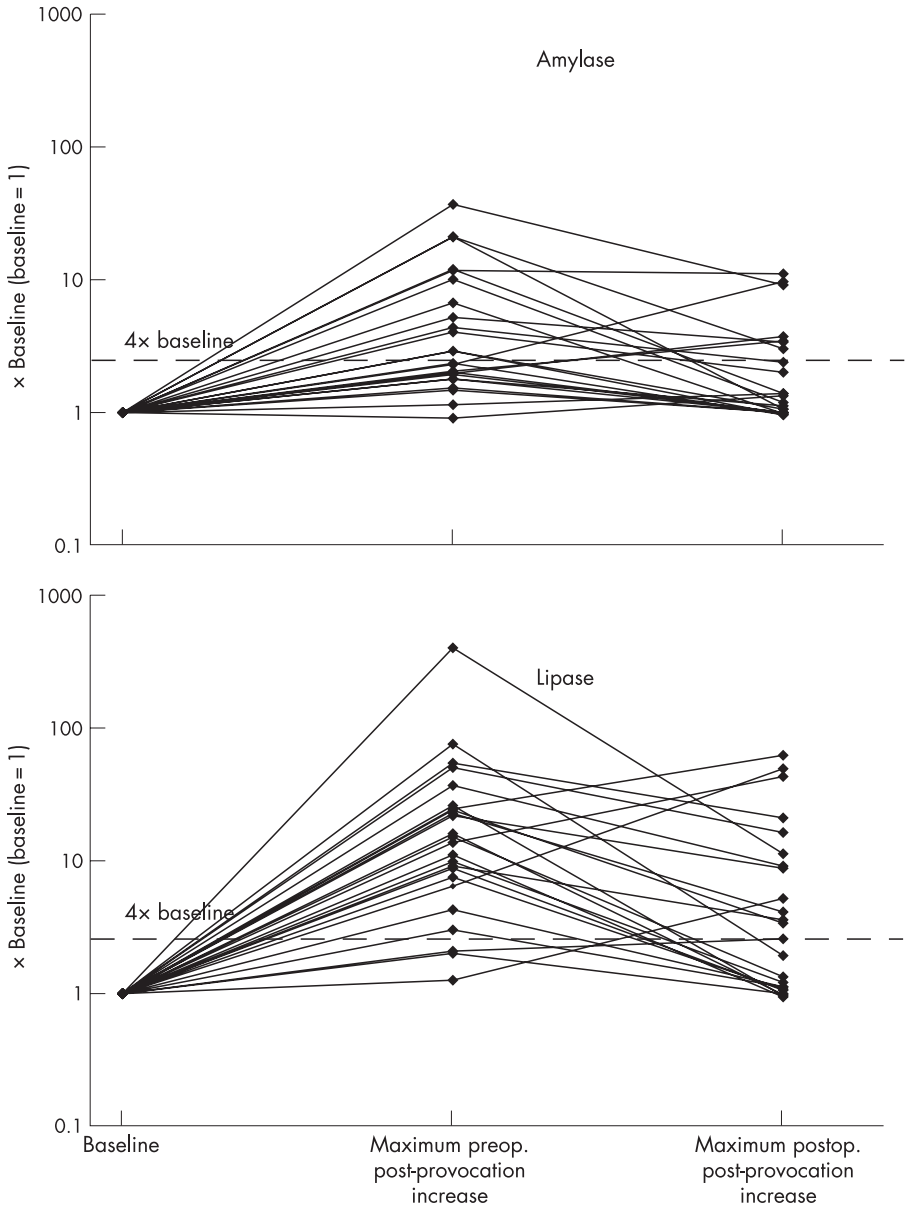

Figure 2 Scatter plot showing maximum increase in serum amylase (top) and lipase (bottom) over baseline values after provocation with morphine and prostigmine before and after transduodenal sphincteroplasty and transampullary septectomy in 24 patients with sphincter of Oddi dysfunction. The baseline value has arbitrarily been defined as 1 and increases are shown as multiples of the baseline value. The dashed line represents 4× baseline. The differences between the maximum post-provocation enzyme concentrations in the preoperative period (median (interquartile range) amylase = 155 (92 to 339) U/l, lipase = 1283 (604 to 2994) U/l) and the postoperative period (amylase = 69 (50 to 136) U/l, lipase = 214 (74 to 971) U/l) were significant (p = 0.002 for amylase and 0.005 for lipase, Wilcoxon signed ranks test).

collected hourly for five hours. Abdominal pain was quantified using visual analogue pain scores. Reference ranges were 23 to 300 U/l for serum lipase and 30 to 110 U/l for serum amylase. The 24 patients subsequently underwent TDS/TAS and the Nardi test was repeated 12 months postoperatively. The gastrointestinal quality of life index (GIQLI)[6] was measured preoperatively and 12 months postoperatively. The two studies were approved by the ethics committees of the University of Nottingham Medical School and Nottingham University Hospitals. Informed written consent was obtained.

