TRANSCRIPTIONAL REGULATION OF THE LACTASE-PHLORIZIN HYDROLASE (LPH) GENE BY POLYMORPHISMS IS ASSOCIATED WITH PERISTALSTIC PERSISTENCE OF HUMAN DAILY LACTASE ACTIVITY

The primary mechanism of both the persistence and non-persistence phenotypes is regulation of gene transcription. Considerable effort has been devoted to the elucidation of the molecular mechanisms involved in the transcriptional regulation responsible for these two human phenotypes.

The gene for human LPH, located on chromosome 2q21, comprises 17 exons and covers approximately 49 kb, giving rise to a messenger RNA (mRNA) of slightly more than 6 kb. From initiation codon to stop codon, human LPH mRNA encodes 1927 amino acids forming the complete translation product. Sequence comparisons indicate that the coding region is comprised of four homologous parts, leading to the suggestion that the gene is the product of two duplication events during evolution. Even the development of the small intestine is heavily glycosylated so that the final translation product is about 220 kDa (fig 1). This high molecular mass glycoprotein undergoes intracellular cleavage, dividing regions I and II from regions III and IV. The protein consisting of regions III and IV contains the two active sites and is inserted into the microvillus membrane of the enterocyte as a mature enzyme of approximately 160 kDa. The proximal portion encompassing regions I and II has no enzymatic activity, but has been shown to function as a signal for correct folding of the enzyme. Initial analyses of the gene identified several single base polymorphisms (SNPs) within both the coding and flanking regions of the coding region and the 5′ flanking region. None was considered to have functional significance.

Subsequent analysis has led to the identification of additional SNPs and several other features unique to the human gene that may be of relevance to the mechanisms of LPH persistence/non-persistence. The first 100 bp of the proximal promoters of the mammals analysed to date (rat, mouse, pig, and human) are virtually identical and appear to be similarly regulated. Studies in transgenic animals have indicated that approximately 1 kb of the 5′ flanking sequence in the pig, and 2 kb of the 5′ flanking sequence in the rat, are sufficient to direct appropriate tissue, cell, and villus expression as well as the developmental decline at weaning. Comparative studies in humans have not been carried out. In contrast with the other mammals analysed, the 5′ flanking region of the human LPH gene contains five inserted stretches of repetitive DNA, two Alu sequences of approximately 300 bp each, and three other short repetitive sequences, making a direct comparison of the more distal regulatory region to those of other mammals difficult. Whether or not these inserted repetitive DNA segments affect LPH expression is currently unknown. Furthermore, exon

Figure 1 Model of the molecular forms of lactase-phlorizin hydrolase during synthesis and processing in the human villus enterocyte. The early changes in apparent molecular size are due to glycosylation, as indicated in the diagram. Note that the two active sites are located in domains III and IV. The subsequently removed domains I and II are important for correct folding of the nascent protein. Although not indicated on this drawing, the enzyme forms a homodimer during processing. The final N terminal cleavage of a small segment is depicted by the elimination of the terminal loop in the microvillus form of the enzyme.
17 of MCM6, a cell cycle regulatory gene, ends 3.5 kb from the start site of the human LPH gene. The transcriptional start site of the MCM6 gene lies approximately 39 kb 5′ of the LPH transcriptional start site. The two genes are close together but the available evidence indicates that their regulation is independent. Two polymorphisms associated with LPH non-persistence originally identified by Enattah and colleagues were examined further here, lie within introns 13 and 9 of the MCM6 gene (fig 2).

The report by Enattah and colleagues mapped the DNA changes responsible for lactase persistence (or its converse, adult-type hypolactasia) to a region 13−22 kb upstream of the LPH gene. Using traditional linkage analysis, they first narrowed the region to approximately 4 million bases between two genetic markers named D2S114 and D2S2385. They then hypothesised that the allele causing lactase persistence arose once in the recent past on a particular chromosome. In this scenario, recombination events in the subsequent history of the population would separate the persistence allele from alleles in other parts of the chromosome, but in the immediate vicinity the persistence alleles would still be inherited together (in linkage disequilibrium) with nearby alleles from the ancestral chromosome. Thus recombination events can be used to narrow the region of interest.

