Challenges and prospects for pharmacotherapy in functional gastrointestinal disorders

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Abstract: Functional gastrointestinal disorders, such as irritable bowel syndrome and functional dyspepsia, are complex conditions with multiple factors contributing to their pathophysiology. As a consequence they are difficult to treat and have posed significant challenges to the pharmaceutical industry when trying to develop new and effective treatments. This review provides an overview of these difficulties and how the industry is reshaping its drug developmental strategies. It describes some of the more significant and encouraging advances that have occurred, and discusses how future research might embrace the opportunities provided by advances in genetic and in particular, epigenetic research.

Keywords: animal models, epigenetics, IBS, mechanisms, pharmacotherapy

Introduction

There is no doubt that functional gastrointestinal disorders (FGIDs), such as irritable bowel syndrome (IBS) and functional dyspepsia (FD), are an enormous worldwide burden on healthcare, inflicting serious reductions in quality of life (QOL) for those patients who are unfortunate enough to suffer from such a disorder. At the extreme, for example, patients no longer enjoy or tolerate the simple pleasures of eating and may have their lives governed by the need to have immediate access to toilet facilities. It seems surprising, therefore, that there is not a greater universal sense of urgency to accelerate research funding and drug development in this area. Indeed, recent reductions in both preclinical and clinical gastrointestinal (GI) research within the pharmaceutical industry (especially where the focus was on FGIDs) serve both to highlight and exacerbate the issue. These changes are not common to all pharmaceutical companies, but notable recent events range from the closure of focused preclinical GI research (with any development opportunities in GI therapeutics arising only from ‘generic’ research into mechanisms such as pain or inflammation) to entrepreneurial ‘spin offs’ of GI research from large pharmaceutical companies, into small biotechnology companies.

Many different factors can be argued to contribute to this relative paucity of research into the causes and treatment of FGIDs. These range from the simple failure to recognize the severity of damage inflicted by these disorders on QOL, to more complex issues that relate to the difficulties associated with diagnosis, pathophysiology, patient reported outcome measures, and drug development.

The purpose of this review is to describe some of the significant and encouraging advances that are now helping to reshape and hopefully, regrow the research that needs to be directed towards GI disorders and FGIDs in particular. These advances include the recent registration of lubiprostone in the USA (for chronic idiopathic constipation, then constipation-predominant IBS) and prucalopride in the EU (chronic idiopathic constipation), improvements in drug development strategies for non-FGIDs (a ‘spin off’ from the recent difficulties), better patient reported and physiologic endpoints, new approaches to the symptomatic treatment of FGIDs and, most excitingly, the beginnings of GI research to embrace the opportunities provided by advances in epigenetic research. Together, these developments offer the possibility of discovering more effective treatments for this

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group of patients, who live with conditions that are frequently difficult to manage.

Significant difficulties in drug discovery for FGIDs

FGID is a term which covers different groups of patients, each with a high probability of subgroups and, by definition, each having no recognized lesions and no specific reliable biomarker [Drossman *et al.* 2006]. Not surprisingly, therefore, there are significant challenges in trying to develop new treatments for these disorders, with a trend developing to first demonstrate efficacy in a non-FGID group of patients (where there is a well-defined clinical endpoint that is directly relevant to the mechanism under study), before exploring the efficacy of that compound in a subgroup of FGID patients (e.g. for new drugs affecting GI motility [Sanger and Alpers, 2008]). These difficulties have been discussed in detail in previous reviews [Camilleri and Chang, 2008; Mayer *et al.* 2008], but a brief description of some of the difficulties is given in the following.

Patient-reported outcomes

Patient-reported outcomes are used in clinical trials due to a lack of a reliable biomarker in FGIDs, such as IBS and FD. Previously used primary endpoints such as binary or global improvement endpoints have recently fallen out of favor due to the perception by regulatory agencies that they lack content validity and have not been tested adequately in the target population. There are ongoing collaborative efforts to develop and validate outcome measures to be used in human clinical studies as mandated by the US Food and Drug Administration (FDA) in their Patient Reported Outcome (PRO) Guidance Document released in 2006 [Talley, 2009; US Department of Health and Human Services FDA Center for Drug Evaluation and Research; US Department of Health and Human Services FDA Center for Biologics Evaluation and Research; US Department of Health and Human Services FDA Center for Devices and Radiological Health, 2006]. Patient-derived outcome measures which capture the patient's experience are currently being explored, but the hope in the future is to determine objective measures which can reliably diagnose and measure treatment response in FGID patients. For a detailed discussion, see Camilleri and Chang [2008].

