dilated glands and surface erosion, as well as polyps with a predominantly *epithelial* phenotype (Figure 1a and 1b). Several features best describing the encountered phenotypes were then grouped together creating 2 categories as is shown in Table 1. Subsequently, all polyps were classified according to these 2 categories. In case polyps displayed heterogenic or intermediate features, overall crypt density served as decisive, most discriminatory feature. In addition, all polyps were graded for dysplasia according to the standard criteria [11].

## Count stereology

To better appreciate our histological findings a quantitative evaluation of crypt density was performed. A crypt-stroma ratio was determined by means of count stereology on H&E stained slides of all juvenile polyps. Using the Q-prodit software fields of vision were systematically distributed throughout the entire polyp at a 10X magnification. An overlying four points Weibel-grid was used to score epithelial or stromal counts. A discriminator of 1.00, describing equal counts of stroma and epithelium, was chosen arbitrarily to determine the predominant feature. A ratio <1.00 indicating a low crypt density was designated type A and a ratio ≥1.00 was designated type B. All polyps were categorised according to these criteria and results were compared to the histological classification.

## Immunohistochemistry and scoring

Tissue was formalin-fixed and paraffinized in accordance with standard procedures. Immunohistochemistry was performed using a monoclonal antibody for Ki67 (DAKO MIB-1, Cat.no. M7240, 1:200). Briefly, 4 μm sections were deparaffinised and blocked for endogenous peroxidase activity by immersion in 0.3% $H_2O_2$ in methanol for 20 min. Antigen retrieval was performed in Tris/EDTA buffer (10 mM/1 mM; pH 9.0) for 10 min at 120°C. Nonspecific binding sites were blocked in PBS with 10% normal goat serum for 10 min, followed by antibody incubation for 1h at room temperature. Antibody binding was visualized using the Powervision+poly-HRP detection system (ImmunoVision Technologies, Co, Daly City, CA, USA) with 3,3-diamino-benzidine (DAB, Sigma D5637) as chromogen. Slides were counterstained with hematoxylin.

Ki67 is a nuclear proliferation marker expressed during all phases of actively growing cells but not in quiescent cells. Normal colon mucosa shows a distinctive nuclear Ki67 staining pattern with positive cells limited to the bottom third of the crypt, i.e. the proliferative compartment. Although in juvenile polyps the glands are often distorted, proliferative activity may remain confined to a restricted crypt compartment underneath the adjacent differentiated – non-proliferative – epithelium and thus retaining a compartmentalized phenotype. Loss of compartmentalization was defined as an overall increase in proliferative cells and the dissemination thereof throughout the epithelium resulting in loss of a clear distinction between the proliferative zone and the overlying differentiated epithelium. Slides were scored in a dichotomous manner describing retention or loss of compartmentalisation, the latter indicating an expansion of cell cycle activity.

### Statistical analysis

Statistical analysis was performed using the SPSS 15.0 software package. The chi-square test was utilized to determine whether correlation between phenotypes and Ki67 expression were statistically significant at a p-value <0,05.

### Laser microdissection and DNA isolation

Dysplastic epithelium was manually isolated from 8 μm sections counterstained with haematoxylin. DNA was obtained using TK buffer [400 μg/ml of proteinase K and 0.5% Tween 20, 50 mmol/l Tris (pH 9), 1 mmol/l NaCl, 2 mmol/L EDTA]. After overnight incubation in 50 μl TK buffer at 56°C, proteinase K was inactivated through incubation at 95°C for 10 minutes [4].

