ANORECTAL DISEASE

Rectal sensorimotor dysfunction in patients with urge faecal incontinence: evidence from prolonged manometric studies

C L H Chan, P J Lunniss, D Wang, N S Williams, S M Scott

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See end of article for
authors' affiliations

Correspondence to:
Dr S M Scott, Centre for
Academic Surgery, GI
Physiology Unit, 3rd Floor
Alexandra Wing, Royal
London Hospital,
Whitechapel, London E1
1BB, UK; m.scott@
qmul.ac.uk

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Background and aims: Although external anal sphincter dysfunction is the major cause of urge faecal incontinence, approximately 50% of such patients have evidence of rectal hypersensitivity and report exaggerated stool frequency and urgency. The contribution of rectosigmoid contractile activity to the pathophysiology of this condition is unclear, and thus the relations between symptoms, rectal sensation, and rectosigmoid motor function were investigated.

Methods: Fifty two consecutive patients with urge faecal incontinence, referred to a tertiary surgical centre, and 24 volunteers, underwent comprehensive anorectal physiological investigation, including prolonged rectosigmoid manometry. Patients were classified on the basis of balloon distension thresholds into those with rectal hypersensitivity ($n = 27$) and those with normal rectal sensation ($n = 25$). Automated quantitative analysis of overall rectosigmoid contractile activities and, specifically, high amplitude contractions and rectal motor complex activity was performed.

Results: External anal sphincter dysfunction was similar in both patient groups. Overall, phasic activity and high amplitude contraction frequency were greater, and rectal motor complex variables significantly altered, in those with rectal hypersensitivity. Symptoms, more prevalent in the rectal hypersensitivity group, were also more often associated with rectosigmoid contractile events. For individuals, reduced compliance and increased rectal motor complex frequency were only observed in patients with rectal hypersensitivity.

Conclusions: We have identified a subset of patients with urge faecal incontinence—namely, those with rectal hypersensitivity—who demonstrated increased symptoms, enhanced perception, reduced compliance, and exaggerated rectosigmoid motor activity. Comprehensive assessment of rectosigmoid sensorimotor function, in addition to evaluation of anal function, should be considered in the investigation of patients with urge faecal incontinence.

Maintenance of bowel continence involves coordination between anorectal and colonic function, and psycho-behavioural factors. Faecal incontinence is a major cause of social and psychological disability, reported in approximately 2% of the adult population,¹ although this likely represents an underestimate as sufferers are often reluctant to volunteer such symptoms.^{2–3} Pathophysiology may be multifactorial.⁴ Urge faecal incontinence (UFI), where incontinent episodes occur against the patient's will, due to lack of voluntary control, is the most common presenting symptom.⁴ External anal sphincter (EAS) dysfunction, either secondary to compromised structural integrity, neurological injury, or a combination of both, is recognised as the major cause of UFI.^{5–7} Nevertheless, patients with an anatomically intact and normal functioning EAS also experience episodes of UFI,⁸ indicating that other pathophysiological mechanisms may contribute to symptoms. It is known that alterations in suprasphincteric mechanisms influence continence⁹ but their precise role in UFI remains undetermined. The reservoir function of the colorectum may be compromised, for example, by disturbance of sensorimotor function.^{10–12} Furthermore, as visceral sensory and motor mechanisms of the anorectum and colon are themselves inextricably linked, alterations in the motor component may effect change in sensory function, and vice versa.¹³ This interaction may be further modulated and modified by higher cortical centres.¹⁴

Evaluation of rectal sensory function in patients with UFI has demonstrated that up to ~50% of patients have evidence of rectal hypersensitivity (RH) to simple volumetric balloon distension—that is, reduced sensory thresholds.^{5–15} We have

recently shown that in patients with UFI, RH is associated with increased bowel frequency, reduced ability to defer defecation, increased pad usage, and negative lifestyle effects.⁵

Motor function of the colon and rectum is an integrated process involving myoelectrical and contractile activity, tone, compliance, wall tension, and stool transit.¹⁶ Although some information is available regarding the influence of alterations in certain motor components, such as compliance,^{10–12} tone,¹⁸ and transit¹⁹ in UFI, little is known about the contribution of colorectal contractile activity to the pathophysiology of this condition. Numerous studies have used prolonged ambulatory manometry to investigate colonic contractile activity in normal subjects and patients with constipation,^{19–23} but few studies have used this technique to address possible colorectal dysmotility in faecal incontinence.^{19–24} Colorectal motility comprises a number of distinct phasic contractile activities, both isolated and in recognisable patterns.²⁵ Two components, high amplitude contractions and rectal motor complexes (RMC), have been shown to be functionally important in patients with faecal incontinence.

Abbreviations: ANOVA, analysis of variance; AUC, area under the curve; CS, control subjects; EAS, external anal sphincter; DDV, defecatory desire volume; HAC, high amplitude contraction; HADS, hospital anxiety and depression scale; HAPC, high amplitude propagated contraction; IAS, internal anal sphincter; IBS, irritable bowel syndrome; MTV, maximum tolerable volume; NS, normal rectal sensation; PNTML, pudendal nerve terminal motor latency; RH, rectal hypersensitivity; RMC, rectal motor complex; SCID, structured and clinical interview for DSM-III-R; UFI, urge faecal incontinence

In one study, a strong correlation between the urge to defecate (represented by depression of an event marker) and high amplitude propagated contractions (HAPC) was demonstrated in both healthy individuals and in the six patients with faecal incontinence.¹⁹ All episodes of urge incontinence were associated with propagation of HAPCs from the transverse colon to the rectum.¹⁹ HAPCs are the major motor correlates of mass intraluminal movement,²⁶ and there is a clear association with both the urge to defecate^{22–27} and faecal expulsion.^{28–29} In a separate study, Santoro *et al* showed RMC frequency to be increased in a proportion of patients with idiopathic faecal incontinence.²⁴ The RMC is a subconscious intrinsic motor programme comprising regular cyclical bursts of phasic pressure waves.^{19–23–30–32} The function of the RMC remains unclear but is thought to represent localised segmental activity.^{31–32} It now appears that the RMC, although predominant in the rectosigmoid region, is indeed manifest throughout the colon, where it has been termed the colonic motor complex.^{20–23–31–32} Like the small bowel migrating motor complex, which has been characterised to a much greater extent over the past three decades,^{16–33} it has been proposed that the RMC may also be used as a marker of enteric neuromotor function, as its presence is independent of intact extrinsic innervation.^{34–36} Prolonged ambulatory manometry, which is now a well recognised clinical tool for the investigation of small intestinal dysmotility,^{37–38} as well as a research tool for the study of colorectal motility,²⁵ has demonstrated that both qualitative and quantitative abnormalities of cyclical motor activity may be of pathological significance.^{21–38–40}

The primary aim of this study was to evaluate rectosigmoid motor activity over a prolonged period in a large cohort of patients with the specific symptom of UFI. The secondary aim was to investigate the relationships between rectal sensation and colorectal motor function, to test the hypothesis that the exaggerated symptoms observed in patients with rectal hypersensitivity are associated with differences in contractile activity.

MATERIALS AND METHODS

Subjects

Patients with urge faecal incontinence (UFI)

The study population consisted of 52 patients with UFI (38 females; median age 45.5 years (range 18–72)) referred consecutively to a tertiary surgical coloproctology centre. All patients had a detailed clinical history taken and underwent investigations to exclude organic gastrointestinal pathology. From their histories, details regarding symptom onset, bowel frequency, and frequency of incontinence episodes were recorded. All patients also underwent a structured clinical interview (SCID) for DSM-III-R (Diagnostic and Statistical Manual of Mental Disorders) to screen for psychopathology⁴¹ and completed validated screening questionnaires: the bowel disease questionnaire⁴² and the hospital anxiety and depression scale (HADS).⁴³ These questionnaires were used to identify the presence of the irritable bowel syndrome (IBS), as defined by the Rome II criteria,⁴⁴ and anxiety or depressive disorders. A score of up to 7 in either the anxiety or depression scale of the HADS is regarded as normal, 8–10 as mild anxiety or depression, 11–14 as moderate anxiety or depression, and 15–21 as severe anxiety or depression.⁴³

Normal healthy volunteers

Twenty four healthy volunteers (16 females; median age 29 years (range 18–55)), recruited by advertisement, were used as control subjects (CS). There was no evidence in any of the subjects of organic or functional gastrointestinal disorder as assessed by detailed clinical history, bowel symptom questionnaire,⁴² or physical examination. Anxiety

and depressive disorders were excluded through the same structured clinical interview (SCID),⁴¹ and completion of the HADS.⁴³

Approval for these studies was obtained from the East London and City Health Authority Research Ethics Committee (P01/84), with written informed consent obtained from all patients.

Anorectal physiological investigation

Standard techniques

All subjects underwent detailed standard anorectal physiological investigations, which included station pull through manometry of the anal canal, evaluation of rectal sensory thresholds using a volumetric based balloon distension technique, assessment of pudendal nerve terminal motor latencies, and endoanal ultrasonography.

Manometry was performed using a single channel side hole catheter linked to an Arndorfer-type pneumohydraulic water perfusion system; a pull back technique allowed assessment of functional anal canal length, maximum resting tone, and maximum voluntary squeeze pressures.⁴⁵ Anal resting tone and squeeze pressures were considered abnormal if they were below 50 cm H₂O, which are the lower limits of normal for our unit, as determined from investigation of the 24 control subjects involved in this study plus 32 further controls (56 in total). Rectal sensation was tested by inflating a latex balloon with air at 1 ml/s and determining the threshold volumes for first constant sensation, defecatory desire volume (DDV), and maximum tolerable volume (MTV).⁴⁶ Patients were considered to have RH if MTV was <100 ml in females or <80 ml in males (determined in 56 healthy control subjects). Pudendal nerve terminal motor latencies (PNTML) were recorded with the St Mark's pudendal stimulating electrode (Dantec Electronics Ltd, Bristol, UK).⁴⁷ PNTML are known to increase with age⁴⁸; patients were considered to have a pudendal neuropathy (either unilateral or bilateral) if PNTMLs exceeded 2.3 ms in those <40 years of age, and exceeded 2.5 ms in those ≥40 years of age. These values represent the upper limit of normal for our unit. Endoanal ultrasound (10 MHz transducer; B&K Medical, UK) was used to assess sphincter integrity.⁴⁹

Advanced techniques

Barostat study

An electronic barostat (Synectics Visceral Stimulator; Synectics Medical, Stockholm, Sweden) was used to measure rectal compliance. Employing a stepwise isobaric distension protocol,⁵⁰ analogue signals from the barostat were amplified and digitised by an interface converter (PC Polygraph HR; Synectics Medical, Enfield, Middlesex, UK) and transmitted to a PC at a sampling rate of 32 Hz for online display and subsequent storage to hard disk. A dedicated software program (Polygram for Windows version 1.1; Synectics Medical, UK) was used for online monitoring and analysis purposes.

With no sedation or bowel preparation, and with subjects lying in the left lateral position, sigmoidoscopy was performed to ensure an empty rectum (all cases). An “infinitely” compliant (that is, within the pressure-volume range studied) barostat bag (maximum capacity 500 ml; Medtronic Functional Diagnostics Zinectics Inc, Utah, USA) mounted on a manometric catheter (internal diameter 3 mm) and fixed at both ends, was then inserted into the rectum, after ensuring that there was no leakage from the system. The catheter was connected to the barostat with an inflation and deflation port. Maximal airflow was 38 ml/s. In an attempt to stabilise basal tone, reduce variability in sensory thresholds and compliance (that is, to improve

reproducibility), and familiarise subjects with the procedure, a conditioning distension protocol was performed.⁵¹ When inflated, the bag became spherical with a length of 8 cm.