Physiologic subgroups in patients

There is increasing evidence that FGIDs are complex conditions with multiple factors contributing to their pathophysiology. In patients with IBS, for example, genetic predisposition, infection, and early adverse life events may each predispose individuals to developing the disorder [Spiller and Garsed, 2009; Chitkara *et al.* 2008; Saito and Talley, 2008]. In addition, chronic stress, psychological symptoms, and maladaptive coping mechanisms can increase symptom exacerbations, illness severity and adverse outcomes in affected individuals [Levy *et al.* 2006]. Such a diversity of possible contributing factors suggests diversity in the pathways that generate symptoms and, hence, a low probability that treatments which target only one pathway will find widespread clinical benefit.

A number of studies conducted in different laboratories, again in patients with IBS, have demonstrated enhanced visceral perception but this is not a finding that is replicated by all [Camilleri and Chang, 2008]. There are also difficulties in interpreting visceral sensitivity studies, which deploy different techniques and do not distinguish between affective, cognitive and true peripheral and/or central mechanisms of increased visceral perception. For example, studies have found that only 40–60% of IBS patients have lowered pain thresholds [Posserud *et al.* 2007; Whitehead and Palsson, 1998] and only 16–37% have increased sensory ratings [Camilleri *et al.* 2008a; Posserud *et al.* 2007] to balloon distension. In addition, whilst recent studies have shown that pain and bloating are highly correlated with measures of sensory ratings [Posserud *et al.* 2007], there is only a modest correlation with perceptual thresholds and symptom ratings [Mayer *et al.* 2008]. Moreover, symptom severity in a single patient does not reliably predict whether they will be hypersensitive or not. Thus, assessment of potential visceral analgesics in patients defined on symptomatology or bowel habit predominance alone, in which a significant proportion will not be hypersensitive, may be unlikely to show beneficial effects on visceral sensitivity. This is supported by the observations that hypnotherapy improves abdominal pain in association with an improvement in rectal sensitivity in patients who are rectally hypersensitive prior to treatment, but not those who are not [Lea *et al.* 2003]. Thus, there is potential for such tests to be able to identify patients with hypersensitivity who may then

respond more favorably to visceral analgesics, but further research is required.

Colonic transit is normal in most patients, with one comprehensive study showing abnormal transit times in only 16% of IBS patients with constipation (IBS-C), 17% of IBS patients with mixed bowel habit (IBS-M), and 46% of IBS patients with diarrhea (IBS-D) [Camilleri *et al.* 2008a]. Similar observations have been made for orocecal transit in IBS [Agrawal *et al.* 2009; Sadik *et al.* 2008]. However, despite the low number of patients appearing to have abnormal transit, drugs which normalize GI transit in phase IIb and III IBS clinical trials have generally been shown to improve stool frequency or consistency along with abdominal symptoms in clinical efficacy trials (e.g. alosetron, tegaserod, prucalopride, lubiprostone, linaclotide [Camilleri and Chang, 2008]). The reasons for this apparent mismatch are not clear, but recent studies in patients with constipation have shown them to exhibit abnormal fasting and/or postprandial motility despite having normal transit [Ravi *et al.* 2009]. Thus, it is possible that these drugs may relieve symptoms by improving bolus transit through an uncoordinated gut or indeed improving the coordination and patterns of motility seen in the GI tract itself. For some (but not all) of these treatments another possible reason for symptom improvement may be that these efficacious therapies not only affect transit but also exert other beneficial effects, e.g. visceral analgesia with alosetron acting at 5-HT₃ receptors and, possibly, tegaserod acting at 5-HT_{2B} receptors (see the 'New developments in drug discovery' section).

Greater efficacy would more likely be achieved if patients were stratified based on pathophysiology (e.g. visceral hypersensitivity, abnormal motility or delayed transit). Indeed the emergence of new and noninvasive technologies such as the wireless capsule that measures motility and pH [Camilleri *et al.* 2008e] and capsule endoscopy using endoluminal image analysis [Malagelada *et al.* 2008], may allow gastroenterologists in the future to better identify those patients most likely to respond to such interventions. However, further validation studies are required (see Camilleri *et al.* [2008e] for further discussion on such technologies), along with trials in appropriate physiologic patient groups with treatments.