### *APC* and *K-ras* mutation analysis

*APC* and *K-ras* mutation analysis was conducted through PCR amplification using Platinum®Taq DNA Polymerase (Invitrogen Corporation, Carsbad, California, USA) of DNA samples. For *APC*, 4 specific primer sets covering the mutation cluster region (MCR) in exon 15 were used [24]. (1For-GAAATAGGATG TAATCAGACG, 1Rev-CGCTCCTGAAGAAA ATTCAAC, 2For-ACTGCAGGGTTCTAGT TTATC, 2Rev-GAGCTGGCAATCGAACGA CT, 3For-TACTTCTGTCAGTTCACTTGA TA, 3Rev-ATTTTTAGGTACTTCTCGCTTG, 4For-AAACACCTCCACCACCTCC, 4Rev-GCATTATTCTTAATTCCACATC). Amplification was performed at a Tm of 55°C for primer sets 1, 2 and 3, and at 58°C for primer set 4. Two primer sets were used for *K-ras* mutation analysis for exon 1 and 2, where mutational hotspots codon 12, 13 and 61 are located [8] (Exon1 For-CTGGTGGAGTATTT GATAGT, Exon1 Rev-ATG GTCCTGCACCAGTAATA, Exon2For-GTGCACTGTAATAA TCCAGAC, Exon2 Rev-CCACCTATAATGGTGAATATCT). Sequencing was conducted using the ABI Prism® 3130 genetic analyzer.

## RESULTS

### Histological characterization

Two categories of individual features were created in order to best describe the 2 phenotypes encountered upon initial evaluation of the slides, namely the *classical* and the *epithelial* type juvenile polyp (Table 1) (Figure 1a and 1b). All polyps were classified according to these 2 categories. Intermediate features notwithstanding, a dichotomous decision with regard to juvenile polyp phenotype was always rendered with *crypt density* serving as decisive feature. The phenotype described as classic juvenile polyp was found in 39 of 65 (60%) JPS polyps and the epithelial variant in 26 of 65 (40%) polyps. The epithelial variant was more common in polyps with a *SMAD4* germline mutation compared to polyps with a *BMPR1A* germline mutation ($p<0.001$). (Table 2) All of the 25 sporadic juvenile polyps were of the classic phenotype.

### Crypt-stroma ratio

Crypt density was considered the most discriminatory in differentiating between the classic juvenile polyp and the epithelial type juvenile polyp based upon initial evaluation of the earlier mentioned features. To better appreciate our histological findings a quantitative evaluation of crypt density was performed. Systematic random crypt and stroma counts were obtained by stereologic methodology to determine a crypt-stroma ratio. (Figure 2a) The crypt-stroma ratio was significantly higher in juvenile polyps with a *SMAD4* germline mutation compared to those with a *BMPR1A* germline mutation ($p=0.001$) (Figure 2b).

Juvenile polyps with a *BMPR1A* germline mutation had a higher ratio than sporadic juvenile polyps ($p<0.001$).

A crypt-stroma ratio <1.00 was designated type A referring to the classic juvenile polyp category, and a ratio ≥1.00 was called type B specifying to the epithelial variant. According to these criteria, 38 out of 65 JPS polyps were of type A (58%) and 27 of type B (42%). Classification according to crypt-stroma ratio confirmed the observations made on histological evaluation that the epithelial variant (type B polyp) is more frequently found in patients with a *SMAD4* germline mutation compared to individuals with a *BMPR1A* germline mutation ($p<0.05$) (Table 2). In 8 JPS polyps, of which 3 had a *SMAD4* germline mutation and 5 had a *BMPR1A* germline mutation, histological classification did not concur with stereological findings (Figure 1c and 1d). Regarding the sporadic juvenile polyps all but one were classified type A.

### Dysplasia

All polyps were graded for dysplasia. The frequency in which different grades of dysplasia were seen was similar for polyps with either a *SMAD4* or *BMPR1A* germline mutation (Table 3). However, evaluation by polyp type revealed a distinct pattern of dysplasia in polyps with a *SMAD4* or *BMPR1A* background. Focal dysplasia in a *SMAD4* setting was found only in type B polyps, but in a *BMPR1A* setting focal dysplasia was seen in both type B and type A polyps. (Table 4) All sporadic polyps were negative for dysplasia.