After allowing the system to equilibrate for a further five minutes, bag pressure was then increased from 0 to 32 mm Hg (or maximum toleration) in 2 mm Hg steps and continued for one minute, followed by a one minute rest. At each pressure step, the mean bag volume over the last 30 second segment was recorded.⁵¹ Static rectal wall compliance (ml/mm Hg) was calculated as the slope ($\delta V/\delta P$) of the compliance curve between the pressure thresholds of first constant and maximum tolerable distension volumes.⁵⁰ From 56 control subjects, the normal range for rectal compliance was taken as 8.6–19.1 ml/mm Hg.

Prolonged rectosigmoid manometry Recording system

Prolonged manometry was carried out using a portable ambulatory recording system (Flexilog 3000; Oakfield Instruments Ltd, Eynsham, Oxon, UK), connected to a Konigsberg solid state manometry catheter (Konigsberg Instruments Inc, Pasadena, California, USA)⁸ incorporating six transducers, spaced 5 cm apart, and calibrated over a range of 0–200 mm Hg.

No prior bowel preparation or sedation was used. All medications known to affect bowel function, such as loperamide, were discontinued for at least 48 hours prior to the study. A strong suture was tied to the tip of the catheter and grasped by biopsy forceps passed down the channel of a flexible sigmoidoscope. With the patient in the left lateral position, the catheter was then introduced into the recto-sigmoid in tandem with the sigmoidoscope, using minimal air insufflation. Under direct vision, the catheter was sited with the most proximal transducer (+25 cm) located in the sigmoid and the most distal transducer (0 cm) in the anal canal. The sigmoidoscope was then carefully withdrawn, ensuring the catheter's position was maintained. This procedure took no longer than five minutes in all cases. The catheter was then secured in place with tape (Mefix; SCA Molyntycke, UK) and connected to the portable recording system, which was secured in a shoulder harness. Recorded data were stored on a memory card and downloaded to a personal computer for subsequent display and analysis.

Study protocol

In general, prolonged manometric studies were commenced between 13:00 and 15:00. Recordings were started immediately once all the equipment was in place and the patient ambulant. If the catheter was not expelled during defecation, recordings were continued for approximately 20 hours until the next morning when the catheter was removed electively using gentle traction. Fluoroscopic examination in a subgroup of patients ($n = 4$) confirmed that the position of the catheter was maintained overnight. Subjects were allowed to go home and were encouraged to engage in their normal daily activities. Fluid intake was allowed ad libitum. Neither meal composition nor subject activities was standardised to avoid inducing further stress which may in turn influence colonic motility.⁵² Instructions were given to retire to bed at 23:00. Subjects were informed to depress an event marker on the recorder when the urge to defecate was perceived. A diary of events recording bedtime, time of wakening, and meal and drink times were carefully recorded.

Data analysis

Overall activity

Automated (quantitative) analysis of overall rectosigmoid contractile activity was initially performed using a validated commercially available computer software program (Flexisoft

III, Data Display & Analysis v 2.6.0; Oakfield Instruments Ltd., Oxon, UK). A pressure wave exceeding a threshold of 5 mm Hg, without a simultaneous pressure event occurring in the other four rectal/sigmoid recording channels, was assessed by the computer algorithm as being the consequence of a sigmoid/rectal contraction. These pressure events were predominantly monophasic elevations that had a discernible onset, peak, and offset, and that did not have the features of strain artefact.

For the purposes of analysis, each recording was divided into nocturnal and diurnal periods. The nocturnal period was defined by diary entries. Given the volume of the data recorded, three of the six catheter recording sites were chosen for analysis, at +25 cm, +15 cm, and +10 cm above the mid anal canal, to represent the sigmoid colon, rectosigmoid, and mid-rectum, respectively. As it is very difficult to maintain a point sensor accurately within the high pressure zone of the anal canal,¹⁹ marked fluctuations in anal pressure were seen to occur secondary to movement artefact. Consequently, a detailed quantitative analysis of anal motility (that is, from the most distal sensor) was not performed. For rectosigmoid activity, recorded measurements included:

- (a) per cent of recording time comprising phasic contractile activity;
- (b) contraction frequency;
- (c) median contraction amplitude;
- (d) maximum contraction amplitude; and
- (e) area under the pressure curve.

Specific contractile events

The automated analysis of two specific contractile events, namely high amplitude contractions (HAC) and RMCs, was performed using a separate computer program that has previously been developed and validated "in house" for the computerised assessment of small bowel motility.^{40–53} Analysis was performed from recording sites at +25 cm, +15 cm, and +10 cm. HACs were defined as individual phasic events which exceeded 50 mm Hg^{34–52–54} in amplitude. For each subject, the frequency of HACs during the recording period was determined. RMCs were defined as bursts of phasic pressure waves lasting ≥ 3 minutes, with a contraction frequency of $\geq 2/\text{min}$.^{23–30–32} Each RMC was identified visually and demarcated manually as a "region of interest" from which RMC frequency (No/h) could be calculated. Automated analysis of contractions within each region of interest allowed calculation of five further separate variables: (i) periodicity, (ii) complex duration, (iii) contraction frequency, (iv) median contraction amplitude, and (v) area under the pressure curve.

Symptoms

The temporal relationship between the symptom of urgency, as defined by an "event" as recorded by the patient, and rectal/sigmoid motor events (HAC, RMC) was assessed visually. A temporal association was defined as occurring within 60 seconds (30 seconds either side) of depression of the event marker. Qualitative analysis was performed on all recordings by two investigators.

Statistical analysis

Two test groups were defined, based on the results of rectal sensory function testing: those with RH and those with normal sensation (NS). Of the 52 patients with UFI, 27 were found to have RH (22 females; median age 49 years (range 28–72)) and 25 had NS (16 females; median age 45.5 years (range 18–63)).

Clinical, anorectal physiology, and barostat data

Data are expressed as mean (SD) or median (range), depending on whether the recorded values assumed a Gaussian distribution. Results were compared between test groups and CS using one way analysis of variance (ANOVA) or the Kruskal-Wallis test for parametric or non-parametric data, where appropriate, using a commercially available statistical software package (Prism 3.0; GraphPad Software Inc., San Diego, California, USA). For each test, the number of individual subjects in whom recorded values fell outside the respective normal ranges was recorded. Whether contingencies differed between groups was tested using the χ^2 or Fisher's exact test.

Prolonged rectosigmoid manometry

Overall contractile activity and HAC

Data were expressed and results compared in the same way as for clinical, physiological, and barostat data.

RMC variables

Due to the cyclical nature of rectosigmoid motility, prolonged manometric studies produce repeated measures of each RMC variable (periodicity, duration, frequency, amplitude, and area under the curve (AUC)) for each individual subject studied. As a result, conventional statistical methods, which assume a simple independent and non-repeated data structure, should not be employed for such data analysis. Failure to take into account the hierarchical structure in the analysis will give misleading results.⁵⁵ To account for the non-independence between such repeated measures, we used a mixed effects model for the data analysis, which is a generalisation of an ANOVA model designed to be especially powerful under these circumstances.⁵⁶ Through application of the mixed effects model, an estimate for the effect of RH or NS patients over CS, its 95% confidence interval (CI), and its statistical significance were calculated.⁵⁷ In order to maintain normal distribution of residuals (random errors) in the mixed effects model, data for each RMC variable were first transformed logarithmically to derive a single summary statistic for each individual, and the expected effect and its 95% confidence interval from the model were then antilog transformed. Using this methodology, the estimated effect for a variable should be interpreted as relative change. To assess whether the effect for a variable depends on time (day and night), an interaction between group (RH, NS, CS) and time was fitted for each variable. Effect was presented separately according to time if the interaction was significant at the 5% level. The mixed effects model was estimated through an SAS PROC MIXED procedure⁵⁶ in SAS version 8.2 (SAS Institute, Cary, North Carolina, USA).

RMC frequency, during both nocturnal and diurnal periods, was compared between groups using one way ANOVA. From previous studies,^{23 24 30–32 39} the upper limit of normal for RMC frequency is reported as 2.0/h during the day and 3.3/h at night. The proportions of individuals in the test groups, with an RMC frequency exceeding the upper limit of normal, were recorded, and contingencies were compared using Fisher's exact test. The other five RMC variables were evaluated using repeated measures ANOVA by means of the mixed effects model.

Correlations with other variables

Direct examinations of the presence of any correlations between prolonged manometric measurement variables and anorectal physiology and clinical history were made. Linear correlation or regression was used to compare the covariation of two numerical variables. When correlation was applied, parametric (Pearson correlation) or non-parametric (Spearman correlation) methods were used as appropriate.

For all tests, a p value of less than 0.05 (two sided test) was considered to be statistically significant. As this was an exploratory study, no adjustment for multiple comparisons were made in the analysis. Caution must therefore be exercised when a p value is near to 0.05, whereas when a p value is small, the observed difference is unlikely to be spurious.

RESULTS

Clinical history

Duration of symptoms was equivalent between those patients with RH and those with NS (48 months (range 9–432) *v* 48 months (range 2–216)). In female patients, parity was equivalent between study groups (RH: median 2 (range 0–10); NS: median 2 (range 1–5)).

Reported bowel frequency (fig 1) was significantly higher in patients with RH (median 4.75 bowel actions/24 hours (range 1–8)) than those with NS (median 2 bowel actions/24 hours (range 0.15–9); *p* = 0.002).

Psychological and psychiatric assessment

The structured clinical interview (SCID) for DSM-III-R⁴¹ did not reveal the presence of any psychological or psychiatric illnesses in any of the patients or healthy volunteers. The bowel symptom questionnaire and application of the Rome II criteria did not identify any patient or healthy volunteer as having IBS.

The anxiety scale was similar for the RH and NS groups (RH median 7 (range 0–17) *v* NS 7 (1–14)). Individually, 37% of patients with RH (10/27) were classified as suffering from anxiety compared with 40% in the NS group (10/25). There were also no significant differences in the depression scale between the RH and NS groups (RH median 4 (range 0–9) *v* NS 4 (0–13)). Again, the proportion of individuals with depression did not differ between the RH and NS groups (11% *v* 20%). There was no evidence of increased anxiety (median 0 (range 0–4)) or depression (median 1 (range 0–3)) in any of the control subjects.

Anorectal physiology

Anal sphincter function

Twenty one patients with RH (78%; data from one subject missing) and 15 patients with NS (60%; data from one subject missing) had reduced anal squeeze pressures (<50 cm H₂O). Only four patients with RH (15%) and four with NS (16%) had both a structurally intact EAS on

Figure 1 Reported bowel frequency in the rectal hypersensitivity (RH) and normal rectal sensation (NS) groups. A significantly higher median stool frequency was reported in the RH compared with the NS group (4.75 *v* 2.0).

Figure 2 Rectal compliance in control subjects (CS) and in those with rectal hypersensitivity (RH) and normal rectal sensation (NS). Compliance of 12 patients with RH and three with NS lay outside the normal range (broken line: 8.6–19.1 ml/mm Hg (mean (2SD))). Rectal compliance was significantly lower in patients with RH compared with NS ($p < 0.001$).

ultrasound and normal PNTMLs. Pathophysiology of EAS dysfunction was similar between the two groups.

Although there was a significantly greater incidence of internal anal sphincter (IAS) defects identified on endoanal ultrasound in those with RH (46%) than in patients with NS (17%; $p = 0.04$), IAS function, as defined manometrically, was similar between those with RH and NS. Anal resting tone was reduced (< 50 cm H₂O) in 58% of patients with RH and 48% of patients with NS.

Rectal sensory function and compliance

By definition, rectal sensory thresholds were significantly lower in the group with RH (mean DDV 48 (SEM 3) ml; MTV 74 (4) ml) than those with NS (DDV 119 (12) ml, $p < 0.001$; MTV 193 (12) ml; $p < 0.001$) and control subjects (DDV 107 (7) ml, $p < 0.001$; MTV 176 (12) ml, $p < 0.001$). There were no differences in rectal sensitivities between patients with NS and CS.