Animal models

It is not possible to model a FGID in animals, given that the causes and pathways in humans are so poorly understood. It has, therefore, been common practice to design potential new drugs to target a particular mechanism, avoiding the need to rely on an animal model of the FGID. However, although animal studies which demonstrate increases or decreases in GI motility usually do translate to human studies, recent history has seen a notable failure of animal models to predict the effects of new drugs on human pain or discomfort [e.g. those acting at neurokinin-1 (NK₁) or corticotropin releasing factor-1 (CRF₁) receptors; see the 'new developments in drug discovery' section] [Mayer *et al.* 2008], the main symptom for IBS and FD. Even complex models of visceral pain (e.g. maternal separation) still suffer from the problem of not knowing what the correct readout should be: these models cannot measure supraspinal aspects, for example. It is, therefore, difficult to escape from the conclusion that to make progress in areas of research focused on hypersensitivity or visceral sensations, we should not wholly rely on any animal model. A more detailed review of the difficulties associated with the translation of animal models to man can be found in Mayer *et al.* [2008].

Lower acceptance of risk relative to benefit for FGID treatments

Although IBS has been shown to be associated with a significant healthcare and economic burden [Longstreth *et al.* 2006; Sandler *et al.* 2002], relatively little risk is accepted by regulatory agencies and many healthcare providers despite the beneficial effects on symptom severity and health-related quality of life (HRQOL). The latter was exemplified most recently by the problems unexpectedly encountered with the 5-HT₃ receptor antagonist alosetron (approved for IBS-D in females in 2000) and the 5-HT₄ receptor agonist tegaserod (approved in the USA and other countries as a treatment for IBS-C and idiopathic chronic constipation). Both of these agents were proven in multicenter, large, high-quality clinical trials to significantly relieve symptoms of these FGIDs [Ford *et al.* 2009]. However, shortly after the introduction of alosetron for clinical use, a small but significant incidence of reversible ischemic colitis (approximately 0.1%) led to its withdrawal from the market, followed later by a reintroduction under a risk-management program [Chang *et al.* 2006; Andresen and Hollerbach, 2004]. While the etiologic mechanism of the ischemic colitis remains

unclear [Camilleri, 2007; Chang *et al.* 2006], it has led to increased scrutiny when evaluating the safety of other 5-HT₃ receptor antagonists being developed for treatment of IBS [Pasricha, 2007]. For example, cilansetron has also been associated with episodes of ischemic colitis [De Giorgio *et al.* 2007] and following phase III trials, USA registration activities were suspended in 2005 and development has now ceased (see <http://www.solvaypharmaceuticals-us.com/newsroom/pressreleases/0,36567-2-0,00.htm>).

The reasons for the withdrawal of tegaserod were less obvious than those for alosetron. For this drug, concern over the risk–benefit profile was initially raised after a cross-study safety analysis found that 13 of 11,614 (0.1%) treated patients had ischemic cardiovascular events, compared with 1 of 7013 (0.01%) on placebo. However, since withdrawal, the real association between tegaserod and cardiovascular adverse events has been questioned, pointing out that these adverse events mainly occurred in older patients with pre-existing cardiovascular disease and/or cardiovascular risk factors [Schiller and Johnson, 2008]. Further, other 5-HT₄ receptor agonists are still in clinical use [Karamanolis and Tack, 2006] and while not being used for treatment of IBS-C patients, the availability of such drugs does question any link between activation of the 5-HT₄ receptor and any cardiovascular adverse event.

These difficulties in new drug development for IBS, and especially the experience with tegaserod, serve to highlight a risk-averse attitude to the treatment of IBS [Schiller and Johnson, 2008].

New developments in drug discovery

The different drug targets proposed for IBS and other functional bowel disorders have been reviewed previously (see, e.g., Camilleri and Chang [2008]). In this section, new developments are summarized.

5-Hydroxytryptamine

New ways of modulating 5-hydroxytryptamine (5-HT) availability or 5-HT receptor function have recently been discussed [Sanger, 2008], along with alternative ideas to examine the functions of other products of tryptophan metabolism. However, the most advanced approaches still remain those exemplified by the selective 5-HT₃ receptor antagonist alosetron and the 5-HT₄ receptor agonist tegaserod. Despite the

difficulties referred to above and consequent reduced interest in future development of other 5-HT₃ receptor antagonists, significant progress has been achieved in bringing forward new 5-HT₄ receptor agonists [Sanger, 2009].