To identify this signal of linkage disequilibrium, they typed several additional markers within the critical 3.4 Mb region. They identified a 47 kb region containing LPH and upstream sequences in which all individuals with lactase persistence carried the same alleles. The localisation was based in part on data from two chromosomes that differ from the ancestral chromosome at only a single marker. These two chromosomes could therefore be derived from a recent mutation at that one marker rather than from a recombination event, meaning that localisation to the 47 kb region might be premature. Nevertheless, they re-sequenced the entire 47 kb region, including the entire LPH gene, and identified only one variant (a C>T SNP located 13910 bases upstream of LPH) that was multiply associated with lactase persistence. All 99 individuals with low lactase activity were found to have this SNP whereas all with high lactase activity were homozygous for the C allele.

Interestingly, other haplotypes had previously been associated with lactase persistence and non-persistence. A report in this issue of Gut by the same group extends these studies by testing whether these SNPs are associated with decreased expression of LPH mRNA levels [see page 647]. As expected, higher levels of LPH mRNA and lactase activity were found in intestinal biopsy samples of subjects whose DNA contained a T at the −13910 SNP and an A at the −22018 SNP. This correlation is perhaps not surprising given the previous very tight correlation between these alleles and lactase persistence, and the tight correlation between lactase persistence and high lactase mRNA levels, as first reported in 1992. However, they also used a clever technique whereby SNPs in the coding region were used to distinguish the transcripts synthesised from the two LPH alleles in an individual heterozygous for one of these coding SNPs. Using allele specific reverse transcription-polymerase chain reaction directed at the coding SNPs, they were able to quantify not only the total levels of LPH mRNA but also the relative levels of expression from the two different transcripts. By this method, they showed that LPH mRNA transcripts are less abundant when synthesised from a chromosome carrying the C at −13910 and G at −22018 than from chromosomes carrying a T and an A at these two sites. Thus, in this population, levels of LPH mRNA were confirmed to be correlated with these SNP patterns.

The reported perfect correlation between T at −13910 and lactase persistence is extremely suggestive, and this paper indicates that lactase persistence is largely or completely explained by a cis acting effect on mRNA levels that is due to either the −13910 SNP or an SNP in perfect linkage disequilibrium with this SNP. In this study, all of the chromosomes carrying a T at −13910 also had an A at −22018. It would be of interest to apply this same technique to individuals in whom these two alleles had been separated (for example, a C at −13910 but an A at −22018) to determine which of these SNPs was more tightly correlated with the cis acting effect on mRNA levels. One could also imagine using this assay in vitro to try to determine whether the −13910 SNP is truly causative or whether a more distant genetic variant might be responsible for the persistence of lactase activity into adulthood.

Except for rare cases of congenital lactase deficiency, reported to be due to a separate gene, every human infant has high levels of LPH expression. If the polymorphisms regulate LPH expression, it is unclear how to account for both universal elevated expression in infants and the later development of lactase persistence/non-persistence in different individuals. While the correlation of the polymorphisms with the LPH phenotypes presented here is excellent, it does not demonstrate causation. The discussion indicates that the polymorphisms are both located within repetitive DNA sequences. As the Alu sequences are unique to primates, this is consistent with a mechanism for LPH persistence unique to humans. However, post-weaning LPH non-persistence is common to all mammals. It is unclear how both pre- and post-weaning human patterns can be accounted for by one or both of these polymorphisms.

In contrast with the previous publication, in which it was suggested that the polymorphisms altered a transcription...
Irritable bowel syndrome

Of actors, bolting horses, and drops in oceans!

F Cremonini, M Camilleri

Does serotonin mediate postprandial symptoms in irritable bowel syndrome?