Rectal compliance (fig 2) was reduced (that is, the rectum was “stiffer”) in patients with RH compared with those with NS (mean 10.8 (1.1) ml/mm Hg ν 17.8 (1.4) ml/mm Hg; $p = 0.001$). Patients with NS had elevated rectal compliance in comparison with control subjects (compliance 13.9 (0.4) ml/mm Hg; $p < 0.05$), with three patients having values above the normal range. Although grouped data for rectal compliance were similar between patients with RH and healthy volunteers, 11/27 RH patients (41%) had a compliance value < 8.6 ml/mm Hg, which is the lower limit of normality (mean $-2SD$), as determined previously in our

unit. No patient with NS had rectal compliance below that of the normal range ($p = 0.0003$ ν patients with RH).

Prolonged rectosigmoid manometry

All subjects tolerated catheter insertion, and no complications were noted during insertion, monitoring, or withdrawal. Although sex matched, control subjects were significantly younger than both RH ($p = 0.001$) and NS ($p = 0.004$) patients.

Recording time

Overall recording time in the 76 subjects was 1243 hours (by study group: CS 445 hours; RH 429 hours; NS 369 hours). Mean recording time was 18.6 (0.5) hours in control subjects, which was equivalent to that in patients with RH (15.9 (1.2) hours) but greater than that in patients with NS (15.1 (0.9) hours; $p < 0.05$). Six studies in patients with RH ($p < 0.03$ ν control subjects) and five studies in patients with NS ($p = 0.05$ ν control subjects) were terminated prematurely because of catheter expulsion during defecation. In none of these was nocturnal activity recorded. In healthy volunteers, all recording catheters were removed electively. The start time of recordings was similar between groups (RH 13:30 $\pm$ 30 minutes; NS 14:30 $\pm$ 20 minutes; CS 14:30 $\pm$ 20 minutes).

Symptoms of urgency

Patients with RH were more frequently symptomatic than either patients with NS or controls. The median number of times per hour the event (symptom) marker was depressed in patients with RH was 0.5 (range 0–4), which was significantly greater than in patients with NS (0.24 (range 0–1.3); $p < 0.01$) and controls (0.05 (range 0–0.3); $p < 0.001$).

Temporal association between event marker and rectal contractile activity

In patients with RH, the event marker was depressed a total of 326 times (median 11 (range 0–57)). Urge to defecate was associated with a HAC in 128 cases (39%), which was significantly higher than in the NS group in whom depression of the event marker correlated with a HAC in 21% of instances (25/117; $p = 0.004$). In controls, the event marker was depressed in association with a HAC in 28% of instances (9/32), similar to those with NS. Urge to defecate was associated with RMC activity in 21% (67/326), 19% (22/117), and 16% (5/32) in the RH, NS, and CS groups, respectively, with no differences between the groups. Overall, in patients with RH, there was an association between the symptom of urgency and rectosigmoid motor events in 72% of cases compared with 45% and 50% of the NS and CS groups, respectively ($p < 0.0001$ and $p < 0.03$, respectively).

Table 1 Parameters of overall contractile activity at the recording site: +15 cm above the mid anal canal (representing the rectosigmoid) in control subjects (CS) and in those with rectal hypersensitivity (RH) and normal rectal sensation (NS)

Parameter		CS	RH	NS
Contractile activity (% of recording time)	+15	22.6 (1.3)	22.8 (1.4)	20.2 (1.2)
Contraction frequency (No/min)	+15	1.6 (0.1)	1.5 (0.1)	1.4 (0.09)
Median amplitude (mm Hg)	+15	9.6 (0.3)*	9.8 (0.4)*	8.4 (0.3)
Maximum amplitude (mm Hg)	+15	124 (9)**	127 (8)**	87 (8)
AUC (mm Hg/min)	+15	84 (6)*	90 (7)**	61 (5)

* $p < 0.05$, ** $p < 0.01$ versus NS.

For activity at +25 cm (sigmoid) and +10 cm (rectum), please refer to the *Gut* website at <http://www.gut.jnl.com/supplemental>.

Table 2 Frequency of high amplitude contractions (>50 mm Hg/h) during the day and night in control subjects (CS) and in those with rectal hypersensitivity (RH) and normal rectal sensation (NS), at the recording site +15 cm above the mid anal canal

Site		CS	RH†	NS‡
Diurnal	+15	1.1 (0.2)*	2.3 (0.6)	1.1 (0.8)**
Nocturnal	+15	0.3 (0.1)*	1.3 (0.3)	1.6 (0.9)

*p<0.05, **p<0.01 versus RH.

†No nocturnal data, n=6; ‡no nocturnal data, n=5.

For high amplitude contraction activity at other levels, please refer to the Gut website at <http://www.gutjnl.com/supplemental>.

Overall contractile activity (table 1)

The percentage recording time taken up by contractile activity and overall contraction frequency were similar between the groups at all three levels studied. However, median contraction amplitude was significantly greater in patients with RH and CS compared with NS patients at +15 cm (p<0.05 and p<0.05, respectively). Maximum contraction amplitude was also significantly greater in patients with RH and CS compared with NS patients at both +25 cm and +15 cm (+25 cm: p<0.01 and p<0.05, respectively; +15 cm: p<0.01 and p<0.01, respectively). Consequently, the AUC was greater in RH patients than those with NS at all levels (+25 cm: p<0.05; +15 cm: p<0.01; +10 cm: p<0.01). AUC in controls was also greater than in patients with NS at +15 cm (p<0.05). AUC was similar between CS and patients with RH.

HACs

Daytime frequency of HAC (table 2) was increased in patients with RH compared with patients with NS at all levels studied (p<0.01). In addition, nocturnal frequency of HAC was higher in RH patients than in NS patients at both +25 cm (p<0.05) and +10 cm (p<0.05). In comparison with control subjects, the frequency of contractions >50 mm Hg was higher in RH patients at both +25 cm and +15 cm during the day (p<0.05, respectively) and at +15 cm at night (p<0.05).

Rectal motor complex (RMC) activity

RMC frequency (fig 3)

For grouped data, RMC frequency in RH patients was significantly increased both during the daytime and at night compared with control subjects at all levels of the study segment (diurnal: +25 cm, p<0.001; +15 cm, p<0.05; +10 cm, p<0.01; nocturnal: p<0.001 at all levels). Likewise, RMC frequency was significantly higher in RH patients than NS patients at all levels during the night (p<0.001) and at +25 cm (p<0.01) and +10 cm (p<0.05) during the day. The

Figure 3 Diurnal (D) and nocturnal (N) rectal motor complex (RMC) frequency in control subjects (CS) and in those with rectal hypersensitivity (RH) and normal rectal sensation (NS), recorded at +15 cm above the mid anal canal. *p<0.05; **p<0.01; ***p<0.001. For data at other recording levels, please refer to the Gut website at <http://www.gutjnl.com/supplemental>.

frequency of RMCs was similar between NS patients and control subjects both in the diurnal and nocturnal periods. Within groups, RMC frequency was greater at night in both control subjects and patients with NS at +15 cm and +10 cm (+15 cm, p<0.01 and p<0.01, respectively; +10 cm, p<0.001 and p<0.05, respectively). In patients with RH, RMC frequency was similar during the day and at night at all levels.

By site of recording, 15 individual patients with RH (56%) had a diurnal RMC frequency elevated beyond the normal range at +25 cm and +15 cm, and 20 RH patients (74%) had an increased RMC frequency at +10 cm. By comparison, the numbers of patients with NS who had an elevated diurnal RMC frequency were: 2 (8%) at +25 cm (p = 0.0007 v RH), 0 at +15 cm (p<0.0001 v RH), and 3 (12%) at +10 cm (p<0.0001 v RH). Because of defecation (that is, catheter expulsion), it should be noted that of the six RH patients in whom the recording was terminated prematurely, four had increased RMC frequency during the day compared with none of those five patients with NS in whom no nocturnal data were acquired.

At night, the numbers of patients with RH who had an RMC frequency elevated above the normal range was 11 (52%) at +25 cm, 5 (24%) at +15 cm, and 4 (19%) at +10 cm. Only four patients with NS (20%) at +25 cm (p = 0.05 v RH)

Table 3 Results from the mixed model analysis of individual rectal motor complex parameters in control subjects (CS) and in those with rectal hypersensitivity (RH) and normal rectal sensation (NS)

Difference	Percentage change in geometric mean (95% CI)		
	AUC	Frequency	Duration
CS–RH	–11 (–23 to 4)	–14 (–22 to –5)**	14 (10 to 18)***
CS–NS	–3 (–30 to 36)	–2 (–22 to 24)	13 (9 to 18)***
RH–NS	9 (–7 to 28)	14 (3 to 27)*	–0.4 (–4 to 3)
Day v night	100 (86 to 115)***	99 (90 to 108)***	–11 (–14 to –8)***

No significant interaction effects between group and time were found.

AUC, area under the curve.

*p<0.05; **p<0.01; ***p<0.001.

For analysis according to level of recording, please refer to the Gut website at <http://www.gutjnl.com/supplemental>.

Table 4 Results from the mixed model analysis of individual rectal motor complex parameters in control subjects (CS) and in those with rectal hypersensitivity (RH) and normal rectal sensation (NS)

Difference	Percentage change in geometric mean (95% CI)	
	Amplitude	Periodicity
Day (CS–RH)	7 (–1 to 14)	93 (76 to 111)***
Day (CS–NS)	–3 (–14 to 10)	15 (3 to 29)*
Day (RH–NS)	–8 (–15 to –1)*	–40 (–46 to –35)***
Night (CS–RH)	–9 (–15 to –2)*	40 (28 to 53)***
Night (CS–NS)	–12 (–22 to –1)*	9 (–2 to 21)
Night (RH–NS)	–4 (–11 to 4)	–22 (–29 to –15)***
CS (day v night)	4 (–3 to 11)	23 (10 to 37)***
RH (day v night)	–11 (–15 to –7)***	–11 (–17 to –5)**
NS (day v night)	–7 (–13 to 0)	16 (4 to 30)***

Significant interaction effects between group and time was found.

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

For analysis according to level of recording, please refer to the Gut website at <http://www.gutjnl.com/supplemental>.

but none at either +15 cm ($p < 0.05$ v RH) or +10 cm ($p = 0.11$ v RH) had an abnormally elevated RMC frequency.

RMC variables

Various differences existed between the study groups with regard to four of the five RMC variables—namely periodicity, duration, contraction, frequency, and median contraction amplitude. These results are summarised in tables 3 and 4. For the principal study group (RH), RMC duration was significantly shorter than in controls (mean decrease in duration 13% (95% CI 9–18%); $p < 0.001$), contraction frequency was higher than in CS (mean increase in frequency 14% (5–22%); $p < 0.01$) or NS (mean increase in frequency 14% (3–27%); $p < 0.05$), and median contraction amplitude was lower during the day than in patients with NS (mean decrease in amplitude 9% (1–15%); $p < 0.05$) but higher at night in comparison with controls (mean increase in amplitude 9% (2–15%); $p < 0.05$). For RMC periodicity, results reflected those of RMC frequency (see above). For a full table of all data relating to RMC variables at the three recording levels for the three groups, please refer to the Gut website at <http://www.gutjnl.com/supplemental>.

Relationship between RMC variables and clinical history

There was no correlation between RMC frequency and bowel frequency, rectal compliance, or rectal sensation.

DISCUSSION

The findings of this study contribute to our knowledge of the pathophysiology of UFI, providing new information regarding the relationship between rectal sensory and motor function and symptomatology in this condition. We have identified that in a subset of patients with UFI, namely those with RH (as defined by “simple” balloon distension),⁵⁸ abnormalities of rectosigmoid motor physiology (reduced rectal compliance, increased high amplitude contractions, and altered cyclical contractile activity) exist that are not present in patients with UFI and normal rectal sensation. Such findings may underlie the clinical severity observed in the RH group, including more frequent sensations of urgency and increased bowel frequency, which is in agreement with a recent audit study of over 250 patients with this condition.⁵

Psychological assessment

It is well documented that increased stress can influence colonic motility,⁵² and thus an elevated state of anxiety could contribute to the increased reporting of urgency in patients with RH. However, in the current study, there were no

differences in the proportions of patients with either RH or NS that suffered from anxiety or depression. RH has been considered to be a marker of IBS⁵⁸ but based on the Rome II criteria,⁴⁴ none of the patients in the present study could be diagnosed as having IBS.