The median (range) age of the 14 male and six female volunteers was 21 (18 to 23) years. Serum lipase and amylase concentrations increased more than fourfold over baseline in 15 (75%) and 13 (65%) healthy subjects, respectively, after morphine–prostigmine provocation. The median (interquartile range) baseline and maximum post-provocation concentrations of serum lipase were 120 (74 to 138) and 2930 (344 to 7824) U/l, respectively; corresponding values for serum amylase were 61 (44 to 81) and 220 (91 to 545) U/l ($p<0.001$, Wilcoxon signed ranks test). Figure 1 shows the maximum increase in enzymes over baseline for each subject, none of whom experienced pain during the study.

The median (range) age of the three male and 21 female patients was 42 (25 to 64) years. The post-provocation enzymatic increases were significantly greater preoperatively than postoperatively (fig 2). Preoperatively, 21 of the 24 patients (87.5%) had a greater than fourfold increase in amylase or lipase or both after morphine–prostigmine provocation, compared with 12 (50%) postoperatively ($p = 0.01$, χ^2 test with Yates' correction). While pain was reproduced after pharmacological provocation in 20 patients (83%) preoperatively, this occurred in only four (17%) postoperatively. The mean (95% confidence interval) GIQLI score improved from 61 (55 to 66) preoperatively to 92 (82 to 102) postoperatively ($p<0.0001$). Twelve months postoperatively, eight patients (33%) were symptom-free and 12 (50%) had reduced analgesia requirement. Four patients (17%) with no improvement also had a positive postoperative Nardi test.

In healthy volunteers increases in serum lipase and amylase after the Nardi test lack specificity. In patients with sphincter of Oddi dysfunction, pain is a universal accompaniment of enzyme elevation after the Nardi test. TDS/TAS significantly obtunds the enzymatic and nociceptive responses to the Nardi test in only 50% of patients with sphincter of Oddi dysfunction. The lack of specificity of the Nardi test makes it unsuitable as an objective diagnostic test for this condition.

D N Lobo, A S Takhar, A Thaper, M G Dube, B J Rowlands
Division of Gastrointestinal Surgery, Wolfson Digestive Diseases Centre, Nottingham University Hospitals, Queen's Medical Centre, Nottingham, UK

Correspondence to: Mr Dileep N Lobo, Division of Gastrointestinal Surgery, E Floor, West Block, Nottingham University Hospitals, Queen's Medical Centre, Nottingham NG7 2UH, UK; Dileep.Lobo@nottingham.ac.uk

doi: 10.1136/gut.2007.126375

Conflict of interest: None declared.

References

1 **Moody FG**, Becker JM, Potts JR. Transduodenal sphincteroplasty and transampullary septectomy for postcholecystectomy pain. *Ann Surg* 1983;**197**:627–36.
2 **Nardi GL**, Acosta JM. Papillitis as a cause of pancreatitis and abdominal pain: role of evocative test, operative pancreatography and histologic evaluation. *Ann Surg* 1966;**164**:611–21.
3 **LoGiudice JA**, Geenen JE, Hogan WJ, *et al*. Efficacy of the morphine–prostigmin test for evaluating patients with suspected papillary stenosis. *Dig Dis Sci* 1979;**24**:455–8.
4 **Steinberg WM**, Salvato RF, Toskes PP. The morphine-prostigmin provocative test – is it useful for making clinical decisions? *Gastroenterology* 1980;**78**:728–31.
5 **Gregg JA**, Taddeo AE, Milano AF, *et al*. Duodenoscopy and endoscopic pancreatography in patients with positive morphine prostigmine tests. *Am J Surg* 1977;**134**:318–21.
6 **Eypasch E**, Williams JI, Wood-Dauphinee S, *et al*. Gastrointestinal quality of life index: development, validation and application of a new instrument. *Br J Surg* 1995;**82**:216–22.