In October 2009 the European Commission approved the 5-HT₄ receptor agonist prucalopride for female patients with chronic idiopathic constipation. Multicenter trials have demonstrated its improvement in constipation symptoms and HRQOL [Tack *et al.* 2009; Camilleri *et al.* 2008b]. There are several points of differentiation from tegaserod. First, the latter was approved by the FDA (but not in Europe) for IBS-C and then for chronic constipation; the difference reflects a new trend for first developing a GI compound in an indication with a clearer mechanism-based rationale, rather than a complex symptom-based disorder such as IBS. Second, prucalopride has higher intrinsic activity at 5-HT₄ receptors within the human colon [Cellek *et al.* 2006] and will be the first clinically available 5-HT₄ receptor agonist to have selectivity for the receptor [Briejer *et al.* 2001]. Tegaserod was recently shown to potently antagonize 5-HT_{2B} receptors [Beattie and Smith, 2008], a pharmacology potentially conferring analgesic properties [Ohashi-Doi *et al.* 2010; O'Mahony *et al.* 2010] but suggested to slow colonic motility [Bassil *et al.* 2009] and further contribute to poor efficacy in IBS-C patients. Finally, extensive evaluation has demonstrated a good cardiovascular safety profile for prucalopride [Camilleri *et al.* 2009]. It will now be interesting to learn if prucalopride will now be evaluated in patients with IBS-C and how other selective 5-HT₄ receptor agonists, such as TD-5108 [Beattie *et al.* 2008; Smith *et al.* 2008] will differentiate themselves from prucalopride.

Intestinal secretion

The third drug recently registered for treatment of IBS-C is lubiprostone, an activator of ClC-2 chloride channels expressed on the luminal surface of intestinal epithelial cells. Subsequent opening of the cystic fibrosis transmembrane conductance regulator secretes chloride and bicarbonate ions into the intestinal lumen, increasing fluid secretion into the small intestine, decreasing colonic fluid absorption and promoting GI transit [Forte, 1999]. Lubiprostone was also initially developed for chronic idiopathic constipation [Johanson and Ueno, 2007].

Subsequent trials [Drossman *et al.* 2009] resulted in USA marketing approval for female patients with IBS-C [Lang, 2008]. It is worth noting that lubiprostone was derived from PGE₁ and may activate prostaglandin E (EP) receptors in the human colon [Bassil *et al.* 2008]. It is not known whether this additional activity contributes to the clinical profile of lubiprostone.

A second but similar approach to stimulating intestinal motility by increasing intraluminal fluid is exemplified by the guanylate cyclase C receptor agonist linaclotide. This compound, which does not achieve systemic exposure after oral administration, has been shown in women with IBS-C to increase colonic transit, stool frequency and consistency, ease of passage and time to first bowel movement [Andresen *et al.* 2007]. Recently, studies have reported that linaclotide significantly improves the other cardinal symptoms of IBS including abdominal pain [Lembo *et al.* 2009] as well as symptoms in chronic functional constipation [Johnston *et al.* 2009].

Pain processing

This is an especially difficult area, marked by great difficulty in translating animal data to humans. Perhaps the failed promise of NK₁ receptor antagonists [Hill, 2000] is now being followed by CRF₁ receptor antagonists. Thus, in two studies, the wealth of animal (and human volunteer) studies that demonstrate positive effects of CRF₁ receptor antagonists did not translate in terms of symptom control in IBS patients [Dukes *et al.* 2009; Sweetser *et al.* 2009]. The difficulty of this area is further highlighted by the tricyclic antidepressants such as desipramine and amitriptyline. Desipramine for example, has shown positive outcomes against pain endpoints in IBS patients associated with mental stress [Kuiken *et al.* 2005]. However, the polypharmacology of tricyclic antidepressants (e.g. amitriptyline may also have muscarinic receptor and sodium channel affinity [Bielefeldt *et al.* 2002; Snyder and Yamamura, 1977]) make it difficult to know which action is best optimized in order to identify a new and more efficacious drug.

New approaches [Bulmer *et al.* 2007] include the gabapentinoids (e.g. pregabalin, gabapentin), thought to bind to the $\alpha_2\delta$ subunit of voltage-gated calcium channels and thereby reduce neurotransmitter release [Davies *et al.* 2007] and

increase pain thresholds to colorectal distension in IBS patients (e.g. Houghton *et al.* [2007]). A new gabapentinoid PD-217014 [Ohashi *et al.* 2008] has undergone phase II trials for IBS [Ashburn and Gupta, 2006], but results have not been reported. A second new approach is highlighted by the ability of a beta₃-adrenoceptor agonist to inhibit visceral pain in rodents, an effect blocked by the somatostatin SST2 receptor antagonist CYN 154806 [Cellek *et al.* 2007]. This represents a novel way of manipulating the analgesic actions of somatostatin, further highlighted by the report of a clinically meaningful increase in adequate relief of IBS pain and discomfort following treatment with the beta₃-adrenoceptor agonist solabegron [Kelleher *et al.* 2008]. Further developments of this class of agent are awaited with interest.