Rectal sensation and anal sphincter function

The definition of RH was based on data from healthy subjects whose ages were significantly younger than those of the study population. It has been shown that rectal sensory thresholds may increase with age,⁵⁹ and therefore there is little risk that patients classified as RH were misclassified in the current study. However, if the control group were older, some patients with NS may have been reclassified as RH. Studies of HAC and RMC activity, stratified by age, are lacking but differences in ages between the control and patient groups may constitute a limitation of this study.

Nearly all patients with UFI were noted to have abnormal EAS function, irrespective of rectal sensory thresholds, supporting the observation that EAS dysfunction is the major pathophysiological factor in this condition.^{5–7} In contrast, 50% of patients with RH had evidence of IAS disruption, three times higher than in patients with NS. Although the proportions of patients with a functionally poor IAS (as reflected by reduced anal resting tone) were similar between groups, recording solely maximum resting tone does not reflect “global” IAS function. The presence of a weakened unstable IAS may lead to an increased number of internal sphincter relaxations⁶⁰ and spontaneous anorectal sampling reflexes,⁶¹ so producing more frequent sensations of urgency.⁶⁰ The IAS is a direct continuation of the circular muscle layer of the rectum and thereby provides a possible link between anal and rectal sensorimotor mechanisms. Such a hypothesis remains to be fully explored.

Rectal sensation and compliance

Although not in accordance with guidelines on standardised practice,⁶² we elected not to administer a rectal enema prior to assessment of compliance on the basis that such action may well disturb the “normal” physiological sensorimotor activity of the rectum, especially in the RH group; indeed, all patients at sigmoidoscopy had essentially empty rectums. As a group, patients with UFI and RH have reduced compliance compared with patients with UFI and NS, with 41% having compliance below the normal range. There was no correlation, however, between compliance and bowel frequency in RH.

That some patients with RH had normal compliance (and normal rectosigmoid contractile activity) suggests that, in a proportion, hypersensitivity may truly involve abnormalities of visceral afferent mechanisms.⁶³ This is supported by the observation that RH patients exhibited enhanced perception of rectal and sigmoid motor events compared with NS and CS, and also from immunohistochemical studies which have suggested the possibility of sensitisation of peripheral mechanisms.^{64–65}

In addition to demonstrating reduced compliance in a proportion of patients with UFI, the finding of compliance greater than normal (that is, hypercompliance) in three patients from the NS group and one patient from the RH group may be a clue to the symptoms of rectal evacuatory difficulty concomitantly reported by some patients with UFI,^{4–66} and following continence restoring procedures.^{8–67}

Prolonged rectosigmoid manometry

Methods

Prolonged manometric recording of enteric phasic contractile activity is becoming an increasingly recognised clinical tool, especially in the evaluation of upper gastrointestinal

function.^{37–38} In this study, the absence of bowel preparation, and a fully ambulant subject, in whom extrinsic influences were minimised, meant that as close to normal physiological conditions were met. In addition, the use of semi- and fully automated quantitative methods of analysis has been shown to be superior in reliability and reproducibility than manual visual analysis of recordings.⁵³ The computer algorithm overcomes not only problems of variable baseline and sudden changes in pressure due to body movements but also permits objectivity and eliminates observer bias.⁵³ Furthermore, appropriate statistical methodology was employed, taking into account the non-independent and repeated nature of the recorded data. One limitation of prolonged manometry is possible catheter migration. However, maintenance of catheter position was confirmed radiologically in four subjects, and since results were similar at the three levels measured, this issue would not seem of great concern in respect of conclusions which may be drawn.

Symptoms

Not only was the frequency of urgency greater in patients with RH but there was a significantly stronger association between rectosigmoid motor events (notably HACs) and symptoms. This suggests that patients with RH may have enhanced perception of gut stimuli. Hypervigilance to intestinal motor events has also been reported in a proportion of patients with IBS,^{68–69} in whom RH is a frequent finding.⁷⁰

Overall activity

The results of this study suggest that the hindgut of patients with RH is hypercontractile compared with those with NS and healthy volunteers. Overall median contraction amplitude, maximum contraction amplitude, and AUC were significantly greater in RH compared with NS, with the frequency of HACs proportionally double in RH compared with NS. High amplitude propagated contractions (because of the short study segment used in the current recordings the term “propagation” was omitted from the description) are the motor correlate of mass intraluminal movement,^{19–26–54} and the urge to defecate.^{22–27} It is possible that exaggerated HAC activity may underlie symptomatology (for example, increased bowel frequency, secondary to more rapid colonic transit) in patients with UFI and RH, as has been suggested in patients with IBS.⁷¹ It must be stated however that in the present study, recording of pressure activity was confined to the rectosigmoid, and thus abnormal “colonic” motility or transit was not assessed. Assessment of “pancolonic” motor activity might provide more information as to whether the changes observed are confined to the distal hindgut or are more generalised.

A methodological concern regarding manometric recording is that recorded amplitudes are dependent on the luminal diameter of the viscus under study²⁵; the above observations therefore may be artefactual in that a less compliant (as in the RH group) and/or narrower calibre rectum would give higher recorded values than a more compliant greater diameter rectum (or one which contains a significant volume of stool).⁷² Prolonged recordings of tonic and phasic activity using the barostat may be considered to overcome this limitation.²⁵

RMC activity

The most striking finding of this study was that RMC activity, notably frequency, was significantly higher in RH compared with NS and controls. Such observations should be independent of the methodological concerns described above. Fifty two per cent of patients with RH had a frequency of RMCs exceeding the upper limit of normal.^{23–30–31} The function and origin of the RMC is still incompletely understood but it has

been reported to be triggered by propagating pressure waves from the proximal colon.^{20–32} It has been further suggested that RMC activity occurs in response to the arrival of stool or gas from the colon. Whether RMCs themselves truly propagate is the subject of debate. RMCs have been observed to move predominantly in a retrograde direction, with less than 5% propagating aborally.³² It is postulated that this would act as a “braking mechanism” to untimely flow of colonic contents³² and so keep the rectum empty.^{27–31} The increased RMC activity seen in patients with RH may represent a form of protective mechanism to increased flow of colonic contents and/or merely reflect proximal colonic dysmotility. RMCs have also been suggested to be involved in the stimulus to defecate,³⁰ and this may contribute to increased urgency in those RH patients who exhibited overall increased RMC frequency. If the RMC is indeed to be considered a marker of enteric neuromotor function, the abnormalities of various RMC variables demonstrated in this study, using appropriate statistical analyses, suggest a true intrinsic hindgut dysmotility, at least in a proportion of patients with UFI and RH.

In healthy subjects, rectal pressure is thought to increase during a RMC contraction.⁷³ This is invariably accompanied by a rise in anal sphincter tone so that anal canal pressure is always greater than rectal pressure, thus maintaining continence.⁷³ In UFI patients with RH, who have compromised anal sphincter function, the more frequent rises in rectal pressure which may occur as a result of increased RMC activity could afford some explanation as to the greater number of episodes of urgency experienced by these patients.⁵

Clinical implications

The importance of this study is identification of a distinct pathophysiological subgroup of patients with UFI who exhibit exaggerated symptomatology, which may have important clinical implications for the management of UFI. It is becoming increasingly recognised that advances in the understanding of pathophysiology, rather than taxonomy based on symptoms alone, remain key to improved management.⁷⁴ In patients with UFI and RH, therapeutic options may include amitriptyline, which has previously been reported to reduce RMC frequency,²⁴ sacral nerve stimulation, which has been shown to reduce urgency,⁷⁵ and the recently described surgical technique of rectal “augmentation”.⁸ By contrast, bowel retraining would seem to be less efficacious in the presence of RH.⁷⁶

In conclusion, this study has attempted to evaluate the relations of rectal sensation and rectal motor activity in patients with UFI through comparison of three homogenous study groups. Only patients with RH have reduced compliance and rectosigmoid dysmotility, as well as enhanced perception of rectal and sigmoid motor events which may be peripherally⁶⁴ or centrally mediated.⁷⁷ This sensory and motor disturbance appears to contribute to the symptomatology in such patients. Demonstration of extrasphincteric dysfunction in approximately 50% of all patients with UFI, in whom traditionally solely an anal pathology has been implicated and addressed, cannot be ignored. The association between RH and internal anal sphincter defects warrants further investigation. Finally, this study may have identified a clinical utility for prolonged rectosigmoid manometry in patients with UFI, in whom optimum management may involve more than simple repair of an anatomically disrupted anal sphincter. Further insight may be gained by evaluation of colonic function proximal to the rectosigmoid. Consideration to such comprehensive investigation should be given to patients presenting with UFI, in whom rectal hypersensitivity is demonstrable.

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Authors' affiliations

C L H Chan, P J Lunniss, N S Williams, S M Scott, Centre for Academic Surgery, Barts and the London, Queen Mary's School of Medicine and Dentistry, London, UK

D Wang, Medical Statistics Unit, London School of Hygiene and Tropical Medicine, London, UK

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EDITOR'S QUIZ: GI SNAPSHOTS.....

Robin Spiller, Editor

An unusual case of hepatosplenomegaly

Clinical presentation

A 21 year old Asian female presented to our clinic with impaired renal function (creatinine level 217 $\mu\text{mol/l}$). There was no history of note. Physical examination elicited hepatosplenomegaly. There were no other positive findings. Urine analysis was unremarkable. Laboratory investigations demonstrated a microcytic anaemia, with depressed total white cell and platelet counts, consistent with hypersplenism. Normal synthetic liver function was noted. Alkaline phosphatase levels were raised (401 iu/l).

Past medical notes (initially not available) described hepatosplenomegaly since birth, first noted in Pakistan. Liver biopsy performed in the UK at the age of two years demonstrated increased collagen deposition around the bile ducts. The patient had been lost to follow up.

Abdominal ultrasound demonstrated normal sized kidneys with hepatosplenomegaly (normal liver echotexture) and a dilated extrahepatic common bile duct (1.1 cm). The gall bladder appeared normal with no calculi. Computed tomography of the abdomen confirmed these findings.

A magnetic resonance cholangiopancreatography scan was performed (fig 1).

Question

What do these scans show and what is the unifying diagnosis?

See page 1331 for answer

This case is submitted by:

D Joshi, J Dunga, A James, M M Yaqoob

Department of Renal Medicine and Transplantation, Barts and The London NHS Trust, London, UK

Figure 1 Magnetic resonance cholangiopancreatography scan of the abdomen (A) and intrahepatic biliary ducts (B).

Correspondence to: Dr M M Yaqoob, Department of Renal Medicine and Transplantation, Barts and The London NHS Trust, Whitechapel, London E1 1BB, UK; m.m.yaqoob@qmul.ac.uk

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PostScript

LETTERS

Plexiform neurofibroma mimicking a pancreatic cystic tumour

Pancreatic neurogenic tumours are extremely rare.¹⁻³ Among benign neurogenic tumours, schwannoma is more frequently encountered.^{2,3} We report here the case of a plexiform neurofibroma, a type of neurogenic tumour in the pancreas, to our knowledge previously unreported.