Urine based detection of intestinal mucosal cell damage in neonates with suspected necrotising enterocolitis

Necrotising enterocolitis (NEC) is a severe gastrointestinal disease with a mortality of 20–40%, affecting predominantly premature neonates.[1] In the early phase of the disease NEC continues to present a diagnostic challenge

for the attending clinician. The signs and symptoms are often non-specific, including gastrointestinal problems such as abdominal distension and feeding intolerance, which are also among the most prevalent presenting features of neonatal sepsis.[2] The diagnosis is further hampered by the limited diagnostic accuracy of the laboratory and radiological tests currently in use.[3 4]

Histopathologically, NEC is characterised by intestinal coagulative or ischaemic necrosis, starting at the mucosa and extending into the submucosa and muscularis externa.[1] We sought a non-invasive test to find evidence of enterocyte cell death in infants with gastrointestinal symptoms suspicious of NEC, in order to differentiate NEC from other neonatal diseases that present with abdominal signs.

Intestinal fatty acid binding protein (I-FABP) has been reported to be a useful plasma marker for early enterocyte cell death.[5 6] The small (14–15 kDa) cytosolic I-FABP is specifically present in mature enterocytes of small and large intestine and is released as soon as cell membrane integrity is compromised. I-FABP is present in very small amounts in the plasma of healthy individuals, probably representing the normal turnover of enterocytes, but levels rise rapidly after episodes of acute intestinal ischaemia and inflammation, including NEC.[5–8]

Because of its low molecular weight, I-FABP present in the systemic circulation passes through the glomerular filter (fractional renal excretion 28%; half life time 11 minutes) and can readily be detected in the urine.[9] I-FABP is not expressed in the urinary tract mucosa. Thus urinary values of I-FABP provide specific information about the number of dying intestinal epithelial cells. Given the age and birth weight of our population of interest, there are important advantages to sampling urine instead of plasma.[10 11]

Over an 18 month period, 17 consecutive neonates were identified in the neonatal intensive care unit at the University Hospital Maastricht in whom NEC was suspected. Inclusion criteria were the presence of at least one clinical gastrointestinal sign (abdominal distension or discolouration, increased gastric residues, bloody stools), causing sufficient clinical concern to require an abdominal *x* ray or to stop enteral feeding or both.

Written consent was obtained from both parents before inclusion, and the study was conducted with approval from the local medical ethics committee.

Urine was collected daily using a bag catch method for at least one week once appropriate consent was obtained. All neonates produced urine at a minimal rate of 1.9 ml/kg/h. Samples were centrifuged at 4000×*g* for 15 minutes, aliquoted, and stored at −20°C until analysis. Urinary I-FABP was measured using a commercially available I-FABP enzyme linked immunosorbent assay (ELISA) that selectively detects human I-FABP (standard, 20–5000 pg/ml), kindly provided by HBT (Uden, Netherlands). Values were expressed as a ratio (units pg/nmol) of I-FABP (pg/ml) to creatinine (Cr, μmol/l), in order to compensate for variations in the concentration of the urine. Urinary I-FABP:Cr levels were compared between neonates with NEC and those with other final diagnoses using the Mann–Whitney test.

Five of the 17 infants with suspected NEC subsequently developed NEC stage IIa (pneumatosis intestinalis on abdominal *x* ray, confirmed by a paediatric radiologist) or higher. Of the remaining 12 patients suspected of NEC, there were seven cases of sepsis, one of ileal atresia with intestinal necrosis, two patients for whom a diagnosis of constipation was ultimately made, one patient with Hirschsprung's disease, and finally one patient born with gastroschisis who later developed signs and symptoms of NEC after gastroschisis repair. In the neonates who developed NEC, the average gestational age was 32 weeks and 3 days, the average age at diagnosis was 14 days, and the average birth weight was 1500 g. For the neonates who subsequently received different diagnoses, the average gestational age was 30 weeks and 4 days and the average birth weight was 1487 g.

In the first urine sample, the median urinary I-FABP:Cr ratio was significantly higher in neonates who ultimately developed NEC or intestinal necrosis (3.9 pg/nmol, range 2.3 to 6.6) than in those without NEC (1.2 pg/nmol, range 0.1 to 1.8) ($p = 0.001$) (fig 1). A value of 2 pg/nmol discriminated between neonates with NEC or intestinal necrosis and the remainder. None of the patients without either NEC or intestinal necrosis was found to have an I-FABP:Cr ratio in excess of 2 pg/nmol over the first week.