Postprandial symptoms are a common feature in patients with irritable bowel syndrome (IBS). In one study, one half of patients presenting with IBS reported symptom occurrence or exacerbation following a meal.1 This effect of meals on gastrointestinal symptoms has been attributed to an increased colonic contractile response to meals in IBS patients. This colonic response has several components. The first and most rapid component occurs within a few minutes of distension of the stomach by the meal and is mediated by gastric mechanoreceptors that evoke colonic contraction through a vagally mediatedafferent pathway. A second phase, mediated by chemo-receptors in the small intestine, results in colonic contraction that may last up to two hours after meal ingestion.2 Prolonged manometry3 and barostat studies4 demonstrated that the increase in colonic motility after meals was almost immediate, and subsequently we and others reported that patients with diarrhoea and mucosal predominant IBS experienced these symptoms in association with repetitive high amplitude propagated contractions that induce mass movements in the colon.5-7 The third phase of the colonic contraction after the meal results from ileal stimulation by chyme and has been documented best in animals as it occurs 2–6 hours post-meal ingestion, a time when humans are often ingesting another meal and stimulating the first two components.

The first two phases of the colonic response to food involve serotoninergic pathways: thus, antagonism of the serotonin (5-hydroxytryptamine (5-HT3)) receptor reduces both components of the colonic response to meal ingestion.8 In this issue of Gut, Houghton and colleagues9 provide further support for the role of serotonin in mediating this response [see page 663]. They report increased postprandial serotonin levels in patients with diarrhoea predominant IBS and meal related symptoms; serotonin levels were higher than those of patients with IBS without meal related symptoms. There were also higher fasting levels of serotonin in IBS patients compared with controls and increased intraplatelet concentrations of serotonin, but no differences in the area under the curve of postprandial plasma serotonin between IBS patients and healthy controls.

WHAT IS THE SIGNIFICANCE AND INTERPRETATION OF THESE FINDINGS? This paper extends prior observations in a pilot study of five IBS patients whose

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serotonin levels were high relative to healthy controls. The observations are of interest as they relate postprandial exacerbation of symptoms to serotonin levels in both plasma and platelets. Several questions arise from consideration of the data.

Firstly, is the peak in postprandial serotonin really responsible for meal related symptoms in these patients? The timing of symptoms and that of serotonin would be expected to coincide if there was an association between serotonin and symptoms. The peak serotonin concentration in plasma was reached 2–3 hours after the meal in all study groups, well after the occurrence of postprandial symptoms. Peak serotonin levels appear to coincide with the later, chemoreceptor mediated or ileal, phase of the delayed appearance of symptoms. The timing of postprandial symptoms is earlier and is more likely attributable to a neural or hormonal response, that may also be mediated by other mechanisms initiated by gastric mechanoreceptors or upper intestinal chemoreceptors stimulated in response to feeding. It would be reasonable to infer that the horse has bolted long before circulating levels of serotonin peaked! Other mediators released within the foregut or midgut, such as gastrin, cholecystokinin, secretin, pancreatic polypeptide, motilin, the vagus or enterochromaffin polypeptide family (including PHI/PHM), and neurotensin may be candidate mediators of the colonic response and the associated postprandial symptoms. To date, one small study in humans evaluated concurrently colonic motility and circulating neuropeptides or hormones, and has shown no significant differences in postprandial levels of these mediators between IBS patients and controls. The size of the sample of IBS patients was not sufficient to characterise any differences between IBS patients with or without postprandial symptoms, in whom an exaggerated sensory response to the meal has also been proposed.11

The similarity in areas under the curve of plasma serotonin likely reflects the integrity of the enterochromaffin cells, and the fact that mechanical and chemical stimuli produce similar integrated responses to the response to several hours. The integrated responses would be less sensitive to differences in the time or level of the serotonin peak concentration. One might therefore conclude that in the presence of an essentially intact gastrointestinal mucosa in IBS patients, release of mucosal peptides into the circulating peripheral (rather than portal) plasma is a relatively insensitive method to evaluate their potential mechanistic role because of the immense dilution of released mediator in the large plasma volume. . . . it is a mere drop in the ocean!

