The role of Cdx genes in the mammalian gut
F Beck

Cdx genes are important in pattern formation during the development of the gut and may well contribute to the balance between differentiation and cell renewal in the mature intestine.

Organisation of multicellular animals involves the action of genes that impart “positional information.” All vertebrates are built on a segmental pattern that is most obviously expressed by the appearance of somites during embryonic development.

A common feature of genes that impart individual identity (and therefore positional information) to specific segments is the possession of a “homeobox” DNA binding motif coding for a consensus sequence of 60–63 amino acids that acts as a transcriptional regulator of “downstream” genes. The most widely researched homeobox genes are the so-called homeotic selector genes of the Antennapedia complex (the defining gene is named Antennapedia). In the fruit fly *Drosophila*, these are situated on chromosome 3 as part of the HOM cluster. HOM-C genes are strongly conserved during evolution and in mammals have been replicated to appear on separate chromosomes in four paralogous complexes called Hox clusters. They are expressed principally in developing ectodermal and mesodermal tissues and in general terms are responsible for segmental specification of the dermatomes, musculoskeletal, and nervous systems. However, Hox genes are not expressed in the greater part of the gut endoderm but in their place, mammalian members of the ParaHox genes—an “evolutionary sister” of the Hox clusters—seem to play an important role in gut patterning. Members of this group are Pdx1 which is required for the correct development of the pancreas and duodenum and three homologues of the *Drosophila* gene Caudal which in mammals are called Cdx1, Cdx2, and Cdx4. In addition to their own unique domains, the Cdx genes exhibit significant topographical overlap of expression during development, as well as in the adult, and it is reasonable to assume that in such areas a degree of compensation may occur in the event of single gene deficiency.

Cdx2 is expressed in the endoderm of the entire postgastric epithelium from the time of its initiation at the stage of hindgut invagination throughout development and adult life. Cdx1 is not expressed in the early definitive gut endoderm but appears at postsomite stages just before transition of the multilayered intestinal endoderm to a single layered epithelium at 14 days post coitum (dpc). Cdx4 is expressed in the earliest hindgut invagination but little is known of its distribution after 10 dpc.

It is important to note that in addition to their role in the gut, Cdx genes are active at multiple other sites during early development where, inter alia, they regulate the extent of ectomesodermal expression of Hox genes and thus also indirectly influence non-intestinal anteroposterior patterning. However, this review is principally concerned with their role in the gut.

Much insight into the function of a gene may be obtained by studying the morphology of mice in which it has been inactivated by homologous recombination. Knockout of the Cdx1 gene produced anterior homeotic shifts (that is, a posterior structure such as a vertebra or rib exhibiting the morphology of a more anterior element) of the axial skeleton but no gut abnormalities. Inactivation of Cdx2 on the other hand produced not only axial homeotic shifts in heterozygotes (one affected allele) but was also found to prevent trophoblast maturation and consequently blastocyst implantation if present in the homozygous state (two affected alleles). Most importantly in the context of this commentary, Cdx2+/− embryos exhibited multiple polyps in the caecum and adjacent ileum and proximal colon (that is, in the midgut). Histological examination showed the presence of a polypoid epithelium in the sub-stance of the polyps and this was interpreted as evidence of an anterior homeotic shift involving the gut endoderm analogous to the mesodermal shift involving the axial skeleton. In other words, disturbance of “positional information” has resulted in anterior structures (forestomach epithelium) occurring ectopically at more posterior sites. While initially defined as adeno- matous polyps, critical histological analysis together with studies on timed development of the polyps established an interesting process of so-called intercalary growth. It was concluded that the function of Cdx2 was to direct the “default state” forestomach endoderm towards a caudal phenotype and that lower levels of expression of Cdx2 in the developing distal intestine of Cdx2+/− heterozygotes lead to reversion to a more anterior phenotype (that is, to forestomach epithelium). Subsequently, intercalary growth around the gut lesions results in “filling in” of tissue types at the discontinuity between the ectopic gastric and surrounding colonic epithelia and an orderly succession of histologically normal epithelia characteristic of cardia, corpus, antrum, and small intestine, in that order, appeared all around the forestomach epithelium at the junction with colonic epithelium. The molecular basis of the intercalary growth is unclear but probably involves local intercellular communication.