In this study urinary I-FABP, a specific marker for intestinal mucosal damage, was measured using ELISA to identify patients with NEC. This study is the first to show that measurement of urinary I-FABP offers valuable additional information in the early diagnosis of NEC. Furthermore, while previous studies have assessed the feasibility of using plasma I-FABP or L-FABP to identify patients with NEC compared with healthy controls,[7 8] this is the first study which prospectively identified neonates with NEC among a population of preterm infants who presented with gastrointestinal symptoms.

In our small series, a value of 2 pg/nmol for the urinary I-FABP:Cr ratio was capable of distinguishing the group with NEC or intestinal necrosis from the other diagnoses.

NEC is a complex and probably multifactorial disease for which the aetiology remains incompletely understood. However, intestinal epithelial cell death is an important early hallmark[1] which is in line with the observed increase in urinary I-FABP. Moreover, normalisation of the urinary I-FABP:Cr ratio correlated well with clinical improvement, at least in the short term.

Given the ease and frequency with which urine can be collected without adverse consequences for the baby, the feasibility of urinary I-FABPs as a *screening* tool for NEC needs to be further evaluated in a prospective trial. Finally, as the cumulative release of I-FABP can be used to estimate the extent of intestinal damage, it may also help to guide treatment strategies, such as the timing of surgery (if required), the duration of antibiotic treatment, and selection of the ideal time for reintroducing enteral feeding.

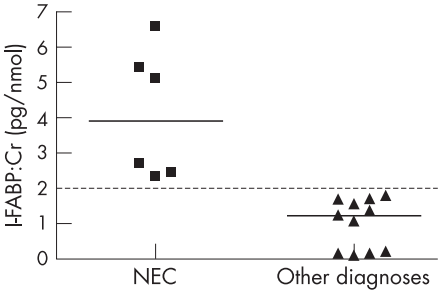

Figure 1 In the first urine sample of neonates with suspected necrotising enterocolitis (NEC), the mean urinary I-FABP:Cr ratio was significantly higher in neonates who ultimately developed NEC or intestinal necrosis (3.9 pg/nmol, range 2.3 to 6.6) than in those without NEC (1.2 pg/nmol, range 0.1 to 1.8) ($p = 0.001$). Cr, creatine; I-FABP, intestinal fatty acid binding protein.

Acknowledgements

This research was supported by AGIKO-stipendium 920-03-438 (to JPMD) from the Netherlands Organisation for Health Research and Development.

J P M Derikx, N J Evennett
Department of Surgery, University Hospital Maastricht, and Nutrition and Toxicology Research Institute (NUTRIM), Maastricht University, Maastricht, Netherlands

P L J Degraeuwe, T L Mulder
Department of Paediatrics; University Hospital Maastricht, Netherlands

A A van Bijnen, L W E van Heurn, W A Buurman, E Heineman
Department of Surgery, University Hospital Maastricht, and Nutrition and Toxicology Research Institute (NUTRIM), Maastricht University, Maastricht, Netherlands

Correspondence to: Professor Erik Heineman, University Hospital Maastricht, P Debeyelaan 25, 6202 AZ, Maastricht, Netherlands; e.heineman@surgery.azm.nl

doi: 10.1136/gut.2007.128934

Conflict of interest: None declared.

References

1 **Lin PW**, Stoll BJ. Necrotising enterocolitis. *Lancet* 2006;**368**:1271–83.
2 **Fanaroff AA**, Korones SB, Wright LL, *et al*. Incidence, presenting features, risk factors and significance of late onset septicemia in very low birth weight infants. *Paediatr Infect Dis J* 1998;**17**:593–8.
3 **Hällströma M**, Koivistob A, Janasa M, *et al*. Laboratory parameters predictive of developing necrotizing enterocolitis in infants born before 33 weeks of gestation. *J Pediatr Surg*. 2006;41: 792– 8).
4 **Tam Alda L**, Camberos A, Applebaum H. Surgical decision making in necrotizing enterocolitis and focal intestinal perforation: predictive value of radiologic findings. *J Pediatr Surg* 2002;**37**:1688–91.
5 **Kanda T**, Fujii H, Tani T, *et al*. Intestinal fatty acid-binding protein is a useful diagnostic marker for mesenteric infarction in humans. *Gastroenterology* 1996;**110**:339–43.
6 **Lieberman JM**, Sacchettini J, Marks C, *et al*. Human intestinal fatty acid binding protein: report of an assay with studies in normal volunteers and intestinal ischemia. *Surgery* 1997;**121**:335–42.
7 **Edelson MB**, Sonnino RE, Bagwell CE, *et al*. Plasma intestinal fatty acid binding protein in neonates with necrotizing entercolitis: a pilot study. *J Pediatr Surg* 1999;**34**:1452–7.
8 **Guthmann F**, Börchers T, Wolfrum C, *et al*. Plasma concentration of intestinal- and liver-FABP in neonates suffering from necrotizing enterocolitis and in healthy preterm neonates. *Mol Cell Biochem* 2002;**239**:227–34.
9 **Poll MCG van de, Derikx JPM, Buurman WA, et al**. Liver manipulation causes hepatocyte injury and precedes systemic inflammation in patients undergoing liver resection. *World J Surg*, (in press)..
10 **Smoller BR**, Kruskall MS. Phlebotomy for diagnostic laboratory tests in adults. Pattern of use and effect on transfusion requirements. *N Engl J Med* 1986;**314**:1233–5.

