PostScript

LETTERS

Reduced microbial diversity in inflammatory bowel diseases

Intestinal microbiota have become the subject of intense investigation in inflammatory bowel disease (IBD) over the past years after some groups demonstrated that significant alterations of the composition of enteric bacteria might be related to the underlying inflammatory process (*Gut* 2006;**55**:205–11).[1–4] However, the complexity of the intestinal microbiota and the availability of a variety of different experimental approaches generated sometimes conflicting and inconsistent data. Manichanh *et al* (*Gut* 2006;**55**:205–11) recently published an extensive study using metagenomic libraries, a novel molecular technique allowing the recruitment of full molecular information of complex microbial habitats. In metagenomic clone libraries with more than 25 000 clones that were generated from faecal samples of healthy subjects and active Crohn's patients, significant loss of indigenous bacteria was found.

The article confirms our report that reduced bacterial diversity seems to be a hallmark of the biofilm in IBD.[4] Using colonic biopsies we found loss of bacterial diversity of the mucosal microbiota in a large cohort of patients with IBD using different 16S rDNA based detection techniques.[4] In contrast with Manichanh *et al*, the taxa of the bacterial phylotypes were determined in our study by sequence homology analysis in clone libraries and not by single strand conformational polymorphism alone.[4] The metagenomic approach used by Manichanh *et al* is likely to be the most informative way of collecting microbial data of complex bacterial communities. Notably, this demonstrates that assessment of 16S rDNA based signals, especially when using large scale clone libraries as in our paper, has sufficient power to determine bacterial richness and diversity. This is not surprising because the taxonomical classification of metagenomic fragments is based mainly on 16S rDNA anchor genes.

In some aspects there are discrepancies between the different molecular studies. Manichanh *et al* only demonstrated alterations of the faecal microbiota. As previously demonstrated, different compartments in the intestine contain complex ecological systems that are distinctly different.[5] Therefore, the composition of the bacterial consortia in human faeces, which contain a high number of transient bacteria, does not fully represent the mucosal microbiota.[6 7] Mucosa related microbes (including intracellular microorganisms) however, seem to be a functionally relevant part of the intestinal microbiom directly interacting with the host immune system.

Generating and analyzing metagenome libraries is very expensive. Therefore, the number of patients that can be analysed will remain relatively small. Confirmation of reduced bacterial diversity however could now be followed by a deeper analysis of the functional capacities of the bacterial communities. These next steps could convert descriptive approaches into a mechanistic understanding. Manichanh *et al* have introduced the metagenomic approach as a novel technique of collecting data from complex human biofilms.

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Analysis of the c-*kit* gene in patients with slow transit constipation

Although slow transit constipation (STC) may not be a congenital disease, the frequent onset in adolescence and strong female predominance suggest that STC could be a result of a sex modified multifactorial disorder of the gastrointestinal tract with a genetic basis. Several genes such as RET proto-oncogene and the neurturin gene have been analysed in STC. Unfortunately, few mutations were found to be associated with STC.[1 2] Our previous studies described a decrease in volume in interstitial cells of Cajal (ICC) in patients with STC, and down-regulation of *c-kit* mRNA and c-kit protein expression in the colonic tissues of STC patients.[3 4] At present, we do not know why ICCs are lost from colonic tissues of patients with STC. Evidence suggests that the c-kit/SCF signal pathway plays a crucial role in ICC development and maintenance of its phenotype. An example of loss of function mutations of the *c-kit* gene is mice that lack the network of ICCs and show abnormal intestinal pacemaker activity.

To date, no study has explored whether the *c-kit* gene is a candidate for STC. Therefore, we screened a series of patients with chronic idiopathic STC for germline mutations of *c-kit*.

The STC group included 23 patients who had a history of longstanding intractable constipation, with bowel movements ranging between once per five and 15 days. Colon transit time, determined by radio-opaque marker tests, was markedly increased by more than 120 hours, and conventional medical therapy had failed in all cases. The control group included eight patients undergoing partial colectomy for non-obstructive carcinoma (T1-T2) or adenoma. Genomic DNA extracted from the resected colon of patients and controls was screened by direct DNA sequencing using the fluorescent dideoxy terminator method. The coding region between exon 9 and exon 21 of the *c-kit* gene was fully sequenced in both directions, including some intron and intron–exon boundaries. Results are summarised in table 1.

The results were compared with published sequence data and eight control DNAs. Seven genovariation sites were detected. Only one mutation was found in one case at the *c-kit* gene 75515T→C, which resulted in codon 531-Ile to Thr in exon 10 but allele frequency was comparable between patients and controls. Two polymorphism sites in the intron region (base substitution at 75794T→A and the heterozygous mutation at 86548T→A)

Table 1 Polymorphisms in the *c-kit* gene (exon 9 to exon 21)

Position	Nucleotide	Amino acid	Allele frequency		χ^2	p Value
			Controls	STC		
Exon 10	75515T→C	531Ile→Thr	0	0.022	0.748	0.387
Exon 10	75561A→G	546Lys→Lys	0	0.022	0.748	0.387
Intron 11	75794T→A	—	0	0.174	6.570	0.018*
Intron 16	81240G→A	—	0	0.063	2.304	0.129
Intron 17	81517C→T	—	0.118	0.261	2.506	0.159
Intron 19	85240A→G	—	0	0.109	3.942	0.069
Intron 20	86548T→A	—	0.029	0.196	4.940	0.038*

—, No amino acid changes.
*$p<0.05$ versus controls.