Clonal analysis experiments have been performed to establish the origin of the secondarily generated tissue between ectopic forestomach and colonic epithelia. Y chromosome painting in wild-type/Cdx2−/− chimaeric mice of opposite sex indicated that once differentiated to express Cdx2, host colonic epithelium can only contribute small intestinal epithelium to the secondarily generated tissue while the Cdx2 mutant chimaeric cells give rise to the succession of gastric-type tissue but never to small intestinal morphology. These findings have interesting implications in the context of intestinal regeneration.

No information concerning the role of Cdx4 in gut development is currently available as knockout or RNAi knockdown experiments have not been performed and no spontaneous mutants are available.

Having shown that Cdx2 is necessary for the establishment of the midgut phenotype, we must now ask whether it is sufficient to convert the “default” stomach phenotype to that of colon. The question has been investigated by making transgenic mice in which Cdx2 is expressed in the stomach (which in wild-type mice is Cdx2 negative). This is achieved by introducing a Cdx2 expressing “transgene” into the genome of *Chimaeric* mice are mosaics produced by injecting genetically distinct cells into recipient embryos at the blastocyst stage so that the resultant mice are a mixture of the donor and recipient cell lines.
of wild-type mouse embryos under the control of a promoter that specifically causes expression of Cdx2 in the stomach. The answer appears to be “yes” as Silberg and colleagues, using cis regulatory elements of the Hnf3γ promoter to drive ectopic expression of Cdx2 in the stomach, described the presence of alcalain blue positive intestinal-type goblet cells in the gastric mucosa of transgenic mice as well as induced expression of intestine specific genes in the relevant areas. Interestingly, Mutoh and colleagues produced similar results using the promoter of the non-catalytic β-subunit gene of rat H+K+ ATPase to drive the Cdx2 transgene. This group described complete replacement of gastric mucosal cells in the body of the stomach at postnatal day 37 by goblet cells, endoendocrine cells, and absorptive cells expressing alkaline phosphatase together with the establishment of the proliferative zone at the base of the glands rather than at the isthmus. As partial cell function is established postnatally, it is reasonable to assume that the Cdx2 transgene was expressed in differentiated cells rather than in stem cells; the H+K+ ATPase promoter being activated with the onset of proton pump function. This suggests that either some partial cells retain characteristics of stem cells—unlikely but possible as some partial cells high up in the gastric glands may not have undergone a terminal differentiative event and may retain proliferative potential— or else Cdx2 expression directly or indirectly causes “dedifferentiation” of parietal cells and their establishment as intestinal stem cells in the basal region of the mucosa whence they have migrated. This in turn might cause the overlying normal gastric mucosa to disappear in favour of the newly established intestinal phenotype. Another possibility is that the protein pump promoter used may be active at low levels in cells other than fully differentiated oxyntic cells expressing the proton pump. Clearly, these are speculative suggestions that require rigorous investigation.

Subsequently, Mutoh and colleagues have expressed a Cdx1 transgene in the mouse stomach, again driven by the H+/K+ ATPase β-subunit promoter, and their study is described in this issue of Gut (see page 1416). Surprisingly perhaps, they again demonstrated intestinal metaplasia similar in many but not all respects to that seen with Cdx2. As Cdx1 knockout has no apparent effect on differentiation of the midgut, the transdifferentiating effect of Cdx1 transgene expression in the stomach is somewhat unexpected. A possible explanation may lie in the overlap of function between cad homologues although clearly Cdx1 does not compensate for Cdx2 in the knockout model of the latter. The overlap of effects may only be partial and this would explain differences in proliferating cell distribution and distribution of Paneth cells that exist between the two transgenic models.