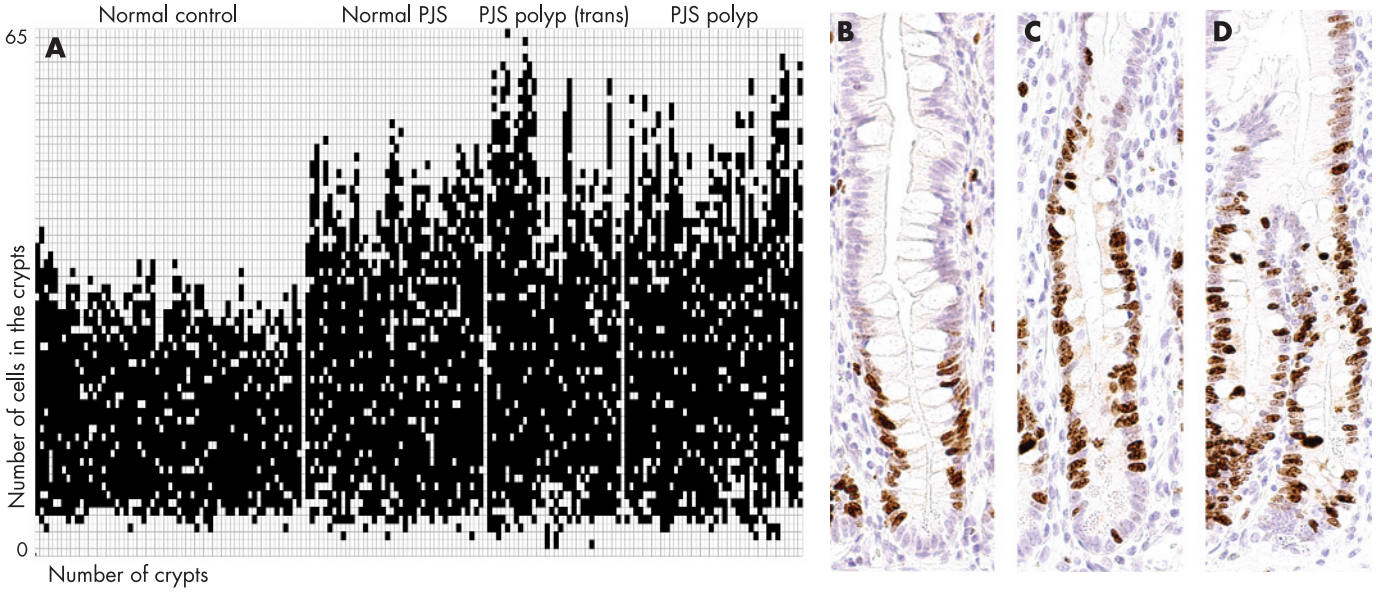

Figure 1 Ki67 staining pattern in intestinal crypts of normal and polyp tissue from patients with Peutz–Jeghers syndrome (PJS) and controls. In all samples the number and location of Ki67 positive cells was scored in 10 completely visible and longitudinally sectioned crypts. The proliferative compartment was defined as the cellular compartment extending between the cell positions of the lowermost and uppermost Ki67 positive labelled cell using the bottom of the crypt as the reference cell position 0.[8] (A) Schematic overview of Ki67 staining, black = positive Ki67 staining. (B) Ki67 staining on normal control intestine. (C) Ki67 staining on PJS normal intestine. (D) Ki67 staining on PJS polyp.

11 **Lin JC**, Strauss RG, Kulhavy JC, *et al*. Phlebotomy overdraw in the neonatal intensive care nursery. *Pediatrics* 2000;**106**:e19–25.