History

A 44 year old Caucasian female patient was hospitalised for epigastric and right abdominal pain lasting for seven months. Abdominal ultrasound and computed tomography showed a cystic lesion located in the superior and anterior part of the pancreatic isthmus, with a maximal diameter of 3.5 cm (fig 1A, B). T2 magnetic resonance imaging demonstrated a trilobar cystic lesion with strong hyperintensity (fig 1B); no communication with the main pancreatic duct was noted at magnetic resonance cholangiopancreatography (fig 1C). Endoscopic ultrasound (EUS) showed a cystic lesion containing heterogeneous fluid (fig 1D). EUS guided fine needle aspiration provided mucoid fluid with no epithelial cells. Fluid pancreatic enzyme concentrations were 423 and 1204 U/l for amylase and lipase, respectively, while CEA, CA 19.9, and CA 72.4 were 17 ng/ml, 9 U/ml, and 140 U/ml, respectively. Despite the low CA 19.9 concentration and lack of mucinous cells in cystic fluid, other findings were consistent with a diagnosis of mucinous cystadenoma. Surgical exploration confirmed a cystic lesion of the superior part of the pancreatic isthmus, distant from the main pancreatic duct (fig 1A, B). Tumour enucleation was performed. On macroscopy there was a well delineated, trilobated, translucent mass, measuring 3.5 cm (fig 1E). The tumour consisted of aggregates of benign spindle cells embedded in a fibrillar matrix (fig 1F). These aggregates formed a thin rim around a large central low cellular zone of oedema and myxoid degeneration. The tumour cells expressed neurofilaments and S100 protein on immunohistochemistry. P53 immunostaining was negative and sparse nuclei were Ki67 positive. These features were consistent with a benign plexiform neurofibroma (PNF). No neurofibromatosis related lesions were found and no mutation of the *NF1* (neurofibromatosis 1) gene was identified on analysis of DNA both from blood lymphocytes and tumour tissue. At follow up, two years after surgical resection, the patient did not present with any complaints and there was no evidence of pancreatic lesions.

Discussion

The presence of PNF in the pancreas has several clinical implications, as indicated by the present case. Firstly, PNF may mimic a pancreatic cyst, as was hypothesised in this case before surgery. The cystic appearance of

neurogenic tumours is frequently encountered, with intratumoral oedematous and myxoid changes probably being the under-

lying lesions.⁴ A bright appearance on T2 weighted magnetic resonance images is a characteristic of PNF.⁵ Secondly, surgical

Figure 1 (A) Enhanced computed tomography. Hypoattenuating round lesion located in the anterior part of the pancreatic isthmus (arrow). (B) The caudal part of the main lesion is encased in the isthmus of the pancreas (white arrow). The pancreatic parenchyma is normal upstream (black arrow). The main portal vein is normal, distant from the lesion (arrowhead). (C) T2 magnetic resonance imaging. The lesion is strongly hyperintense as cysts; another similar lesion was seen on the right side (arrows). (D) Magnetic resonance cholangiopancreatography with thick slice. The two lesions are well visible, with a third one indicated (arrows). The main pancreatic duct is normal (arrowhead) with no obvious communication with the lesions. (E) Endoscopic ultrasound. Anechoic cystic lesion without defined cyst wall or mural nodule (arrow). (F) Surgical specimen consisted of a bilobated, firm, translucent, well delineated mass. (G) On microscopy, at low magnification, the lesion was heterogeneous with a solid cellular part (arrows) and a central oedematous acellular zone (*), giving the pseudocystic aspect of the lesion (haematoxylin and eosin stain, magnification $\times 10$). (H) At high magnification, the solid part of the lesion was composed of a regular spindle cell proliferation. Intratumoral vessels showed a thin fine wall (arrow) (magnification $\times 40$).

resection was necessary to exclude malignancy which is more frequently encountered in PNF compared with classical neurofibromas.² In addition to classical benign features, similar to published data on benign PNF,^{6,7} a high cell proliferation and p53 protein expression were absent in our case. Thirdly, PNF is a morphological variant of neurofibroma, generally considered pathognomic for an NF1 syndrome.⁸ When diagnosed in adult patients, it is frequently a solitary tumour and is considered a mosaic located form of NF1 syndrome.⁹ The absence of detectable genetic abnormalities and other clinical NF1 syndrome associated lesions in the present case could be explained by such a mechanism.⁹ For these patients, there is a low risk of developing other diseases associated with NF1 syndrome.

In conclusion, we have reported an uncommon case of PNF, unique in its pancreatic location. Intratumoral myxoid and oedematous changes that develop in this type of neurofibroma give a cystic appearance which may lead to a misdiagnosis of a pancreatic cyst. Such lesions should be added to the list of benign pancreatic tumours with a cystic appearance.

A Handra-Luca

Department of Pathology, Jean Verdier and Beaujon Hospitals, Assistance Publique-Hôpitaux de Paris, France

D Vidaud

Department of Biochemistry, Beaujon Hospital, Assistance Publique-Hôpitaux de Paris, France

M-P Vullierme

Department of Radiology, Beaujon Hospital, Assistance Publique-Hôpitaux de Paris, France

N Colnot

Department of Pathology, Beaujon Hospital, Assistance Publique-Hôpitaux de Paris, France

D Henin

Department of Pathology, Bichat-Claude Bernard Hospital, Assistance Publique-Hôpitaux Paris, France

P Ruszniewski

Department of Gastroenterology, Beaujon Hospital, Assistance Publique-Hôpitaux de Paris, France

P Bedossa, A Couvelard

Department of Pathology, Beaujon Hospital, Assistance Publique-Hôpitaux de Paris, France

Correspondence to: Dr A Handra-Luca, MD PhD, Service d'Anatomie Pathologique, Assistance Publique-Hôpitaux Paris, Hôpital Jean Verdier, Avenue du 14 Juillet, 93143 Bondy, France; adriana.handra-luca@jvr.ap-hop-paris.fr

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No genetic association between EPHX1 and Crohn's disease

In a case control study on the associations between functional genetic polymorphisms in biotransformation enzymes and Crohn's disease, we found a strong association between the Tyr113His (348T>C) polymorphism in exon 3 of the microsomal epoxide hydrolase (EPHX1) gene and Crohn's disease.¹ The three referees all agreed that the study was interesting and should be published so that other groups can attempt to replicate the results in independent study cohorts. This was done recently by Cuthbert and colleagues (*Gut* 2004;**53**:1386) who investigated 344 controls and 307 patients with Crohn's disease, and who were unable to reproduce our results. In addition, they reported that our data for the EPHX1 exon 3 polymorphism in the control group were not in Hardy-Weinberg equilibrium (HWE), as also noticed previously by Györfy and colleagues.² Our data on EPHX1 exon 3 genotyping were obtained by restricted fragment length polymorphism (RFLP) analyses by applying the method described by Lancaster and colleagues.³

However, recently it was reported that a silent substitution polymorphism (G to A) at codon 119 of the EPHX1 gene may exist, which may flaw the polymerase chain reaction (PCR) RFLP method applied by us, as the presence of this polymorphism may disturb proper binding of the reverse primer, covering the 119 G>A area, resulting in over-classification of His113 alleles.^{4,5} Therefore, we developed a dual colour allele specific discrimination assay for genotyping the polymorphism at codon 113 of the EPHX1 gene. EPHX1 genotypes were detected with the iCycler iQ Multicolour Real Time Detection System (Bio-Rad Laboratories, Veenendaal, the Netherlands) using molecular beacons. PCR was performed with the forward primer 5'-CAA CTC CAA CTA CCT GAA G-3' and the reverse primer 5'-TGA CAT ACA TCC CTC TCT G-3' in the presence of the FAM labelled wild-type beacon (5'-CGC GAT GAT TCT CAA CAG ATA CCC TCA CTT CAA TCG CG-3') and the HEX labelled mutant beacon (5'-CGC GAT ATT CTC AAC AGA CAC CCT CAC TTC AAT CGC G-3'). The 25 µl reaction mixture contained 200 ng of genomic DNA, 10 mM Tris/HCl (pH 9.0), 50 mM KCl, 0.1% Triton X-100, 4 mM MgCl₂, 0.25 mM dNTPs, 50 ng of each primer, 200 nM of each beacon, and 2.5 U Taq-DNA-polymerase. The PCR

conditions were three minutes at 95°C, then 40 cycles of 30 seconds at 95°C, 30 seconds at 59°C, and 30 seconds at 72°C. Fluorescent signals were measured at 59°C. Genotypes were assigned using the iCycler iQ Optical System, software version 3.1. At each PCR run (in 96 well plates) sterile H₂O instead of genomic DNA was added in several wells as a negative control for amplification. As the PCR-RFLP analyses were performed in the first half of 1999, only some of the samples were still available (125 of 149 controls and 149 of 151 cases) and these were re-evaluated by the iCycler method.

Genotype distribution of the EPHX1 Tyr113His polymorphism in patients with Crohn's disease and controls was now in HWE ($\chi^2 = 2.47$, $p = 0.12$ and $\chi^2 = 0.82$, $p = 0.37$, respectively) and genotype distribution was not significantly different between cases and controls ($\chi^2 = 3.5$, $p = 0.17$). The Tyr allele frequencies of 0.70 and 0.68 obtained for cases and controls, respectively, were very similar to the corresponding values of 0.71 and 0.70, as reported by Cuthbert *et al.*

Thus in answer to the question as posed by Cuthbert *et al.*: "Genetic association between EPHX1 and Crohn's disease: population stratification, genotyping error, or random chance?", we can conclude that a genotyping error was responsible for our earlier published association between the EPHX1 Tyr113His polymorphism and Crohn's disease.¹ We now clearly state that our revised data do not support an association between this EPHX1 polymorphism and Crohn's disease. Similar genotyping errors may also be present in several other studies on the EPHX1 exon 3 polymorphism in association with a variety of diseases, as many studies were based on methods using a reverse primer covering the "119 silent mutation area" of the EPHX1 gene.^{3,6–9} This may also have consequences for interpretation of results in the cited papers. However, a rapid literature search by Pubmed revealed more than 100 papers on EPHX1 polymorphisms over the past 10 years, suggesting that many more papers may deal with genotyping problems, as outlined above.

In addition, Cuthbert *et al.* also reported that another polymorphism tested in our study,¹ the CYP1A1 exon 7 Ile/Val polymorphism, was not in HWE in the control group. This is correct but this deviation from HWE may be attributed to random chance, due to the rarity of the Val allele in our population, which makes the χ^2 test inappropriate under such conditions. For instance, genotype distribution is in accordance with HWE when only two individuals less would have been classified as Val/Val homozygotes.

We thank Cuthbert *et al.* and Györfy and colleagues² for their interest in our work. In addition, we conclude that (interpretation of) data in many other published studies on the EPHX1 Tyr113His (exon 3) polymorphism should be critically re-evaluated.

W H M Peters, E M J van der Logt,
R H M te Morsche, H M J Roelofs,
D J de Jong, T H J Naber

Department of Gastroenterology, University Medical Centre Nijmegen, the Netherlands

Correspondence to: Dr W H M Peters, Department of Gastroenterology, University Medical Centre Nijmegen, PO Box 9101 Nijmegen, the Netherlands; w.peters@mdl.umcn.nl

Conflict of interest: None declared.

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Transcriptional downregulation of the lactase (LCT) gene during childhood

Adult-type hypolactasia, characterised by bloating, gas formation, and diarrhoea after ingestion of lactose containing food, affects half of the world's population.¹ The molecular background of lactase non-persistence/persistence trait has been shown to associate with a single nucleotide polymorphism (SNP) C/T₋₁₃₉₁₀ residing 13910 base pairs upstream from the 5' end of the lactase (LCT) gene in an intron of the minichromosome maintenance 6 (MCM6) gene.^{2–4} We have demonstrated a trimodal distribution of lactase activity in the intestinal mucosa in adults, with low lactase activity (4–9 U/g protein) in those with the C/C₋₁₃₉₁₀ genotype.³ The C₋₁₃₉₁₀ and T₋₁₃₉₁₀ allele show differential regulation of lactase promoter activity and binding capacity for the nuclear proteins in electromobility shift assay.^{3–6} Our recent analysis in a paediatric population demonstrated that the main time period for lactase downregulation in Finns and in Somalis is from five to 10 years of age.⁴

To further assess the role of the C₋₁₃₉₁₀ allele in downregulation of lactase activity during development, we isolated lactase RNA from intestinal biopsy samples with verified disaccharidase activities.⁷ The study group comprised 15 subjects aged 10 months to 23 years, 12 with the C/T₋₁₃₉₁₀ genotype and three with the C/C₋₁₃₉₁₀ genotype. All subjects were heterozygous for the G/A₄₅₉₃ polymorphism residing in exon 1 of the LCT gene. Relative expression of lactase mRNA transcribed from the C₋₁₃₉₁₀ and T₋₁₃₉₁₀ allele was assessed by quantitative minisequencing using the G/A₄₅₉₃ polymorphism on the LCT gene as a marker. The methods used

Figure 1 Relation of age to relative expression of lactase LCT mRNA from the C₋₁₃₉₁₀ compared with that from the T₋₁₃₉₁₀ allele. Actual relative expression of the C allele in the biopsy sample was obtained by relating the results of the minisequencing to an 11 point standard curve ($y = 0.0135x - 0.9714$ up to 82%; $y = 1 \times 10^{-5}e^{0.1129x}$ for 82–100%) obtained based on information of the relative amounts of G and A in a G/A₄₅₉₃ heterozygous genomic DNA sample (for details of methods, see Kuokkanen *et al*⁸).

are described in detail by Kuokkanen and colleagues.³ The study was approved by the ethics committee at the Helsinki University Central Hospital. All families gave their informed consent.