Mammalian cad homologues and in particular cad2x2 are multifunctional genes and the picture presented above is simplistic. In the mature gut, Cdx1 is expressed principally in intestinal crypts while Cdx2 is demonstrable in cells lining the villi where it regulates production of many gut enzymes, such as lactase-phlorizin hydrolase. Apart from its importance in pattern formation during the development of the gut, Cdx2 in conjunction with Cdx1 may well contribute to the balance between differentiation and cell renewal in the mature intestine and recently it has been suggested that in this respect it is controlled by PTEN/phosphatidylinositol 3 kinase signalling and tumour necrosis factor α signalling via nuclear factor κB dependent pathways. Furthermore, Cdx2 functions as a tumour suppressor in the adult mouse. In one study, Cdx2+/−/ mice developed adenocarcinoma in the distal colon in response to low doses of azoxymethane compared with a significantly smaller effect of the same dose on wild-type controls. The tumours differed histologically as well as in geographical situation from non-neoplastic heterotopic gastric polyps found in the peri- caecal region of these animals.

Another group used Apc mutant/Cdx2+/−/ compound mice. They found that levels of both Apc and Cdx2 were significantly lower in the distal colon and this was correlated with the development of adenomatous polyps in the distal colon whereas these lesions were predominantly present in the small intestine in mice with Apc lesions only. The authors conclude that reduced Cdx2 expression is important in colonic tumorigenesis. Both studies reported lower apoptotic rates in the colonic mucosa thus allowing greater survival of azoxymethane compromised cells or of cells with loss of heterozygosity at Apc.

Clearly, much remains to be done before the role of cad homologues in mammalian gut development and function is fully elucidated. It is certain that they are critically important during development in defining gut pattern and that in the adult they contribute to the complex mechanisms of cell turnover and phenotypic differentiation of stem cells. Future challenges include elucidation of upstream and downstream genes and the possibility of modifying gene expression for therapeutic purposes.

References

Irritable bowel syndrome

Is there a SERT-ain association with IBS?

M Camilleri

The homozygous short genotype of SERT-P may be a candidate gene for diarrhoea predominant irritable bowel syndrome in women

In this issue of Gut, Yeo and colleagues\(^1\) report on the association between a functional polymorphism in the serotonin transporter gene and diarrhoea predominant irritable bowel syndrome (D-IBS) in women (see page 1452). In a study of 194 North American female participants with D-IBS in a clinical trial programme and 448 female controls, there was an association between the homozygous short genotype of SERT-P (serotonin reuptake transporter gene) and the D-IBS phenotype, with an odds ratio of 2.25 (95% confidence interval 1.51–3.31). The fact that the confidence interval does not cross the value of 1 suggests that the association is statistically significant, and the authors suggest that the SERT-P may be a candidate gene for D-IBS in women.

To place these interesting observations in perspective, the reader may wish to address the following. What is SERT? What is the theoretical consequence of a polymorphism of the SERT-P gene? What happens to animals when the SERT gene is knocked out? Have other studies addressed the association of this polymorphism and IBS? Are there any pitfalls in the interpretation of such association studies?

WHAT IS SERT?

Serotonin (5-hydroxytryptamine, 5-HT) is secreted in copious amounts from gut enteroendocrine cells and serves as a critical messenger for gastrointestinal fluid secretion and gut motility.\(^2\) There are seven subclasses of serotonergic receptors, differentiated on the basis of structure, molecular mechanism, and function.\(^3\) In contrast with the remarkable diversity of 5-HT receptors, only a single protein, the 5-HT transporter (or SERT), mediates reuptake of 5-HT from the synaptic cleft and thus termination of its action. The approved gene symbol for SERT is SLC6A4 (solute carrier family 6 member 4); this abbreviation is used in other papers and this may be confusing to readers. Human SERT is encoded by a single gene on chromosome 17q11 and is composed of 14–15 exons. Transcriptional activity of the SERT gene, SERT availability, and ultimately 5-HT reuptake is modulated by a polymorphic repetitive element unique to humans and simian primates, the 5-HT gene linked polymorphic region (5-HTLPR) upstream of the transcription start site. Additional variations have been described in the 5’ untranslated region (5’UTR), in intron 2, and 3’UTR (reviewed by Reif and Lesch\(^7\)).

Neurotransmitter transporters are channel-like proteins that are involved in chemical signalling in the brain and periphery; in fact they are considered to do the heavy lifting in neurotransmitter activation.\(^4\) SERT in the gut is similar to that in the brain of the same species.\(^5\) To control 5-HT actions in the gut and limit 5-HT receptor desensitisation, both neurons and crypt epithelial cells synthesise SERT proteins.\(^6\)

WHAT IS THE THEORETICAL CONSEQUENCE OF A POLYMORPHISM OF THE SERT-P GENE?