Peutz–Jeghers syndrome polyps are polyclonal with expanded progenitor cell compartment

Peutz–Jeghers syndrome (PJS) is an autosomal dominant cancer susceptibility syndrome characterised by mucocutaneous melanin pigmentation, hamartomatous polyps, and an 18-fold increase in intestinal and extraintestinal cancer risk.[1] PJS is caused by a germline mutation in *LKB1*, a gene that plays a role in cellular polarity.[2] Proper cellular polarity is critical for accurate asymmetrical stem cell division.[3] The pathogenesis and neoplastic risk, if any, of hamartomatous Peutz–Jeghers polyps remain unclear.

Based on rare observations of neoplastic changes in PJS polyps and the finding of biallelic inactivation of the gene involved in PJS, the existence of a unique hamartoma–carcinoma sequence has been proposed in this disorder.[4 5] This concept suggests that PJS polyps are clonal premalignant lesions responsible, at least in part, for the high rates of gastrointestinal cancer in these patients. However, dysplastic changes have been found rarely. Clarification of the risk of neoplastic transformation in PJS polyps would assist in the design of polyp surveillance strategies for PJS patients.

We studied the clonality of laser microdissected epithelium from two fresh frozen PJS polyps obtained from each of two female PJS patients from different well characterised PJS families, employing X chromosome inactivation of the *HUMARA* gene.[6] Diagnosis of PJS in these two patients was confirmed by identifying the *LKB1* germline mutations—two new mutations resulting in premature truncations (c.829-830insGGGCG, p.Asp277Glyfs12 and c.718C→A, p.Ser240X). The clonality assay showed no significant change in the shift of ratios between the small and large *HUMARA* allele for all polyps after digestion of the unmethylated allele, indicative of a polyclonal nature of these polyps. Furthermore, a loss of heterozygosity (LOH) analysis of four markers surrounding *LKB1* showed no LOH in any of the polyps. As LOH would be indicative of monoclonal cell expansion, its absence is consistent with the polyclonal nature of the polyps as found in the clonality assay.

The polyclonal nature and lack of *LKB1* LOH is compatible with our previous hypothesis that elongation of the progenitor zone, caused by a germline defect in asymmetrical stem cell division and subsequent mucosal prolapse, may play a role in polyp formation.[3 7] To investigate whether the length of the progenitor zone in PJS patients is altered compared with the length of the progenitor zone in normal intestinal mucosal controls, immunohistochemical Ki67 analysis was undertaken to obtain proliferation indices. Six PJS polyps from the PJS patients studied in the clonality assay were divided into three areas: (1) normal flat intestinal mucosa adjacent to the stalk of the polyp; (2) the area that forms the transition between flat intestinal mucosa and hamartomatous mucosa; and (3) the head of the polyp consisting of hamartomatous mucosa. The length of the proliferative zone in PJS flat intestinal, transitional, and polyp head mucosa combined was elongated compared with the crypts in the normal intestinal mucosa of the controls ($p<0.001$) (table 1, fig 1). However, the labelling index—defined as the percentage of positive Ki67 cells within the proliferative compartment—was not statistically different, showing that the percentage of proliferating cells in the crypt remains unchanged, but that in PJS mucosa the length of the proliferative zone is expanded.

In conclusion, we have shown that PJS polyps are polyclonal expansions, arguing against the presence of a hamartoma–carcinoma sequence in PJS. Furthermore, the progenitor zone in intestinal crypts in PJS patients is expanded compared with normal individuals. We postulate that this is caused by disruption of the delicate balance between symmetrical and asymmetrical stem cell divisions, possibly leading to altered stem cell lineage turnover rates and protracted clonal

Table 1 The length of the progenitor zone in cell positions and the labelling index for normal intestine and Peutz–Jeghers syndrome (PJS) polyps and statistical analysis (*t* test)

	n	Mean length ofprogenitor zone	SD	Labelling index (,%)	p Values	
Normal	60	30	3	73.30		
PJS						
total	110	46	7	74.10	Normal vs PJS intestine:	<0.001
flat	30	43	5	73.30	PJS flat vs transition:	0.023
					PJS flat vs head:	0.012
transition	40	48	9	75.50	PJS transition vs head:	0.655
head	30	47	7	73.50		

Flat, normal flat intestinal mucosa adjacent to polyp stalk; head, head of polyp; transition, transition zone between flat mucosa and hamartomatous mucosa.