Subjects with the C/T₋₁₃₉₁₀ genotype (age range 10 months to 17 years) had high lactase activity, ranging from 21 to 113 U/g protein (mean activity 47 U/g protein; sample not available n=2) except for one child presenting with low lactase activity (6 U/g protein). In this case the indication for endoscopy was exclusion of gastro-oesophageal reflux. Due to the very low lactase activity and the C/T₋₁₃₉₁₀ genotype, mutations underlying congenital lactase deficiency (CLD)⁸ were screened for this patient and he was shown to be a carrier of a CLD mutation (unpublished data). Of the three subjects with the C/C₋₁₃₉₁₀ genotype, the oldest subject aged 23 years had low lactase activity (6 U/g protein) as expected; the five year old subject had high lactase activity (24 U/g protein). Lactase mRNA was transcribed in a 1:1 ratio from the C₋₁₃₉₁₀ and the T₋₁₃₉₁₀ allele in children younger than five years of age. In children over six years of age, relative lactase mRNA expression from the C₋₁₃₉₁₀ allele was reduced to 18% and 16% compared

with that from the T₋₁₃₉₁₀ allele (fig 1, table 1).

Our results show an increasing imbalance in relative mRNA expression levels of the C₋₁₃₉₁₀ and T₋₁₃₉₁₀ alleles in children aged >5 years. These results support the earlier findings on transcriptional regulation of the lactase gene^{9–10} and the finding in our own laboratory that the persistent T₋₁₃₉₁₀ allele was shown to represent a mean of 92% of expressed lactase mRNA in C/T₋₁₃₉₁₀ heterozygous adults.³ The decline in lactase mRNA expression transcribed from the C₋₁₃₉₁₀ allele in the intestinal mucosa occurs in parallel with the time period of the decline in lactase enzyme activity, indicating a causative role for the intronic region containing the C₋₁₃₉₁₀ allele. Characterisation of the transcriptional regulators at the C/T₋₁₃₉₁₀ enhancer element and the exact mechanism underlying C₋₁₃₉₁₀ allele specific downregulation of lactase activity awaits elucidation.

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H Räsänen

Department of Medical Genetics, University of Helsinki, Finland

M Kuokkanen

Department of Medical Genetics, University of Helsinki, Finland, and National Public Health Institute, Department of Molecular Medicine, Helsinki, Finland

K-L Kolho, H Lindahl

Hospital for Children and Adolescents, University of Helsinki, Finland

N S Enattah

Department of Medical Genetics, University of Helsinki, Finland, and National Public Health Institute, Department of Molecular Medicine, Helsinki, Finland

E Savilahti

Hospital for Children and Adolescents, University of Helsinki, Finland

Table 1 Lactase activity, L/S ratio, and allelic ratio of the study subjects

Age (y)	C/T ₋₁₃₉₁₀ genotype	Lactase activity (U/g protein)	L/S ratio	Allele ratio (%) C/T ₋₁₃₉₁₀ *
0.8	CT	85	1.11	48/52
1.1	CT	113	1.02	52/48
4.0	CT	31	0.49	48/52
4.3	CT	53	0.48	42/58
4.7	CT	40	0.62	40/60
4.9	CT	–	–	42/59
5.1	CT	6	0.08	48/52†
6.7	CT	22	0.28	18/82
7.6	CT	–	–	16/84
11.1	CT	29	0.54	13/87
14.9	CT	21	0.40	17/83
17.0	CT	29	0.62	24/76
1.1	CC	–	–	52/48
5.0	CC	24	0.28	51/49
22.8	CC	6	0.08	49/51

*Defined by assessing cSNP G/A₄₅₉₃ in exon 1 of the lactase LCT gene.

†Carrier of a CLD mutation (unpublished data).

A Orpana

Department of Medical Genetics, University of Helsinki, Finland, Helsinki University Central Hospital, Laboratory of Molecular Genetics, Helsinki, Finland, and Department of Clinical Chemistry, University of Helsinki, Finland

I Järvelä

Department of Medical Genetics, University of Helsinki, Finland, and Helsinki University Central Hospital, Laboratory of Molecular Genetics, Helsinki, Finland

Correspondence to: Dr I Järvelä, Helsinki University Central Hospital, Laboratory of Molecular Genetics, Haartmaninkatu 2, PO Box 140, FIN-00290 Helsinki, Finland; irma.jarvela@hus.fi

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Cystic fibrosis transmembrane regulator gene carrier status is a risk factor for young onset pancreatic adenocarcinoma

Pancreatic adenocarcinoma is the fourth leading cause of cancer death in the USA. Although predominantly a cancer of the elderly, approximately 20% of patients are diagnosed under the age of 60 years. Younger patients are likely the best candidates for early surgical intervention, and patients at risk for young onset cancer comprise a logical focus for screening or prevention.

Carriers of mutations in the gene that encodes the cystic fibrosis transmembrane conductance regulator (*CFTR*) are associated with chronic idiopathic pancreatitis.¹ Chronic pancreatitis, in turn, increases the risk for pancreatic cancer by 26-fold.² Therefore, we hypothesised that mutations in *CFTR* may confer a higher risk of pancreatic cancer.

From October 2000 to April 2004, pancreatic cancer patients seen at the Mayo Clinic were ultra rapidly recruited to our study, with more than 75% of all such patients seen at the Mayo Clinic enrolled in the registry. This

Table 1 Comparison of *CFTR* mutation frequencies detected in the young onset pancreatic cancer cohort versus the clinical database

	Young onset pancreatic cancer cases (<60 y old at diagnosis, n = 166)		Mayo Clinic clinical database reference group (n = 5349)	
	No	%	No	%
<i>CFTR</i> mutation non-carriers	152	91.6	5132	95.9
<i>CFTR</i> mutation carriers	14	8.4	217	4.1
Mutation distribution				
ΔF508	12	85.7	155	71.4
R177H	1	7.1	28	12.9
G551D			6	2.8
2789+5G>A			6	2.8
G542X			4	1.8
N1303K	1	7.1	3	1.4
1717-1G>T			2	0.9
3849+10kbC>T			2	0.9
A455E			2	0.9
R1162X			2	0.9
R347H			1	0.5
R553X			1	0.5
3905insT			1	0.5
621+1G>T			1	0.5
W1282X			1	0.5
1898+1G>A			1	0.5
R560T			1	0.5

Young onset pancreatic cancer cases were more frequent carriers of the *CFTR* mutations compared with patients in the control database (odds ratio 2.18 (95% confidence interval 1.24–3.29); $p = 0.006$).

represents a substantial improvement over population based pancreatic cancer epidemiological studies, with participation rates ranging from 34.6%³ to 45.6%.⁴ Informed written consent and institutional review board approval were obtained.

As a pilot study, 33 patients were selected in whom a pathological diagnosis of pancreatitis was also noted at the time of pancreatic cancer surgery. The patients ranged in age from 41 to 81 years (median 65), and seven of the 33 had a diagnosis of pancreatitis made at least one year prior to cancer diagnosis. These patients were screened for variants in *CFTR* using the Tag-It Mutation Detection Kit, a clinically available kit testing for 40 mutations.

Of 33 samples tested, two patients (6%) were noted to have mutations in *CFTR*, both of which were the most common mutation identified in the *CFTR* gene, ΔF508. Both patients had young onset disease (ages 42 and 50 years). In total, seven patients in our pilot sample were below the age of 60 years, making the carrier rate 29% in this young onset subgroup.

Therefore, we designed a larger study to test the remainder of young onset cases in our registry, comprising a sequential unselected sample for mutations in *CFTR* (Cystic Fibrosis v3.0 ASR, Celera/Abbott), totalling 166 patients under the age of 60 years. Smoking status and family history were obtained from questionnaires. Personal history of chronic pancreatitis was identified by a single physician review of the medical records.

For a comparison group, the clinical database of *CFTR* analyses performed at the Mayo Clinic from November 2003 to May 2004 was utilised. Ethnic composition of cases and controls were highly comparable.

As shown in table 1, 14 of the 166 (8.4%) young onset pancreatic cancer cases were carriers for *CFTR* mutations, compared with 217 of 5349 (4.1%) patients in our control database ($p = 0.006$, odds ratio 2.18 (95% confidence interval 1.24–3.29)). There was no

significant difference in age of onset, pancreatitis, family history of pancreatic cancer, or smoking in carriers versus non-carriers of *CFTR* mutations.

Several cases of patients with cystic fibrosis (CF) and pancreatic adenocarcinoma have been reported,^{5–6} and two cohort studies have shown an increased risk for pancreatic cancer among CF homozygotes.^{7–8} Two studies have investigated *CFTR* mutation frequencies in pancreatic cancer patients, with negative results. However, both series only investigated one mutation (ΔF508), and neither focused on young onset patients.^{9–10}

Our study represents the first positive association of pancreatic cancer risk with *CFTR* carrier status, with mutations conferring a twofold risk for cancer before the age of 60 years. The finding that only one of the *CFTR* carriers had an antecedent history of pancreatitis is intriguing, as either pancreatitis is subclinical or the presence of one mutant *CFTR* allele may increase the risk for pancreatic cancer through a mechanism independent of chronic pancreatitis. A larger study to confirm these results is ongoing.

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R McWilliams

Department of Oncology and Department of Medicine, Mayo Clinic, Rochester, Minnesota, USA

W E Highsmith

Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota, USA

K G Rabe, M de Andrade, L A Tordson

Department of Health Sciences Research, Mayo Clinic, Rochester, Minnesota, USA

L M Holtegaard

Department of Laboratory Medicine and Pathology,
Mayo Clinic, Rochester, Minnesota, USA

G M Petersen

Department of Medicine, Department of Health
Sciences Research, and Department of Medical
Genetics, Mayo Clinic, Rochester, Minnesota, USA

Correspondence to: Dr R McWilliams, Department of
Medicine, Mayo Clinic, Gonda 10, 200 First St, SW,
Rochester, MN 55905, USA;
mcwilliams.robert@mayo.edu

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Distal intestinal obstruction syndrome in the early postoperative period after lung transplantation in a patient with cystic fibrosis: morphological findings on computed tomography

Distal intestinal obstruction syndrome (DIOS) occurs in 15.9% of adults with cystic fibrosis (CF).¹ Usually the diagnosis is based on history, physical examination, and plain abdominal roentgenogram. The increased risk of gastrointestinal complications such as DIOS is well known after lung transplantation.^{2–3} Due to the added risk of gastrointestinal surgery in the postoperative period⁴ and the generally good response to conservative treatment, it is necessary to distinguish DIOS from other gastrointestinal complications. Nevertheless, descriptions of computed tomographical patterns of DIOS in the international literature are rare.⁵

We present the case of a 34 year old male suffering from end stage CF. Because of gastrointestinal manifestations of CF, the patient had exocrine pancreas insufficiency. As a consequence of deterioration in respira-

Figure 1 (A–C) Abdominal plain film showing remarkably little abdominal gas and poor delineation of the abdominal organs. Markedly distended small bowel loops and right hemicolon (white arrow) completely filled with a homogenous mass. Swelling of the intestinal wall with increased contrast medium enhancement. Thin transverse and descending colon with only few faeces (black arrows).

tory function, lung transplantation was performed. Despite enzymatic and propulsive medical treatment the patient developed an acute abdomen during the postoperative period. To determine the cause of his symptoms abdominal radiographs and computed tomography were performed. Abdominal plain films showed remarkably little abdominal gas and poor delineation of the abdominal organs (fig 1A). Contrast enhanced computed tomography showed massive dilatation of the small bowel and proximal colon with marked swelling of the intestinal wall (fig 1B, C). The lumen of the small intestine and proximal part of the ascending colon were filled with a homogeneous mass (fig 1B) with increasing roentgen opacity from the duodenum (approximately 19 Hounsfield units (HU)) to the right hemicolon (approximately 39 HU). Isolated air fluid levels were seen in the small bowel. The transverse, descending, and sigmoid colon were thin with only little faeces. There was no evidence of external compression. Based on these findings a diagnosis of DIOS was made. Laparotomy, performed due to failure of medical treatment, confirmed the diagnosis.