In elegant functional studies, Lesch \textit{et al.} showed that, compared with the homozygous long genotype, polymorphic homozygous short and heterozygous SERT-P were associated with reduced function of the transporter protein in a lymphoblastoid cell line.\(^9\) Figure 1 illustrates the consequence of a less effective reuptake process for 5-HT, as might occur in a patient with an s/s homozygous or s/l heterozygous polymorphism.

WHAT HAPPENS TO ANIMALS WHEN THE SERT GENE IS KNOCKED OUT OR IS DECREASED BY DISEASE?

SERT deficient mice display increased anxiety related behaviours based on increased serotonergic neurotransmission resulting in desensitised and down-regulated 5-HT\(_{1A}\) and 5-HT\(_{2A}\) or 5-HT\(_{3C}\) receptors.\(^10\) Gastrointestinal motility is also abnormal in SERT knockout mice.\(^11\) Adaptive changes occur in the subunit composition of enteric 5-HT receptors in these knockout mice. Such changes are reflected in altered 5-HT receptor affinity and desensitisation in the response of the receptor to 5-HT released from enteroendocrine cells.\(^12\)

An intriguing recent observation suggests that experimental colitis alters 5-HT signalling by increasing 5-HT availability while decreasing 5-HT reuptake.\(^13\) The authors speculated that altered 5-HT availability might contribute to the dysmotility of inflammatory bowel disease, possibly due to desensitisation of 5-HT receptors.

HAVE OTHER STUDIES ADDRESSED THE ASSOCIATION OF THIS POLYMORPHISM AND IBS?

There have been five studies that explored the association of SERT...
polymorphisms and IBS; results from the three genetic association studies are summarised in fig 2.

The first report was a study from Turkey in 54 IBS patients (18 with D-IBS) and 91 healthy controls. Pata et al examined possible associations between SERT polymorphisms and the different clinical patterns of IBS. They observed that the l/s genotype was present in 18 D-IBS patients with a frequency of 88% whereas none of the patients had the l/l genotype. Overall, the distribution of SERT polymorphisms were similar in healthy subjects and the whole group of IBS patients.16

The second report by Kim et al at the Mayo Clinic, which studied people residing in the Mid-West in the USA, was recently published in Gut.17 The study explored the distributions of genetic polymorphisms for SERT-P, 2C Del 322–325, and 2C A–1291 C→G polymorphisms in 274 patients with lower functional gastrointestinal disorders (FGID) and 120 controls. The distribution of SERT polymorphisms was not significantly different between the lower FGID patients versus controls (an observation that replicated the result in the whole IBS group studied in Turkey) or between the subset with the phenotype of D-IBS (n = 128) versus controls. In the final analysis of 90 C-IBS patients reported by Kim et al, the odds ratio for SERT-P l/s or s/s polymorphisms was not significant (0.7; 95% confidence interval 0.4, 1.2). This study included a post hoc analysis to determine whether clinically meaningful associations of the three candidate genotypes could have been detected with at least 80% power. Thus it was reported that the sample size of the study could detect a difference in prevalence of wild type (l/l) versus polymorphic (l/s or s/s) genotypes of 12% for all lower FGID, and 19–20% for IBS-C and IBS-D. On the other hand, the study identified that combinations of polymorphisms were associated with high somatic symptom scores: 2C Del 322–325 with SERT-P (odds ratio 5.0 (95% confidence interval 1.11, 22.22)).17 This suggests that the SERT-P genotype may predispose to somatisation or other complex behavioural traits (including fibromyalgia, anxiety, and depression, as discussed by Yeo and colleagues1) and that studies of interactions between candidate genes that modify motor, sensory, or behavioural functions would be of interest.

A third report from Korea18 showed no differences in genotype distributions between IBS, IBS subgroups, and healthy controls.

The fourth report from Italy was centred around the function of platelet SERT. SERT is found in peripheral sites, including platelets.19 As the SERT protein displays the same molecular properties at all known cellular locations7 and, in accordance with the general receptor theory,20 Bellini et al proposed that it is conceivable that similar alterations in 5-HT uptake efficiency may also occur at the intestinal level. SERT was found to be expressed on platelet membranes of 12 D-IBS female patients at a low density (decreased Bmax) as well as to display a low degree of affinity (increased Kd) at its ligand binding site compared with 12 healthy female volunteers.21 They also suggest a possible correlation between the reduced capacity of serotonin reuptake and the severity of D-IBS symptoms.21 However, the association with genotype was not formally explored.