### Immunohistochemistry

To investigate whether variations in crypt density in juvenile polyps could be attributed to differences in proliferative activity, immunostaining of the Ki67 proliferation marker was performed. Focal loss of compartmentalisation of Ki67 indicating expanded cell cycle activity, was observed in 12 of 21 (57%) juvenile polyps with a *SMAD4* germline mutation and in 25 of 44 (57%) juvenile polyps with a *BMPR1A* germline mutation. Evaluation of the immunostaining per polyp type per germline defect showed a correlation between a B phenotype and focal loss of Ki67 compartmentalisation especially in juvenile polyps with a *SMAD4* germline mutation ($p=0.006$), but not in those with a *BMPR1A* mutation ($p=0.131$) (Table 5). However, when stratified by presence or absence of dysplasia no correlation between de-compartmentalisation of Ki67 and the B phenotype was seen in juvenile polyps with either a *SMAD4* ($p=1.000$) or *BMPR1A* ($p=0.668$) germline mutation. Focal loss of compartmentalisation was found in 2 sporadic juvenile polyps.

### *APC* and *KRAS* mutation analysis

To explore the role of the conventional adenoma-carcinoma sequence in the development of neoplastic change in JPS patients, *APC* and *K-ras* mutation analysis was performed. Only those polyps graded for dysplasia were analysed. Of the 16 tissues available, 2 polyps showed a *K-ras* point-mutation in exon 1 (GGT ➔ GAT). The *K-ras* mutations were found in areas of low grade dysplasia in one type A polyp from a patient with a germline *BMPR1A* mutation and in one type B polyp from a patient with a *SMAD4* germline mutation. None of the polyps showed a mutation in the MCR of the *APC* gene (data not shown). In addition 10 nonsyndromic juvenile polyps were found negative for *K-ras* mutation.

## DISCUSSION

JPS is caused by a germline defect in *SMAD4* or *BMPR1A*. [16,17] Transgenic mice develop distinct JPS-like phenotypes depending on which of the JPS causing genes is targeted. *Smad4* mutant mice show hyperplastic or serrated epithelium and minor stromal overgrowth whereas *Bmpr1a* mutant mice or mice with inhibited BMP signalling through transgenic

expression of noggin show polyps with reactive changes of the epithelium, crypt dilatation and a prominent stromal compartment.[13–15,27] We investigated and compared the histological phenotype of human juvenile polyps from patients with a *SMAD4* or *BMPR1A* germline mutation.

Consistent with earlier reports, histological evaluation revealed a subset of JPS polyps featuring an epithelial phenotype (40%), deviating from classic juvenile polyps characterised by a prominent stromal compartment (60%).[10,19] The epithelial phenotype was more prevalent in cases with a *SMAD4* germline mutation, whereas, juvenile polyps with a *BMPR1A* germline mutation predominantly had the classic juvenile polyp phenotype ($p<0.001$).

Interestingly, quantitative evaluation of the crypt-stroma ratio confirmed our initial histological findings. Juvenile polyps with a *SMAD4* germline mutation had a significantly higher crypt-stroma ratio compared to those with a *BMPR1A* germline mutation indicating a higher crypt density in the former and confirming the epithelial phenotype. These results underscore the relevance of crypt-density as discriminatory feature between the classic and epithelial type juvenile polyp.

Nevertheless, 8 JPS polyps showed a discrepancy between histological and stereological classification and may thus be considered to display intermediate features (Figure 1c and 1d). Re-evaluation of these polyps revealed that massive crypt dilatation may result in a juvenile polyp initially labelled as classic phenotype to be considered a B type polyp by stereologic means. On the other hand, surface erosion and subsequent inflammation in juvenile polyps with a proliferative core may cause a polyp to be classified histologically as an epithelial phenotype yet stereologically be scored as type A.