DIOS is unique to patients with cystic fibrosis. Intestinal obstruction developed due to accumulation of highly viscous mucofaeculent material in the terminal ileum and right hemicolon. Pancreatic insufficiency is a prerequisite for DIOS but is not its only pathophysiological cause. Other factors such as reduced intestinal water content, lower luminal acidity of the foregut, accumulation of intraluminal macromolecules, dehydration of the mucus layer due to altered intestinal secretion, and slow intestinal transit contribute to the development of DIOS.^{1–6}

Plain films are only of limited value in differentiating DIOS from other causes of acute abdomen. In the case of DIOS, they usually show typical signs of a small bowel ileus but other frequent reasons for ileus in patients with CF (for example, adhesions,³ intussusception,⁷ paralytic ileus due to perforated appendicitis,⁸ or Crohn's disease⁹) cannot be excluded without further investigation. In our case, abdominal plain films showed no typical signs of small bowel ileus but little abdominal gas with poor delineation of the abdominal organs leading to the differential diagnoses of ascites, colitis, mesenteric infarction, and proximal bowel obstruction. In contrast with the plain abdominal radiograph, computed tomography showed the criteria of DIOS. The small bowel was completely filled

with a homogenous mass with increasing roentgen opacity from the duodenum (approximately 19 HU) to the right hemicolon (approximately 39 HU), suggesting increasing viscosity of the intestinal content due to water absorption. In accordance with previous descriptions of DIOS,³ obstruction occurred in the right hemicolon.

Our case showed that abdominal plain films, as used in previous studies, are not adequate for the diagnosis of DIOS. Computed tomography can reveal the characteristic signs of DIOS and exclude inherent differential diagnoses. We have demonstrated for the first time that DIOS causes increasing opacity of intestinal contents during small intestinal passage, suggesting increasing viscosity.

K Nassenstein, B Schweiger

Department of Diagnostic and Interventional
Radiology and Neuroradiology, University Hospital
Essen, Germany

M Kamler

Department of Cardiothoracic Surgery, University
Hospital Essen, Germany

J Stattauss, T Lauenstein, J Barkhausen

Department of Diagnostic and Interventional
Radiology and Neuroradiology, University Hospital
Essen, Germany

Correspondence to: Dr K Nassenstein, Department for
Diagnostic and Interventional Radiology and
Neuroradiology, University Hospital Essen,
Hufelandstrasse 55, D-45122 Essen, Germany;
kai.nassenstein@uni-essen.de

doi: 10.1136/gut.2005.075994

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Association of a new cationic trypsinogen gene mutation (V39A) with chronic pancreatitis in an Italian family

Predisposition to hereditary pancreatitis has been associated with mutations in three genes: protease, serine, 1 (PRSS1), which codes for cationic trypsinogen,¹ cystic fibrosis transmembrane conductance regulator (CFTR),² and serine protease inhibitor Kazal type 1 (SPINK1).³

We have identified a novel PRSS1 mutation in seven subjects with chronic pancreatitis (CP) from three generations of an Italian family. The index patient was a 57 year old man with CP referred to our hospital for ductal adenocarcinoma of the pancreatic head. Eleven relatives were examined, and an uncle, also with CP, had died in an accident.

Congenital malformations and alcoholic, biliary, obstructive, and autoimmune pancreatitis were ruled out. Eleven subjects gave their written consent to the study.

The cystic fibrosis assay (CF-OLA; Applied Biosystems, California, USA) was used to look for 31 frequent CFTR mutations in all subjects. The five exons of the PRSS1 gene were sequenced with the oligonucleotides described by Nishimori and colleagues.⁴ The four SPINK1 exons were investigated by

denaturant gradient gel electrophoresis (DGGE). No CFTR or SPINK1 mutations were found although subject III-8 (with CP) carried the N1303K mutation in heterozygosis in the cystic fibrosis gene.

The PRSS1 exon 2 sequence of the index patient revealed a T>C change at nucleotide 116 (c.116 T>C) causing a valine to alanine substitution at codon 39 (V39A). This mutation was present in another six subjects with CP, diagnosed from exocrine insufficiency and computer tomography and magnetic resonance imaging demonstrations of typical ductal alterations and parenchymal calcifications. Two of these patients were also diabetic. In a further two patients, the genetic analysis was not performed, but CP was confirmed by clinical and morphological findings. The remaining four subjects had a normal pancreas and did not carry the V39A mutation (fig 1).

The lod score calculated for the association between V39A and CP was $z = 3.0$ at $\theta = 0.0$. This mutation was not found in a DGGE investigation of 130 patients with sporadic CP.

Mean age of the patients was 47.22 (± 13.64) years (median 54 (range 25-60)). Mean age at onset was 30.0 (± 7.35) years (median 32 (range 19-40)) whereas in patients displaying other PRSS1 mutations, onset was typically during childhood or adolescence.^{5,6}

An acute attack requiring hospitalisation formed the clinical overtone in six of the nine CP patients. The other three (III-4, III-5 and IV-2) presented morphological and functional evidence of CP at the time of the study but were asymptomatic. It is clear therefore that damage to the pancreas may occur prior to the clinical onset of CP.

In hereditary CP, the mechanism of the R122H mutation has been elucidated.¹ This substitution removes a hydrolysis start site and makes both trypsin and trypsinogen autolysis resistant. A similar mechanism has been proposed for the N29I mutation which alters protein conformation and masks the R122 site.⁷

Valine 39 is evolutionarily conserved in the trypsinogen gene of all terrestrial vertebrates⁸ and would thus seem of importance in the protein's structure and function. As V39 is only 10 amino acids distant from N29, its replacement by alanine may result in abnormal conformation of the peptide and mask arginine 122 against enzymatic degradation. Further work is needed to define the mechanism and confirm this interpretation.

In conclusion, the presence of the V39A mutation in seven of the CP patients, its absence in their healthy relatives, the 3.0 lod score, and the strong evolutionary conservation of V39, all indicate that the novel mutation is the cause of CP in this family.

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C Arduino

SC Genetica Medica, ASOS Giovanni Battista, Torino, Italy

P Salacoe

SC Gastroenterologia, ASO San Luigi Gonzaga, Orbassano (TO), Italy

B Pasini, A Brusco

Università di Torino, Dipartimento di Genetica, Biologia e Biochimica, Torino, Italy

Figure 1 Pedigree showing the age of subjects, and for those with pancreatitis (black symbols) their age at onset (where known). WT, wild-type (that is, subjects without pancreatitis and without the V39A mutation); black triangle, index patient; ?, no clinical or genetic data available.

P Salmin

SC Genetica Medica, ASOS Giovanni Battista, Torino, Italy

E Bacillo

SC Gastroenterologia, ASO San Luigi Gonzaga, Orbassano (TO), Italy

A Robecchi, L Cestino

Università di Torino, Dipartimento di Discipline Cliniche e Chirurgiche, Chirurgia 5, ASOS Giovanni Battista, Torino, Italy

S Cirillo, D Regge

SC Radiologia, IRCC, Candiolo (TO), Italy

N Cappello

Università di Torino, Dipartimento di Genetica, Biologia e Biochimica, Torino, Italy

E Gaia

SC Gastroenterologia, ASO San Luigi Gonzaga, Orbassano (TO), Italy

Correspondence to: Dr E Gaia, Gastroenterology Unit, S Luigi Gonzaga Hospital, Regione Gonzole 10, 10043 Orbassano, Italy; eziogaia@iscali.it

doi: 10.1136/gut.2004.062992

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ITPA genotyping is not predictive for the development of side effects in AZA treated inflammatory bowel disease patients

We read with interest the letter by Colombel *et al* on the non-predictive value of ITPA genotyping for the development of myelosuppression after azathioprine (AZA) treatment (*Gut* 2005;54:565).

The level of thiopurine methyltransferase (TPMT) activity is determined by a common genetic polymorphism.¹ It was shown that low TPMT activity is linked to a higher relative risk of development of myelosuppression after AZA treatment.² Testing for TPMT genotype before the start of AZA treatment is of limited clinical value as myelosuppression resulting from TPMT mutations occurs in less than one third of patients with myelosuppression.³

Polymorphisms in genes encoding inosine triphosphate pyrophosphatase (ITPase), another enzyme involved in metabolism of AZA, have also been suggested to be associated with the development of side effects in AZA treatment.⁴ Colombel *et al* show that there was no difference in the frequency of ITPA polymorphisms in 41 patients who developed AZA related myelosuppression in comparison with a previously published control population.⁵ Unfortunately, this still leaves the question of other side effects such as flu-like symptoms, rash, and pancreatitis unanswered. In addition to the TPMT genotype, we determined the 94C>A ITPA polymorphism. All (109) patients with inflammatory bowel disease who started AZA treatment from January 2003 onwards were included, and side effects were determined. There was a mean follow up time of 13 months (range 4–24). The frequency of side effects was compared with the frequency of side effects in AZA treated patients without any (ITPA or TPMT) polymorphism. Notably, for patients with a heterogeneous TPMT or ITPA polymorphism, no preventive adjustments of AZA dosing were made.

In a patient group of a total of 109 patients, we found 10 who had a TPMT polymorphism and 12 who had a 94 C>A ITPA polymorphism. Eighty eight patients had none of the studied polymorphisms in TPMT or ITPA genes. Of the 12 patients who had an ITPA heterozygous polymorphism only two had side effects (17%). One had a rash and the other had complaints of arthralgia. In patients without any of the investigated

polymorphisms, 34 of 88 (39%) had side effects (summarised in table 1). There was one patient, receiving a normal dose of AZA, who had both a TPMT*3A and an ITPA 94 C>A heterozygous polymorphism. Interestingly, this patient did not develop any side effects.

Our data confirms the results of Colombel's research by showing that an ITPA heterozygous polymorphism is not associated with an increased risk for the development of leucopenia. Additionally, we also found that there was no increased risk for the development of other side effects.

No conclusions can be drawn for patients who are homozygous for the ITPA 94 C>A polymorphism as none was included either in our study or in Colombel's. Marinaki *et al* included three patients with a homozygous 94 C>A polymorphism for ITPA and all three had side effects.⁴ Therefore, further research on the risk of developing side effects in homozygous 94 C>A ITPA patients is desirable.

J M van Dieren, A J van Vuuren, J G Kusters, E E S Nieuwenhuis, E J Kuipers, C J van der Woude

Erasmus MC, University Medical Centre Rotterdam, the Netherlands

Correspondence to: Ms J M van Dieren; Erasmus MC, University Medical Centre Rotterdam, Dr Molewaterplein 40 room, Ba 393 Rotterdam, the Netherlands; j.vandieren@erasmusmc.nl

Conflict of interest: None declared.