The fifth report is the paper in the current issue of Gut. Yeo and colleagues1 observed a significant odds ratio of having the s/s genotype in D-IBS patients relative to controls in North America. This observation contrasts with the significant association with the l/s genotype in Turkey; however, from the function studies performed in vitro,10 both l/s and s/s genotypes would be predicted to produce SERT molecules with reduced function, and therefore result in higher synaptic levels of 5-HT. On the other hand, the odds ratio provides statistical evidence of an association although it does not prove a disorder in the function of the SERT or that it is causatively related to the development of D-IBS. In fact, the proportion of the l/l, l/s, and s/s genotypes in D-IBS were virtually equivalent in the 194 patients from North America.1

In summary, these data show that the distribution of the l/s genotype seems very heterogeneous in different populations. A polymorphism which is associated with a disorder in one population may not be in another ethnic group. This is a problem that is increasingly being appreciated in behavioural genetics. This may explain, at least in part, a number of apparently contradictory findings gathered on specific genetic variations in different studies.

**INTERPRETATION OF GENE-ASSOCIATION STUDIES**

There are several potential pitfalls that need to be considered in the interpretation of these studies.

**Ethnic differences**

Difficulties in interpretation of population based association studies arise due to ethnic differences in SERT-P allele frequencies which may also explain some conflicting results. The frequency of the l/l genotype was 6% in Japanese subjects,19 25 5% in Koreans,18 34% in

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**Figure 2** Proportion of different SERT-P genotypes in patients with different irritable bowel disease (IBS) phenotypes and healthy controls; note that the diarrhoea predominant (D-IBS) patients in Turkey have predominantly the l/s genotype in contrast with none of the 18 patients with the l/l genotype. Note also that controls in the two series from North America have very similar distributions but the proportions differ from controls in Turkey, suggesting significant racial variation. Finally, note that in the series of Yeo et al from North America, there are relatively more D-IBS patients with the s/s genotype compared with controls but the actual proportion of the three genotypes in D-IBS was very similar. C-IBS, constipation predominant irritable bowel syndrome.
European-Americans, 16 and 24% in Turks. 14 This variation in background prevalence of a genotype clearly influences the statistical power to detect a genotype related difference.

**Racial homogeneity of control and disease groups:**

In the current paper by Yeo and colleagues, 1 there were no differences in the proportion with the 1/1 genotype in D-IBS patients and controls. In contrast, they observed differences in the distribution of s/s and l/s between controls (17.2% and 47.8%, respectively) and D-IBS (31.4% and 31.9%, respectively). Regrettably, the authors did not provide critically important data on the racial derivation of patients and controls participating in this study. They included a larger number of controls to attempt to suppress confounding effects such as population stratification and admixture. The proportions in predominately European-Americans of the s/s and l/s genotypes of 19% and 49%, respectively, 20 and 20% and 49%, 26 suggest that the controls in the paper by Yeo and colleagues 1 were similar to two other independent control cohorts. This is reassuring as the 448 control DNA sources. In contrast, the study by Kim et al. which did not show an association between SERT-P polymorphisms and D-IBS (n = 128) or other IBS phenotype, drew patients (n = 276) and controls (n = 120) from the same geographical region in the Mid-West of the USA and provided detailed information about the sex and race of participants. Thus female participants predominated in both lower functional gastrointestinal disorder patients (82%) and controls (79%), and European Americans were 89% of controls and 97% of patients; there were 6% Asian and 3% Hispanic among healthy participants. Control for ethnic differences is critically important in appraising disease associations between genotype and phenotype.