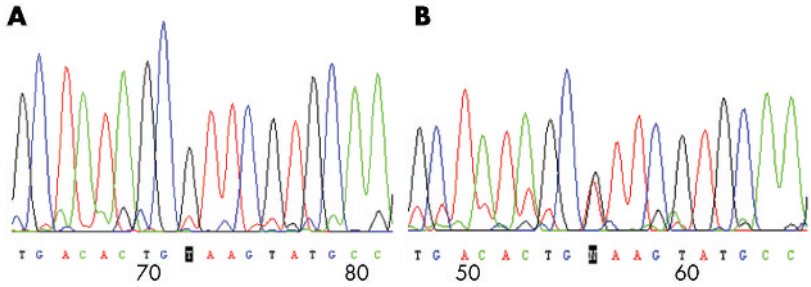

Figure 1 DNA sequencing of the *c-kit* gene at intron 20. (A) Controls. (B) STC patients with the mutation at base 86548T→A.

exhibited a significantly higher frequency compared with controls (fig 1).

This study evaluated for the first time whether c-kit gene variations were related to the pathogenesis of STC. Seven genovariation sites were detected; only two (81240G→A, 85240A→G) had been published previously. The only missense mutation was found in one case at *c-kit* gene 75515T→C, which result in codon 531-Ile to Thr in exon 10. This point mutation may be pathological for individuals but its significance in the aetiology of STC needs further evaluation in large samples. Moreover, two polymorphism sites in the intron region were remarkable. The base substitution at 75794T→A (just six bases downstream of intron 11) and the heterozygous mutation at 86548T→A (located at 48 bases upstream of intron 21) are expected to affect exon–intron splicing, and are thus worthy of further exploration. The unique published missense single nucleotide polymorphism site of the *c-kit* gene at base 75544 A→G, the codon 541-Met to Val, were not detectable in all subjects.[5] This implies that the codon 541 polymorphism is not the cause of STC. Moreover, exons 1–8 were not detected in this study. It is also possible that the extracellular region of the *c-kit* gene and other candidate genes, especially the gene encoding SCF, may be involved. Future studies should address these hypotheses.

In conclusion, mutation of the *c-kit* gene is not a frequent cause of STC but some polymorphisms in the intron region are worthy of further study.

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Does longitudinal muscle contraction of the oesophagus hold important secrets?

Tutuian *et al* (*Gut* 2006;**55**:584–5) performed high resolution oesophageal manometry in a patient with achalasia and ''chest pressure'' following swallowing. They observed a sustained increase in intra-oesophageal pressure with maximum pressure at the time of maximal oesophageal shortening. Oesophageal shortening is attributed to longitudinal muscle contraction. The mechanism that they propose for this pressure increase is ''pump gun'' (after the classic side action firearm first patented in Britain by Alexander Bain in 1854). They also suggest that reduction in oesophageal pressure during this episode is due to oesophageal emptying that clears the oesophagus and results in resolution of chest pain.

Although these observations are important, we propose an alternative interpretation based on our recent description of a similar phenomenon in association with gastro-oesophageal reflux[1] where the so called ''common cavity pressure'' (a simultaneous increase in oesophageal pressure along the length of the oesophagus) was found to more closely relate to longitudinal muscle contraction of the oesophagus, rather than gastro-oesophageal reflux (as commonly believed). We suggest that longitudinal muscle contraction results in two actions: (1) it renders the oesophagus stiff or non-compliant, and (2) it causes a reduction in oesophageal length. Both of these factors are critical and result in an increase in intra-oesophageal pressure, based on the physical principle of Boyle's law (pressure in a non- compliant cylinder increases as its volume decreases). Tutuian *et al* suggest that the decrease in oesophageal pressure is due to oesophageal emptying that clears the oesophagus. We believe their explanation is unlikely because lower oesophageal sphincter pressure is fairly high (obstructive) during the entire period of oesophageal shortening. It is more likely that the observed decrease in oesophageal pressure is related to lengthening of the oesophagus and increase in oesophageal compliance as longitudinal muscle contraction dissipates. Accordingly, the tracing provided by Tutuian *et al* shows a close temporal correlation between the decrease in oesophageal pressure and lower oesophageal sphincter descent.

Tutuian *et al* suggest that the increase in oesophageal pressure due to longitudinal muscle contraction is the cause of the patient's chest pain and the latter is relieved as oesophageal pressure decreases. We described longitudinal muscle contraction of long duration (which we initially called a sustained oesophageal contraction (SEC)) in association with oesophageal pain.[2] We suggest that this patient's symptoms of chest pressure and pain are related to a prolonged episode of longitudinal muscle contraction and its relief related to dissipation of longitudinal muscle contraction.

In summary, this important event captured by Tutuian *et al* provides a novel insights into the mechanism by which longitudinal muscle contraction may increase intra-oesophageal pressure and at the same time cause symptoms. Our explanation of the various events in this record may be slightly different than that described by Tutuian *et al*, but we agree that this brief episode holds an important secret as to what causes spontaneous oesophageal pain.

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Duration of lamivudine prophylaxis in inactive hepatitis B virus carriers with haemato/oncological malignancies who receive chemotherapy

I read with interest the article by Hui *et al* reporting hepatitis B reactivation after withdrawal of pre-emptive lamivudine in patients with haematological malignancy on completion of cytotoxic chemotherapy (*Gut*