The hormonal gut–brain axis associated with eating

Different hormones are released from the gut during fasting or after eating, to help regulate appetite and the ways the gut responds to these situations. Compounds which regulate these actions might help treat certain functional bowel disorders [Sanger and Lee, 2008]. Unfortunately, in the first of three current examples, the long-awaited promise of cholecystokinin-1 (CCK₁) receptor antagonists (for treatment of FD, IBS-C or critically ill patients receiving lipid-enriched enteral feeding) has yet to be translated into a new drug. A second example stems from the widespread use of the antibiotic and motilin receptor agonist erythromycin as a gastric prokinetic agent; new, selective motilin receptor agonists are currently being developed [Sanger *et al.* 2009; McCallum *et al.* 2007]. Finally, TZP-101 represents the most advanced ghrelin receptor agonist, being an intravenously administered compound shown to increase gastric emptying [Ejskjaer *et al.* 2009] and achieve positive phase II results in patients with gastroparesis or postoperative ileus (see <http://www.tranzyme.com>); an orally available compound (TZP-102) is also under evaluation. The role of motilin and ghrelin receptor agonists in functional dyspepsia has not yet been explored, although for at least some FD patients with delayed gastric emptying, a gastric prokinetic may be effective (e.g. improvements in symptoms noted after treatment with tegaserod in patients with dysmotility-like symptoms [Vakil *et al.* 2008]).

Colonic microbiota

An area of research currently receiving extensive attention is the idea that changes in this enormous depository of bacteria might influence the development of the gut, its susceptibility to stress (see Shanahan and Kiely [2007] and references therein) and major disorders such as obesity [Turnbaugh *et al.* 2006], inflammatory bowel [Frank *et al.* 2007] and IBS [Kassinen *et al.* 2007]. With respect to IBS, many of the early studies were of poor design, only assessed small numbers of patients and generally showed limited efficacy [Brenner *et al.* 2009; Moayyedi *et al.* 2008; Spiller, 2008]. More recently, however, larger randomized placebo-controlled trials of higher quality with *Bifidobacterium infantis* have reported efficacy in IBS [Moayyedi *et al.* 2010; Brenner *et al.* 2009; Spiller, 2008]. A particularly interesting animal study is the demonstration that oral administration of *Lactobacillus acidophilus* reduced butyrate-induced hyperalgesia in rats, to a level approximately similar to that of a single dose of morphine (1 mg/kg sc) [Rousseaux *et al.* 2007]. In the same study the expression of mu-opioid and cannabinoid CB₂ receptors was also increased within an intestinal epithelial cell line exposed to the same bacteria. However, as in pharmaceutical development caution is required when extrapolating effects seen in animal models to man. For example, whilst both *B. infantis* 35624 and *Lactobacillus salivarius* UCC4331 were shown to have an anti-inflammatory effect in animal models, only *B. infantis* 35624 was shown to have beneficial effects on symptoms and the ratio of serum anti-inflammatory (interleukin-10 [IL-10]) to a proinflammatory (IL-12) cytokine in patients with IBS [O'Mahony *et al.* 2005]. In addition, caution is required when extrapolating findings of one to another probiotic, as no two are the same, or to a probiotic combination. Other notable findings include the reduction in abdominal distension experienced by patients with IBS-C (which directly correlates with transit time [Agrawal *et al.* 2009]) following administration of the probiotic *Bifidobacterium lactis* DN-173 010 which accelerates both orocecal and colonic transit [Agrawal *et al.* 2008]. Although, such studies do not easily lend themselves to new, small molecule drug discovery, there are clear opportunities for new probiotic or bacterial approaches [Shanahan and Kiely, 2007].

An alternative strategy to modulate the gut microflora in IBS is the use of antibiotics such

as neomycin, metronidazole and rifaximin. Rifaximin is a broad-spectrum antibiotic with very low systemic absorption that has shown some efficacy in the treatment of IBS. Two initial placebo-controlled studies conducted in IBS patients demonstrated that a 10-day course of rifaximin (400 mg bid or tid) was associated with a greater improvement in global symptoms [Pimentel *et al.* 2006] or bloating [Sharara *et al.* 2006]. In a phase IIb, multicenter, placebo-controlled study, treatment of IBS-D patients with rifaximin was associated with significantly greater adequate relief of global IBS symptoms (52% versus 44%) and bloating (46% versus 40%) which was maintained at the end of the 12-week follow-up period [Lembo *et al.* 2008]. Phase III trial results preliminarily appear to look promising in nonconstipated IBS patients, but complete data are pending. Further investigations are needed including those to see whether the patients who benefit from such treatments truly fulfill the definition of IBS (no recognizable 'lesion') or represent a different disorder which can now be defined by aberrant bacterial function.