**Interpretation of data from surrogate measurements**

Although the structure and biochemistry of SERT molecules at different sites are similar within the same species, it is unclear whether the results obtained in binding studies of SERT in platelets really reflect the function of enteric SERT. For example, binding studies of SERT in brainstem nuclei may be normal in diseases such as major depression whereas other studies suggest that platelet SERT function is abnormal in depression. 25

**Genetic dissections of complex diseases are extremely complex**

Investigations of gene-gene and environment interactions in humans and animals are revealing genes that may underlie behavioural variations. However, studies also lead to the notion expressed by Reif and Lesch that a probabilistic rather than deterministic impact of the genes can be identified on the phenotypic expression of these behavioural or functional disorders. 1 To understand the probabilistic impact or contributions each genetic variation in a semi quantitative manner requires studies with large sample sizes. Such studies are laborious and difficult, particularly in IBS where there is no diagnostic test.

**CONCLUSION**

A hypothesis proposed by Blakley 26 is not that IBS is equivalent to SERT dysfunction but that SERT dysfunction may present with a gastrointestinal phenotype or as a behavioural disorder. Anxiety disorders are commonly observed to be comorbid with IBS. 27 28 SERT dysfunction may contribute to behavioural and functional gut disorders; however, as previously noted by others, the influence of a single polymorphism on continuously distributed traits is likely to be small in humans. Much work needs to be done to assess the influence of genetic variation of SERT in the manifestations and response to therapy 29 of irritable bowel syndrome before we can be certain of the role of SERT genetics in D-IBS. Acquired changes in SERT mRNA in animal models of colitis 30 and in patients with ulcerative colitis and IBS 31 suggest that there specific molecular alterations deserve further study.

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Diet and relapsing ulcerative colitis: take off the meat?

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Dietary factors such as red meat, high protein intake, and alcohol are associated with relapse in ulcerative colitis, probably mediated via hydrogen sulphide production.

Major advances in the understanding of the aetiopathogenesis and genetics of inflammatory bowel disease (IBD) have been accompanied by an increase in the therapeutic armamentarium, including immunosuppressants and anticytokine drugs. Whereas patients are in many cases highly motivated to use prescribed drugs in those chronic disorders, they would be even more willing to change lifestyle and dietary habits so they could actively influence the course of their disease. Therefore, one of the most common questions physicians treating patients with IBD are asked is whether changing diet could positively affect the course of their disease. So far, and this has been especially true for patients with ulcerative colitis (UC), our answer had been “we do not know and there are no special recommendations”.

This may now change as Jowett and colleagues1 in this issue of Gut present interesting and clinically novel data studying the role of dietary factors on the clinical course of UC (see page 1479). In this prospective cohort study, they investigated the effects of habitual diet on relapses of disease. Impressively, 96% of patients (n = 191) completed the study. Dietary factors such as red and processed meat, protein, and alcohol, as well as sulphur and sulphate intake were positively associated with relapses. Even though dietary factors in this study were less important than measures of prior disease activity in determining the risk of relapse,2 clinically this information is extremely valuable as it may open the perspective of lifestyle modification in the treatment of UC. As discussed in detail by the authors, these effects may be related to sulphur and sulphate contents of food. Most importantly, to continue to prove this important work, in a next step an intervention study is needed before it can be concluded that sulphur and sulphate rich foods definitely increase the risk of relapse. However, caution with regard to interpretation of the data of Jowett et al is also warranted. Habitual diet was assessed only once and therefore might not a priori reflect food intake over the entire observation period, especially the period immediately preceding relapse. Furthermore, based on energy intake estimates by physical activity level (PAL), 23% of patients over- or under-reported their food intake. When these patients were excluded from the analysis, only high oil meat intake (particularly red and processed meat) and high alcohol consumption were associated with relapse while all other associations were no longer statistically significant. Provocatively, therefore, it may well be speculated that other constituents of meat besides sulphur compounds (see below) might play a role in relapsing UC.

Pre-illness diet and subsequent development of UC

As the rising incidence of IBD in the last decades coincides with profound changes in diet pattern, various lifestyle/dietary aspects have been addressed. Available studies mainly investigated the effects of pre-illness diet and subsequent development of UC. Russel et al performed a case control study in recently diagnosed cases with UC.3 They showed a positive correlation between consumption of soft drinks and chocolate and UC. In another study, a high fat intake was associated with an increased risk for UC whereas a negative correlation with vitamin C and fruit consumption was found.4 A third study also showed a positive correlation with fat intake and development of UC.5 All of these findings however may also be an expression of modern lifestyle involving other risk factors for the development of IBD.