Similar frequencies of indefinite, low grade and high grade dysplasia were found in juvenile polyps from patients with either a *SMAD4* or *BMPR1A* germline defect, contradicting earlier reports of a more dysplasia prone intestinal phenotype in polyps with a *SMAD4* germline mutation.[12,26] Interestingly, 50% of all foci of low or high grade dysplasia in juvenile polyps with a *BMPR1A* germline defect were found in type A polyps, whereas, none of the type A polyps with a *SMAD4* germline defect contained dysplasia.

To investigate whether the neoplastic change in the juvenile polyps could be attributed to mutations in the conventional adenoma-carcinoma sequence, the dysplastic areas were investigated for *APC* and *K-ras* mutations. Our results revealed only 2 polyps with *K-ras* mutations, consequently, prior reports of *APC* mutations in dysplastic polyps [31] could not be confirmed. These data suggest that the conventional adenoma-carcinoma sequence may not play a distinct role in JPS tumour formation, as has been concluded by other investigators.[32] Moreover, the different phenotypes could not be attributed to either an *APC* or *K*-ras mutation. Evaluation of the Ki67 proliferation marker demonstrated that focal loss of compartmentalisation of Ki67 i.e. expanded cell cycle activity, could not be linked to an A or B phenotype when stratified by dysplasia. This finding is consistent with the concept that loss of compartmentalisation of the proliferative zone is a general feature of dysplasia regardless of the underlying genetic defect.

Few prior studies have been dedicated to the evaluation of a relation in genetic make-up and histological phenotype in juvenile polyps. Handra-Luca et al analyzed a series of juvenile polyps for percentage and morphology of epithelial and stromal components, blood vessels, level of inflammation, hyperplasia and dysplasia.[12] They characterized several distinctive features of polyps with a *SMAD4* mutation: association with various grades of dysplasia, upper digestive tract location and malformative vessels in the stroma. *BMPR1A* polyps were exclusively of the lower digestive tract and were not associated with dysplasia or

malformative vessels. As mentioned, our results showed no distinction with regard to presence and/or grade of dysplasia between juvenile polyps in the setting of a *SMAD4* or *BMPR1A* germline mutation. Although presence of vascular malformation was evaluated in the initial screening it was not a discriminatory feature in our series of juvenile polyps (data not shown). No polyps of the upper digestive tract were present in our cohort.

Also, Aretz et al describe various histological phenotypes ranging from juvenile polyps to hyperplastic polyps and pseudopolyps albeit with adenomatous components or even adenomas in juvenile polyposis patients with an established germline mutation in one of the associated genes but no correlation between genotype and histological phenotype is provided.[1] Nevertheless, our results confirm the wide array of histological phenotype variations encountered in the setting of juvenile polyposis.

*SMAD4* and *BMPR1A* are both key components of the TGF-β/BMP signalling pathway maintaining homeostasis of the intestinal lining through processes of cellular proliferation (TGF-β) and differentiation and apoptosis (BMP). Signal transduction takes place through phosphorylation of the type 1 transmembrane receptor kinase (i.e. BMPR1A) by the type 2 receptor. The activated type 1 receptor phosphorylates the pathway restricted SMAD2 and 3 (TGF-β) or SMAD1,5 and 8 (BMP) which, in complex with the common mediator SMAD4, is translocated to the nucleus where target gene transcription is regulated.[23]

Individuals with germline defects in *SMAD4* or *BMPR1A* and consequent disrupted TGF-β/BMP signalling develop multiple hamartomatous malformations in the gastrointestinal tract. These hamartomas are often characterized by an abnormal stromal component suggesting a prominent role for the stroma in polyp formation. The polyp epithelium initially shows normal maturation, although inflammation is common and may cause reactive changes. Subsequent dysplastic progression of the epithelium has been proposed to be the result of the altered microenvironment.[21]

Recent studies provide evidence that conditional inactivation of *Bmpr2* in the intestinal mesenchyme leads to mice developing hamartoma-like polyps, whereas, conditional deletion of *Bmpr1a* in the epithelium showed elongation of the villi, but no de-novo crypt or polyp formation.2,[3] Consistent loss of heterozygosity (LOH) of the *BMPR1A* locus has thus far not been detected in the epithelium or stroma of JPS polyps from patients with a *BMPR1A* germline mutation [16]; although one study reports somatic loss of the 10q22 region exclusively in the lamina propria and not in the epithelium suggesting inactivation of *BMPR1A* might be a stromal event.[18]