Where to next: genetics and epigenetics

It is timely to consider a novel approach to drug treatment in patients with FGIDs. Thus, as eluded to throughout this review, we increasingly recognize the heterogeneous symptomatology observed in FGID patients (Table 1) and the array of pathophysiological mechanisms that could contribute to this battery of symptoms [Drossman *et al.* 2006]. There is also an as-yet unresolved debate over the initiating/etiological/contributing factors underlying this spectrum of conditions. Finally, experience of trying to develop novel therapeutic agents for these patients (see previous sections) leads to the conclusion that targeting one or even a few of the late-stage mediators is likely to deliver, at best, a therapeutic with limited efficacy, in a defined subpopulation of these patients.

Epidemiologic studies have supported a genetic contribution to the development of IBS and its phenotypic expression. Two population-based studies found that the presence of IBS symptoms is significantly associated with a first-degree relative with GI symptoms compatible with IBS [Kalantar *et al.* 2003; Locke *et al.* 2000]. In addition, twin studies for the most part have demonstrated a significantly greater concordance of IBS between monozygotic twins (identical; 100%

Table 1. Summary of numerous potential etiologies and pathophysiological mechanisms underlying the spectrum of symptoms in functional bowel disorders. A key challenge is understanding the contribution of each in a given patient to ultimately help guide patient selection for experimental clinical studies with new agents. Each initiating factor or pathological mechanism is likely to underlies several of the described symptoms.

Etiology	Pathophysiology	Symptomatology
Infection/inflammation Childhood stress/abuse Antibiotic treatment Genetic polymorphisms and epigenetic modifications	Altered epithelial permeability Increased neuroimmune interactions Sensitized spinal afferents Altered vagal modulation Altered HPA function Altered compliance Altered microbiota Altered pain modulation Altered motility/transit Altered secretion	Pain Bloating Urgency Referred pain Diarrhea and/or constipation Hypervigilance/anxiety Comorbid fibromyalgia, migraine
HPA, hypothalamic–pituitary–adrenal.		

shared genes) than dizygotic twins (fraternal; 50% shared genes) which further support a genetic component in IBS [Lembo *et al.* 2007; Bengtson *et al.* 2006; Mohammed *et al.* 2005; Levy *et al.* 2001; Morris-Yates *et al.* 1998].

Studies by Levy and colleagues suggest that while there is likely a genetic predisposition to developing IBS, environmental factors may make an even stronger contribution [Levy *et al.* 2000, 2001]. For example, both the probability that a dizygotic twin (15.2%) or a monozygotic twin with IBS (17.1%) will have a mother with IBS is significantly greater than the probability that a dizygotic twin with IBS will have a cotwin with IBS (6.7%) [Levy *et al.* 2001]. In another study, these investigators found that children of adult IBS patients make more healthcare visits for GI and non-GI complaints than children of non-IBS parents [Levy *et al.* 2000]. These studies suggest that environmental factors, e.g. nurturing effects or learned illness behavior, have a significant effect on the development of IBS.

The possibility that symptoms of FGIDs might be derived from a genetic polymorphism is beginning to be explored. A number of genetic association studies have been performed in order to determine whether particular candidate genes are associated with phenotypic expression in IBS. These studies are discussed in a recently published comprehensive review [Camilleri, 2009]. As mentioned in this review, one of the challenges in this line of research is that large numbers of patients are required when studying the association of genotype with clinical phenotype due to multiple contributing factors including environmental and other biological effects.

In addition, comparable numbers of healthy controls from similar racial backgrounds are needed since race can impact prevalence of genetic polymorphisms. Candidate genes that are involved in a number of potentially relevant pathways along the brain–gut axis have been studied in IBS and/or FD. This includes gene polymorphisms involved in the function of the anti-inflammatory cytokine IL-10 [van der Veek *et al.* 2005; Gonsalkorale *et al.* 2003], 5-HT transporter [Niesler *et al.* 2009; Van Kerkhoven *et al.* 2007], catechol-o-methyltransferase (COMT) [Tahara *et al.* 2008], α -adrenergic 2A and 2C receptors [Kim *et al.* 2004], G-protein coupled receptors, [Grudell *et al.* 2008; Camilleri *et al.* 2008c; Saito *et al.* 2007; Holtmann *et al.* 2004], neuropeptide S receptor 1 [Camilleri *et al.* 2010], enzymes involved in endocannabinoid metabolism [Camilleri *et al.* 2008d] and a variant of the 5-HT_{3E} receptor gene which appears to affect microRNA-regulated gene expression of the receptor [Kapeller *et al.* 2008]. The association of genotype and symptoms, response to treatment or biomarker has been assessed. With regard to the latter, a biomarker with a clear genetic link has been termed endophenotype. Potentially, smaller sample sizes would be sufficient if evaluating a clearly defined endophenotype. Studies up to this point are of limited value given that there are some significant but small associations in IBS and occasionally conflicting (e.g. SLC6A4 gene) results. It is conceivable that clusters of genes, rather than single genes, may be of greater relevance in a complex disorder such as IBS or that other factors, such as environmental effects, mediate the association between genotype and phenotype due their ability to alter gene expression.