Hydrogen sulphide: Bad malodorous gas responsible for relapses in UC?

Mercaptides such as sodium hydrogen sulphide (one of the main malodorous compounds in human flatus) are reducing agents that help maintain anaerobic conditions in the colonic lumen. They are produced in the human large intestine by bacterial reduction of dietary inorganic sulphate and sulphite and by fermentation of sulphur amino acids. The acute toxicity of hydrogen sulphide appears to result from inhibition of cytochrome oxidase leading to mucosal damage, loss of barrier function, and histological changes resembling UC. Hence the colonic mucosa has developed a very effective means of detoxifying hydrogen sulphide.6

Mainly exogenous sources contribute to the colonic pool of sulphur, such as red meat, cheese, milk, fish, nuts, and eggs, and as preservatives found in commercial breads, beers, many alcoholic drinks, sausages, and dried fruits. Faecal sulphide levels increase after consumption of increasing amounts of meat,7 providing evidence that meat is an important substrate for sulphide generation by bacteria in the human large intestine.

It has been proposed that sulphide toxicity may be important in the pathogenesis of UC.8 9 The initial evidence in this regard was demonstration that...
experimental exposure of colonic tissue to sulphide causes inhibition of butyrate use (see below), a defect similar to that observed in mucosal biopsies obtained from UC patients. 11 UC patients have significantly higher luminal concentrations of hydrogen sulphide than controls, and disease activity correlates with sulphide production rates. 12 Hydrogen sulphide induces hyperproliferation of colonic mucosa and this effect is antagonised by butyrate. 13 Treatment with 5-aminosalicylates and bismuth subsalicylates has been shown to reduce hydrogen sulphide production in the colonic lumen. 14 Apart from the direct toxicity of hydrogen sulphide, it has been speculated that thiols may react with sulfhydryl containing compounds to form persulfides, which may alter protein function as well as antigenicity, which could theoretical lead to a chronic immune mediated process, as known in UC. 4 In summary, there is evidence in the literature that hydrogen sulphide may play a role in UC and on the other hand certain foods such as meat cause an increase in colonic levels of hydrogen sulphide.

**FAECAL BUTYRATE: KEY METABOLITE IN UC**

Short chain fatty acids, including butyrate, propionate, and lactate, are generated in the colon as result of bacterial fermentation of dietary fibre by luminal bacteria such as *Bifidobacterium*, *Eubacterium*, and *Lactobacillus* species. Roediger et al demonstrated significant inhibition of butyrate but not glucose oxidation by hydrogen sulphide in the ascending colon, splenic flexure, and in the rectosigmoid region. 15 A direct anti-inflammatory effect for butyrate, the most extensively studied of the short chain fatty acids, may be attributable to its inhibition of nuclear factor κB, thus preventing the transcription of pro-inflammatory cytokines. 16 In this study, butyrate also attenuated dextran sulphate sodium (DSS) induced colitis. Furthermore, butyrate has been demonstrated to reduce colonic permeability by enhancing peroxisome proliferator activated receptor γ (PPAR-γ) activation. This is of special interest as PPAR-γ ligands show antineoplastic and anti-inflammatory effects in experimental colitis. 17 Patients with active extensive UC have decreased colonic butyrate oxidation. As remission of disease is associated with normalisation of butyrate oxidation, UC mucosa is not intrinsically altered in butyrate oxidation. 18 Butyrate enemas have been shown to be of benefit in the management of distal UC. 19 20 So, how to increase faecal butyrate levels? In animal and human studies, ingestion of resistant fibre has resulted in an increase in the population of *Bifidobacterium* and *Lactobacillus* in the colon and an increase in faecal butyrate concentrations. Administration of oat bran over three months to UC patients in remission (corresponding to 20 g dietary fibre) has recently been shown to result in increased faecal butyrate levels and in this pilot study no relapses were observed. 21 Alternative strategies of delivering short chain fatty acids to the inflamed colon are by providing a substrate, a “prebiotic”, for short chain fatty acid production by colonic bacteria, or directly delivering probiotics to the intestinal lumen.