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Table 1 Side effects in 109 azathioprine treated inflammatory bowel disease patients related to their thiopurine methyltransferase (TPMT) and inosine triphosphate pyrophosphatase (ITPA) genotypes

Side effect	No polymorphisms (88 of 109)	TPMT polymorphisms (10/109)				ITPA polymorphisms (12 of 109)	
		*1/*3A	*1/*3C	*3A/*3A	*3B/*3B	WT/94C>A	94C>A/94C>A
None	54	5	0	0	0	10	0
Leucocytes <2×10 ⁹ /l	0	0	0	1	1	0	0
Leucocytes 2–4×10 ⁹ /l	9	1	0	0	0	0	0
Hepatotoxicity	5	0	0	0	0	0	0
Pancreatitis	5	1	0	0	0	0	0
Gastrointestinal	7	0	0	0	0	0	0
Other	8	0	1	0	0	2	0
Total	88	7	1	1	1	12	0

One patient was included in both the TPMT polymorphisms column and in the ITPA polymorphisms column as he was heterozygous for the TPMT*3A polymorphism and heterozygous for the ITPA 94 C>A polymorphism. Side effects categorised as "other" included rash, renal function disorders, vertigo, myalgia, and arthralgia.

Lack of serum antibodies to membrane bound carbonic anhydrase IV in patients with primary biliary cirrhosis

Nishimori *et al* have recently reported the presence of autoantibodies against carbonic anhydrase IV (anti-CA IV) in patients with autoimmune pancreatitis (*Gut* 2005;54:274–81). Furthermore, serum antibodies to CA II (anti-CA II) were observed in several autoimmune conditions. We have now investigated the presence of anti-CA IV and anti-CA II in a large series of sera from patients with primary biliary cirrhosis (PBC) and controls.

CA II is known to be expressed in the cytosol of various types of epithelial cells, including those lining bile ducts, renal tubules, and salivary ducts. For this reason, CA II was suggested as a common antigen in conditions characterised by an autoimmune aggression against epithelia. In autoimmune pancreatitis, serum anti-CA II are useful diagnostic tools while in PBC they were first detected by Gordon *et al* in 5/6 sera from patients with antimitochondrial antibody (AMA) negative PBC.¹ Subsequent studies however demonstrated prevalence rates as high as 46% in PBC sera but failed to confirm their specificity for AMA negative sera.^{2,3} Interestingly, anti-CA II were also shown to inhibit enzyme activity.⁴

Apart from cytosolic CA II, the CA family also includes a highly active membrane bound enzyme that was coined CA IV.⁵ Both CA II and CA IV are abundantly expressed in human bile duct epithelial cells. Interestingly, mainly due to the sequence homology between CA II and CA IV and CA IV localisation on cell membranes, Nishimori *et al* hypothesised that the exposed CA IV might be more immunogenic than cytosolic CA II.

Seventy sera from patients with PBC (60 AMA positive; all anti-hepatitis C virus negative; 63 women; mean age 60 (SD 10) years) who attended our tertiary referral centre were consecutively enrolled in the study. Control sera were obtained from 50 healthy subjects matched with patients for sex and age class (≤ 50 v > 50 years). All sera were tested by immunoblotting for anti-CA IV and anti-CA II, as previously described.² Briefly, proteins were denatured and separated (10 µg/lane) on a 1.5 mm sodium dodecyl sulphate-12% polyacrylamide gel. Proteins were then transferred onto nitrocellulose (pore size 0.45 µm) using a semi-dry transfer system. The nitrocellulose membrane was cut into 4 mm strips and, after blocking with 5% non-fat milk, all strips were incubated with serum samples diluted 1:100 and 1:200 for anti-CA IV and 1:100 for anti-CA II. Rabbit horseradish peroxidase conjugated antibodies against human immunoglobulins G, A, and M (Dako, Glostrup, Denmark) was diluted 1:1000 and used as secondary antibody. Peroxidase development was obtained with 0.05% 4-chloro-1-naphthol in Tris buffered saline containing 20% methanol and 0.05% H₂O₂. A rabbit polyclonal antihuman CA IV antiserum⁶ was used as a positive control throughout the study. CA IV antigen and anti-CA IV antisera were provided by Dr William S Sly (St. Louis, Missouri, USA).

Results demonstrated no reactivity against CA IV in any of the PBC or healthy control sera. In contrast, similar to previous reports, anti-CA II antibodies were detected in 6/70 (9%) sera from patients with PBC but were absent in control sera.

In summary, we submit that the hypothesis that antibodies against the membrane bound CA IV may play a role in PBC should be rejected, based on our experimental data on a large series of sera. Our finding may be secondary to a different cellular expression of CA IV in the target organ (that is, pancreatic and bile ducts) but only specific tissue studies can provide these answers. At present, therefore, anti-CA IV should be regarded as specific to autoimmune pancreatitis and research should focus on better defining their possible role in this condition.

P Invernizzi, C Selmi, M Zuin, M Podda

Department of Internal Medicine, San Paolo School of Medicine, University of Milan, Italy

Correspondence to: Dr P Invernizzi, Division of Internal Medicine, Department of Medicine, Surgery, and Dentistry, San Paolo School of Medicine, University of Milan, Via di Rudini 8, 20142 Milano, Italy; pietro.invernizzi@unimi.it

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Association of achalasia and dental erosion

Dental erosion is the dissolution of enamel and dentine caused by organic or inorganic acids.¹ The source of acid is normally either dietary acids² or regurgitation of stomach juice³ into the mouth. Enamel and dentine begin dissolution at a pH of approximately 5.5.⁴ In achalasia, bacterial fermentation of food produces lactic acid, with a minimum pH of approximately 3.5,⁵ which has the potential to demineralise teeth if it reaches the mouth. This study investigated whether regurgitated lactic acid fermented from food lying within an achalasic oesophagus causes dental erosion. The aim of the study was to measure the prevalence of dental erosion in patients referred for management of untreated achalasia and to compare the results with a control group.

Patients referred to the oesophageal laboratory from a variety of medical sources for investigation of achalasia were recruited. Manometry was used to diagnose the presence of achalasia in all subjects. Ethics approval was provided from the local hospital and each patient gave informed consent for assessment of erosion. The distribution and severity of dental erosion was determined using the Smith and Knight tooth wear index (TWI).⁶ All tooth assessments were carried out by the first author under ideal conditions. The index scores

erosion on a five point scale, with 0 representing no erosion and a score of 4 representing pulpal exposure. A control group, without symptoms or history of gastro-oesophageal reflux disease, were selected from the partners of patients attending for oesophageal tests. Inclusion criteria did not take into account the presence or absence of dental erosion. A dietary questionnaire was used to exclude subjects with a high intake of dietary acids. Mann-Whitney U tests were used to compare patients with controls for differences in tooth wear scores. Intraclass correlation showed good agreement for the erosion scores (0.99).

Fifteen patients with achalasia (six males and nine females) with a mean age of 49 years (SD 18.4) were recruited over a two year period and compared with 32 controls (14 males and 18 females) with a mean age of 43 years (SD 16.8). Median percentage of teeth scoring a TWI of 2 or above was 21.4% (interquartile range (IQR) 11.46–30.77) in patients and 7.76% (IQR 0–12.2) in controls, for all tooth surfaces ($p=0.001$). At the moderate level (score 3 and above), with dentine exposed for more than one third of the surface, patients had a median of 0% (IQR 0–16.1) and controls a median of 0% (IQR 0–0; $p<0.001$). The distribution of the erosion was predominantly on the palatal surfaces of the upper incisors.

Achalasia is an uncommon disorder of the oesophagus in which there is failure of normal peristalsis in the body of the oesophagus and the lower oesophageal sphincter fails to relax.^{7,8} The control group were recruited from the partners of the patients attending for oesophageal tests. Unfortunately, it was not feasible to undertake manometry in the controls as this was ethically unacceptable but there remains a possibility that some had asymptomatic reflux but not achalasia. If any controls had asymptomatic reflux they were at more risk of developing dental erosion but the results from the erosion scores seemed not to indicate this.

Ineffective oesophageal motility causes delayed acid clearance and its association with the presence of palatal dental erosion was reported by Bartlett and colleagues.^{9,10} The results of this study support the hypothesis that oesophageal motility disorder has an important role in the development of dental erosion, albeit an extreme example. In this case, an obstructive oesophagus causes food fermentation, and in turn regurgitation of fermented food causes dental erosion. The presence of palatal dental erosion in patients with achalasia strongly suggests that the source of the acid within the oesophagus is lactic acid unlike reflux disease where hydrochloric acid from the stomach is responsible.¹¹ This study shows that in patients with achalasia, particular attention to the condition of their teeth needs to be addressed. In conclusion, achalasia is related to palatal dental erosion and the cause of the erosion is fermented foods and not regurgitated gastric juice.

R Moazzez

Department of Prosthodontics, GKT Dental Institute, London, UK

A Anggiansah, A J Botha

St Thomas's Hospital NHS Trust, London, UK

D Bartlett

Department of Prosthodontics, GKT Dental Institute, London, UK

Correspondence to: Dr R Moazzez, Department of Prosthodontics, GKT Dental Institute, Floor 26, Guy's Tower, St Thomas' St, London Bridge, London SE1 9RT, UK; Rebecca.moazzez@kcl.ac.uk

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BOOK REVIEW

New Techniques in Gastrointestinal Imaging

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Many areas of radiology are rapidly developing new techniques to answer clinical

problems or devising ways of refining current imaging techniques. Gastrointestinal imaging is no exception.

New Techniques in Gastrointestinal Imaging has been edited and written by experts in the field from the international community and encompasses the more recent developments in all aspects of gastrointestinal imaging. The book has been divided into chapters that either concentrate on a particular imaging technique (for example, computed tomography (CT) colonography) or those that cover recent developments in the investigation of a particular area (for example, the rectum). There are very comprehensive chapters covering the new CT and magnetic resonance (MR) techniques available for imaging the colon and small bowel. New CT and MR techniques for hepatic imaging are also included, with special reference to the development of CT angiography. There are excellent chapters on the use of microbubbles in ultrasound (US) and endoscopic US, both of which are good introductions to these techniques for those with limited previous knowledge or experience. Also included is a very useful chapter on positron emission tomography (PET) with a gentle introduction to the physics of the technique and current applications and limitations. New interventional imaging techniques are also covered, with chapters on radiofrequency ablation of liver lesions and on self expanding metallic stents in the colon.

I was however dismayed to find a section on defecating proctography, a technique I had rather hoped had been consigned to history. The current method seems to have changed little from my days as a junior registrar banished to the barium room although new MR techniques are described.

This book has been written to update the general radiologist in areas of gastrointestinal radiology that have changed significantly in recent times. This it does very well, with concise descriptions of the techniques, thorough discussions on clinical use, and handy tips on image interpretation. As such, there are chapters in the book that need some

background knowledge of radiological techniques to appreciate the new developments (for example, CT and MR chapters on liver imaging). However, all chapters provide a good setting for each of the new techniques so that the interested gastroenterologist would find useful information on the current role of each investigation, its performance with relation to more established techniques, and future developments.

A Graham

CORRECTIONS

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In the Editor's quiz: GI snapshot on p1272 of the September issue (D Joshi, J Dunga, A James and MM Yaqoob. An unusual case of hepatosplenomegaly. *Gut* 2005;**54**:1272; doi:10.1136/gut.2005.064824) the second author's name should read Dunga not Dunga.

In the Gut Tutorial on p296 of the February issue the author's name and affiliation was omitted. The details are as follows: Robin Spiller, Professor of Gastroenterology, Wolfson Digestive Diseases Centre, University Hospital, Nottingham NG7 2UH, UK.

In the Gut Tutorial on p555 of the May issue the author names and affiliations were omitted from the original publication. This has been updated on the Gut website. The authors and affiliations are as follows:

S A Khan, A Miras, Liver Unit, Department of Medicine A, Faculty of Medicine, Imperial College London, St Mary's Hospital Campus, South Wharf Road, London W2 1NY, UK; M Pelling, Department of Radiology, Faculty of Medicine, Imperial College London; S D Taylor-Robinson, Liver Unit, Department of Medicine A, Faculty of Medicine, Imperial College London.