Environmental factors such as chronic, sustained stressors are associated with onset and symptom exacerbation in the majority of patients [Mayer *et al.* 2001] and predict the development of post-infectious IBS (PI-IBS) [Gwee *et al.* 1999]. Furthermore, there is increasing evidence that IBS patients have experienced a greater number of early adverse life events compared with healthy individuals and patients with organic GI conditions [Chitkara *et al.* 2008; Ross, 2005; Salmon *et al.* 2003; Talley *et al.* 1994; Drossman *et al.* 1990]. Early adverse life events refers to traumatic experiences including, but not limited to, maladjusted relationships with a parent or primary caregiver, severe illness or death of a parent, and physical, sexual or emotional abuse. In addition, studies suggest that perturbations during the prenatal and perinatal period might contribute to the development of IBS in adults [Chitkara *et al.* 2008]. For example, a twin study demonstrated that IBS was more prevalent and occurred at an earlier age in the twin with the lower birth weight [Bengtson *et al.* 2006]. In another study, gastric suction at birth was associated with a diagnosis of a functional bowel disorder in adulthood [Anand *et al.* 2004].

The association with early life trauma or abuse is not unique to IBS as they are linked with other negative health outcomes and behaviors in the adult, including the presence of multiple somatic symptoms (somatization) and alterations in developing neural networks and the neuroendocrine system [Vidlock *et al.* 2009; Creed *et al.* 2008; Anda *et al.* 2006]. In a recent study, early adverse life events were associated with an enhanced cortisol response to a visceral stressor in both IBS patients and healthy controls. However, a faster resolution of cortisol to basal levels was associated with lower symptom intensity and increased IBS-related QOL, suggesting that hypothalamic–pituitary–adrenal (HPA) axis reactivity has a moderating effect on IBS symptoms [Vidlock *et al.* 2009]. It is possible that a faster resolution reflects a more adaptive system in which expression of the glucocorticoid receptor, which mediates the negative feedback of the HPA axis, is intact and a stress response can be quickly attenuated. This is supported by work by Meaney and colleagues [Meaney *et al.* 2007] in an animal model of IBS (see below) [Coutinho *et al.* 2002]. Rats exposed to perinatal stress (i.e. maternal separation) were found to have methylation of the glucocorticoid receptor promoter

resulting in decreased gene expression and prolonged elevation of corticosterone levels.

Consequently, it is conceivable that early adverse life events render an individual more vulnerable to developing IBS due to a failure of adaptive or coping mechanisms, which enable the individual to better respond to subsequent traumas. This is an area which is now being explored by epigenetics research. Epigenetic programming refers to changes that can be passed down meiotically and permanently alter the expression of genes in somatic cells [Wolffe and Matzke, 1999]. This stems from the considerable literature on the effects of *in utero* and early life conditions on adult health and disease [Gluckman *et al.* 2008]. Gluckman and colleagues have synthesized evidence from several disciplines to support the contention that environmental factors acting during development have a powerful role in influencing later susceptibility to chronic diseases, e.g. cardiovascular, metabolic and osteoporosis [Gluckman *et al.* 2008]. These authors developed a hypothesis that this developmental plasticity is mediated at least in part by epigenetics processes such as DNA methylation and histone modification. Several other reviews explore further the developmental origins of adults' disease [Simmons, 2009; Szyf, 2009; Waterland, 2009; Champagne, 2008; McEwen, 2008; Meaney *et al.* 2007].

DNA methylation patterns are sculpted during development and are responsive to different environmental stimuli throughout life. Szyf and colleagues have hypothesized a mechanism linking the social environment early in life and long-term epigenetic programming elicited by maternal care in rats [Szyf *et al.* 2008]. In these studies, maternal licking and grooming in the rat triggered activation of 5-HT receptors in the hippocampus, activation of the transcription factor nerve growth factor-induced gene A (NGFIA) and acetylation of the promoter of the glucocorticoid receptor (mediated by a histone acetyltransferase). Further, in the offspring of 'low licking and grooming' mothers this process was reduced in comparison with the 'high licking and grooming' mothers, leading to differential epigenetic programming of the glucocorticoid receptor.