**PREBIOTICS IN UC: EFFECTIVE VIA BUTYRATE INDUCTION?**

Prebiotics are defined as non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth or activity of bacterial species already present in the gut. A germinated barley foodstuff (GBF) which contains hemicellulose rich fibre and glutamine rich protein has been shown to attenuate inflammation in DSS, trinitrobenzene sulphonic acid (TNBS), and HLA-B27 transgenic animal models of colitis. 1 In DSS induced colitis GBF suppressed significantly serum interleukin 6 levels and mucosal STAT-3 expression. These effects may be caused by increased faecal butyrate production. 22 GBF has been demonstrated to improve disease activity in a small pilot study in patients with active UC. 23 In a controlled small study investigating 18 patients with active UC, patients were treated with baseline anti-inflammatory treatment with or without GBF. GBF therapy resulted in a significantly better outcome and was associated with increased faecal concentrations of *Bifidobacterium* and *Eubacterium limosum*. 24 Another important study in the area of prebiotic and dietary fibre has been performed by a Spanish collaborative group. 25 In this large study, *Plantago ovata* seeds (dietary fibre 20 g/d) was compared with mesalamine in the maintenance of remission in patients with UC (n = 105). Treatment failure rate was 40% in the *Plantago ovata* seed group, 35% in the mesalamine group, and 30% in the *Plantago ovata* plus mesalamine group. A significant increase in faecal butyrate levels was observed after *Plantago ovata* seed administration. The same preparation was also shown to ameliorate colonic damage in HLA-B27 transgenic rats and this effect was also associated with increased production in short chain fatty acids. 26 Therefore, most of prebiotic products might exert their beneficial effects via modulating short chain fatty acid metabolism.

**FISH OIL SUPPLEMENTATION/ ESSENTIAL FATTY ACIDS: NOT EFFECTIVE IN THE TREATMENT AND MAINTENANCE OF REMISSION IN UC**

Prostaglandin E2 and leukotriene B4 are metabolites of arachidonic acid via the cyclooxygenase pathway and the lipoxigenase pathway, respectively. Increased levels of prostaglandin E2 and leukotriene B4 are found in active UC. Diets containing high levels of n-3 fatty acids such as eicosapentaenoic acid and docosahexaenoic acid are known to modify leukotriene production. Dietary fish oil supplementation has improved patients with other inflammatory disorders, such as rheumatoid arthritis. Five placebo controlled double blind studies have addressed this question in UC. 29–33 Despite reduced levels of leukotriene B4, some histopathological improvement, and a tendency towards a steroid sparing effect, no overall convincing clinical benefit of dietary fish oil supplementation for 4–12 months was seen in the treatment of patients with active UC. Most of these trials involved treatment of active disease whereas one study failed to demonstrate any benefit in maintenance therapy. A recent randomised controlled trial again failed to demonstrate any efficacy of essential fatty acid supplementation in the maintenance of remission in UC. 41 Therefore, despite some modest effects of n-3 polyunsaturated fatty acids in the treatment of active mild to moderate disease, essential fatty acids combination therapies have no benefit in maintaining remission in UC.

**ANTIOXIDANTS AND OXIDATIVE STRESS**

Oxidative stress is believed to play a key role in the pathogenesis of IBD as intestinal inflammation is accompanied by excessive production of reactive oxygen species and nitrogen metabolites. D’Odorico et al showed increased free radical peripheral leucocyte DNA damage and decreased plasma antioxidant defences in both UC and Crohn’s disease patients. 26 27 N-3 fatty acids have been shown to increase antioxidant concentrations, although as mentioned, n-3 polyunsaturated fatty acids are not effective in the therapy of UC. Dietary iron supplementation increases lipid peroxidation, decreases antioxidant vitamins, and enhances DSS induced colitis in rats. 28 Studies investigating the effects of oral iron administration in humans are lacking, as well as clinical studies assessing the

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CONCLUSION
In summary, this provocative and clinically important report by Jowett et al. reopens the topic of diet and relapsing UC. The findings are well taken and may offer a new perspective for potential intervention by practical lifestyle modifications, and as such are eagerly awaited by our patients. Despite this excitement, interventional studies are now needed, setting the scene for specific dietary recommendations and for further defining the role of sulphur/ sulphate which may even lead to novel therapies.

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