Selective loss of Smad4-dependent signalling in T cells leads to a JPS-like phenotype reminiscent of what we described as a type A polyp with cystic spaces lined by columnar epithelium surrounded by abundant stroma.[20] *Smad4* heterozygous mice on the other hand develop polyps with an epithelial phenotype (type B) and show LOH specifically in the epithelium of larger polyps.[27,32] Likewise, LOH of the *SMAD4* locus occurs in the epithelium of juvenile polyps from patients with a *SMAD4* germline mutation.[22,29] The exact role of SMAD4 and timing of *SMAD4* inactivation in polyp initiation and progression remains poorly understood but it seems that these polyps develop mainly through an epithelial defect.[22,30] In addition to phenotype classification, SMAD4 immunohistochemistry may provide a specific marker for the detection of a *SMAD4* germline mutation.[22]

Although the number of polyps in this study is limited, we propose that juvenile polyps with a *SMAD4* germline defect have a higher crypt density regardless of the dysplastic status. On the contrary, juvenile polyps with a *BMPR1A* defect are more often classic juvenile polyps with a prominent stromal compartment. Crypt density in these polyps is initially low but

may increase due to neoplastic change of the epithelium. Investigation of Ki67 immunohistochemistry reveals that the difference in crypt density in juvenile polyps with a *SMAD4* or *BMPR1A* germline mutation is not a result of altered proliferative activity.

We conclude that juvenile polyps in the setting of juvenile polyposis syndrome may exhibit distinct phenotypes. Juvenile polyps with a *SMAD4* germline mutation more likely express an epithelial phenotype with a relatively high crypt density, whereas, juvenile polyps with a *BMPR1A* mutation are usually the classic juvenile polyp phenotype with a prominent stromal compartment. Importantly, we find similar rates for all grades of dysplasia in juvenile polyps with either a *SMAD4* or *BMPR1A* background.

## Abbreviations

| | |
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| **JPS** | juvenile polyposis syndrome |
| **LOH** | loss of heterozygosity |
| **MCR** | mutation cluster region |

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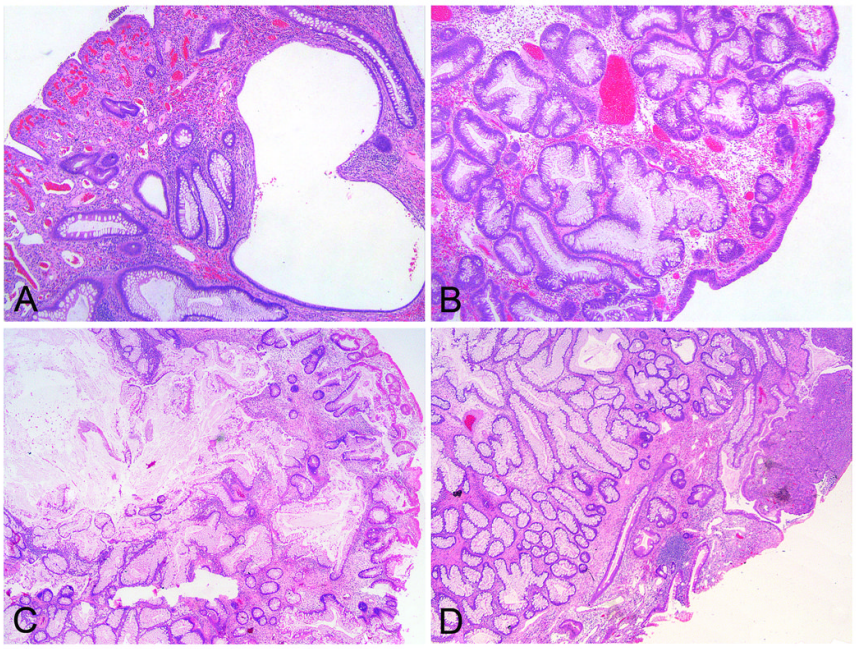