These different acetylation patterns result in long-lasting behavioral changes in adult offspring. Champagne and coworkers have

explained further these long term and transgenerational effects of maternal care [Champagne and Curley, 2009; Champagne, 2008]. These epigenetic modifications may be pharmacologically manipulated (by Trichostatin A and L methionine administration, for example). Since levels of methionine are influenced by diet, these effects also suggest that diet could contribute significantly to this type of behavioral plasticity [McGowan *et al.* 2008]. More recently McGowan and colleagues argued that these results may translate to humans by demonstrating epigenetic changes in hippocampal glucocorticoid receptors of suicide victims who suffered childhood abuse [McGowan *et al.* 2009]. Similarly, Meaney and colleagues demonstrated that repeated maternal separation in the neonatal period in rats was associated with reduced glucocorticoid negative feedback as an adult [Meaney *et al.* 1996]. Maternal separation predisposes adult rats to develop stress-induced visceral hypersensitivity, enhanced defecation, intestinal mucosal dysfunction, increased HPA axis responses and anxiety-like behavior [Gareau *et al.* 2006; Coutinho *et al.* 2002]. Other early life 'stressors' may include exposure to toxins, chronic medication, malnutrition, physical or sexual abuse.

We hypothesize that by modulating such chemical marks on DNA or histone proteins, we are theoretically more likely to deliver a highly efficacious treatment for FGID patients. It is likely that a single such modification will alter the expression of several tens or hundreds of genes, which collectively predispose the individual to these debilitating conditions. For example, Wang and coworkers showed 17 histone modifications in CD4+ T cells, which affected 3286 promoters [Wang *et al.* 2008]. Modulating one out of 3286 gene expression changes is very unlikely to have a meaningful effect on the resultant pathology. However, it can be argued that modulating one of the 17 histone modifications would be much more likely to do so. Consequently, we aim to characterize further these chemical marks on DNA or histone proteins in relevant tissues from FGID patients or appropriate preclinical assay systems, in an endeavor to identify new potential targets for these disorders. Clearly, the selectivity and potential therapeutic index of drugs which modify epigenetic processes will be a key concern. However, we do know that targeting the acetylation of histone proteins with histone deacetylase inhibitors is clinically useful in

cancer [Liu *et al.* 2010; Paik and Krug, 2010; Takai and Narahara, 2010]. There is also an increasing literature on the clinical potential of these drugs even in inflammatory diseases [Grabiec *et al.* 2010; Halili *et al.* 2009]. If similar disease specificity can also be shown for GI-related disorders, the study of epigenetics promises to open up an entirely new approach to the treatment of patients with FGIDs.

Conclusions

Some therapeutic success has been achieved in IBS (by 5-HT₃ receptor antagonism, nonselective 5-HT₄ receptor activation and by CIC-2 channel activation), but so far, not in other FGIDs, such as FD. Paradoxically, however, difficulties associated with some of these achievements have also highlighted the risk-averse environment for new drug development in the treatment of FGIDs, the complexities of the disorders themselves and as a result, encouraged drug development strategies away from FGIDs and towards other disorders which may have common symptoms.

In future we need to continue to study the impact of environmental factors on genotype (nature and nurture) which can lead to phenotypic variability. However, current evidence suggests that any genetic associations are likely to be as complex as the range of FGIDs themselves. A particularly interesting advance is the beginnings of GI research to embrace the opportunities provided by epigenetic research. This line of research is still in its infancy, in terms of new treatments for GI disorders, but it differs from other approaches in that it offers the possibility of discovering real cures within this difficult group of patients.

Conflicts of interest

Dr Sanger has received a research grant from GlaxoSmithKline as well as funding from the Medical Research Council to undertake translational neuropharmacology using human isolated gastrointestinal tissues (Skills Gap Award).

Dr Chang acts as a consultant to Albireo, Takeda, Prometheus, Salix, Ironwood, Forest, Movetis, McNeil, Rose Pharma, and Synergy. She has also received research grants from Rose Pharma and Prometheus as well as funding from the National Institutes of Health for studies in irritable bowel syndrome (R01 AR46122 and P50 DK64539).

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Dr Houghton has served as a speaker, a consultant, and/or an advisory board member for Novartis, Pfizer, Solvay Pharmaceuticals, GlaxoSmithKline, Clasado, Ono Pharma UK Ltd, and the Neurogastroenterology Unit has received research funding from Novartis, Pfizer, Solvay Pharmaceuticals, GlaxoSmithKline, and Danone Research.

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