**Figure 1.**
Histological appearance of the classic juvenile polyp and the epithelial phenotype. A shows histological image of a classic juvenile polyp with prominent stromal compartment, an eroded surface, inflammation and reactive changes of the epithelium, and distortion and dilation of the glands. B is illustrative of the epithelial phenotype devoid of an expanded stromal compartment but with an intact surface and with abundant tall columnar mucus secreting epithelium. C shows an intermediate phenotype initially graded as a classic type polyp, but was rendered type B by stereologic means. Conversely, panel D shows a juvenile polyp that was scored histologically of the epithelial type, yet stereology revealed a type A phenotype.

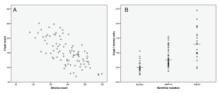

**Figure 2.**
Crypt-stroma ratio. Crypt and stroma counts as obtained by systematic random count stereology (A) and the crypt-stroma ratio displayed by genetic background (B). Juvenile polyps with a *SMAD4* germline defect had a significantly higher crypt-stroma ratio compared to juvenile polyps with a *BMPR1A* germline defect (p=0.001).

**Table 1**

Features of the classic juvenile polyp versus the epithelial variant.

| Classic juvenile polyp | Epithelial juvenile polyp |
|---|---|
| Spherical | Lobulated |
| Eroded and granular surface | Villous-like surface |
| Stromal compartment expanded | Stromal compartment not expanded |
| Low crypt density | High crypt density |
| Flattened reactive epithelium | Columnar hypermucinous epithelium |

## Table 2

Results of the classification based on histology and crypt-stroma ratio.

| Germline mutation | Histological classification | | Crypt-stroma classification | |
|---|---|---|---|---|
| | Classic | Epithelial | A | B |
| *SMAD4* | 6 (29%) | 15 (71%) | 8 (38%) | 13 (62%) |
| *BMPR1A* | 33 (75%) | 11 (25%) | 30 (68%) | 14 (32%) |
| Total | 39 | 26 | 38 | 27 |

**Table 3**

Dysplasia in juvenile polyps.

| Dysplasia | Germline mutation | |
|---|---|---|
| | ***SMAD4*** | ***BMPR1A*** |
| Negative | 8 (38%) | 17 (39%) |
| Indefinite | 4 (19%) | 8 (18%) |
| Low grade | 7 (33%) | 15 (34%) |
| High grade | 2 (9%) | 4 (9%) |
| Total | 21 | 44 |

## Table 4

Dysplasia in juvenile polyps organised by phenotype and germline defect.

| Dysplasia | Germline mutation and polyp type | | | |
|---|---|---|---|---|
| | ***SMAD4*** | | ***BMPR1A*** | |
| | **A** | **B** | **A** | **B** |
| Negative | 7 (88%) | 1 (12%) | 15 (88%) | 2 (12%) |
| Indefinite | 1 (25%) | 3 (75%) | 5 (63%) | 3 (37%) |
| Low grade | - | 7 (100%) | 8 (53%) | 7 (47%) |
| High grade | - | 2 (100%) | 2 (50%) | 2 (50%) |
| Total | 8 | 13 | 30 | 14 |

## Table 5

Results Ki67 immunohistochemistry on juvenile polyps organised by phenotype and germline mutation.

| Compartmentalisation of Ki67 | Germline mutation and polyp type | | | |
|---|---|---|---|---|
| | *SMAD4* | | *BMPR1A* | |
| | A | B | A | B |
| Normal | 7 (88%) | 2 (15%) | 15 (50%) | 4 (29%) |
| Loss | 1 (12%) | 11 (85%) | 15 (50%) | 10 (71%) |
| Total | 8 | 13 | 30 | 14 |