Secondly, circulating plasma serotonin levels have to be interpreted in the context of the dynamic interplay between food mediated release, high or low capacity serotonin reuptake mechanisms, and storage in circulating platelets. Physiological regulation of serotonin levels is complex: there are reuptake mechanisms in neuronal cells, gut epithelial cells, and platelets that utilise a high affinity (but relatively low capacity) serotonin transporter (SERT). The liver and kidneys are other important sites of serotonin uptake through the organic cation transport system, which has a lower affinity but a higher capacity compared with SERT. These are the sites where serotonin is metabolised to 5-hydroxyindoleacetic acid (5-HIAA). The SERT is regarded as a major determinant of plasma serotonin concentration, and it contributes to the prevention of the dangerous effects of abnormally high serotonin levels on vascular tone, fibrogenic effects, and blood clotting.12 The sclerosing effects of high serotonin levels contribute to the cardiac valvular lesions and scleroderma in carcinoid syndrome when the neuroendocrine tumour produces serotonin in excess of the inactivating mechanisms. Differences in circulating serotonin levels in IBS may conceivably result from changes in mucosal enteroendocrine cells number,13 hypersensitivity of chemoreceptors in the mucosa resulting in greater release of serotonin, or altered inactivation or reuptake of the transmitter. In the latter case, differences in serotonin levels could result from functional polymorphisms of the SERT gene, associated with reduced serotonin reuptake in gut epithelia or platelets in patients with postprandial symptoms. Houghton and colleagues found increased platelet levels of serotonin and this suggests there were no deficiencies in reuptake mechanisms. This may also explain why the serotonin area under the curve was not different in the three groups. Differences in fasting serotonin levels in the entire group of IBS patients compared with healthy controls are not easily explained given the fact that serotonin is released by the meal stimulated enteroendocrine cells, and the normal functional capacity of SERT suggested by the normal postprandial area under the curve. Pata et al have reported differences in the prevalence of SERT-P genotypes in diarrhoea predominant IBS relative to controls and other IBS groups in a Turkish population. Specifically, they identified the short homogous or heterozygous polymorphisms to be significantly more prevalent than the homozygous long polymorphism in diarrhoea predominant IBS. Theoretically, these polymorphisms would be associated with reduced reuptake of serotonin by the presynaptic membrane.14 Functional SERT polymorphisms may be responsible for pharmacogenetic differences, as has been demonstrated in the colonic transit response to the 5-HT3 receptor antagonist alosetron.15

However, data from our laboratory (HJ Kim, M Camilleri, R Urrutia, unpublished observation) suggest that such differences in genotype prevalence are not observed in IBS patients (despite a five fold higher sample size) in a US population. Indeed, the sample size needed to detect significant differences in genetic polymorphisms to explain the observed differences in platelet serotonin between IBS patients and a control group would likely be an order of magnitude higher than the size of the group studied.16 Future studies will have to attempt to define the contribution of these polymorphisms to plasma and platelet levels of serotonin.

A third major consideration is that the effects of serotonin may be neurally mediated and unrelated to plasma circulating levels. Thus antagonist studies show unequivocally that 5-HT1A, 5-HT1B/D, and 5-HT3 receptors17–19 are involved in the response to feeding. One can conclude that the observations by Bearcroft and colleagues8 and Houghton and colleagues8 add an interesting piece to the puzzle but the case for the role (at least in part) of serotonin would be no weaker if these data were unavailable. Nevertheless, it is also important to remember that while serotonin is a prominent actor which may contribute to alterations in motor, sensory, and epithelial barrier functions,10 other mediators are available to modulate its actions as well as the postprandial function of the gut. Given the redundancy of mechanisms able to modulate these functions in the gut, it is unlikely that the colonic response to feeding represents a soliloquy or a one act play. Rather, it represents the integrated effects of an orchestra of players that “have their exits and their entrances” at different times on the postprandial stage and present physiological targets for novel therapies.

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Irritable bowel syndrome

Tegaserod and IBS: a perfect match?

W Grant Thompson

IBS patients require diagnosis, advice, and a reassuring doctor. For constipated patients who need it, tegaserod is safe and effective.

Up to recently, there existed little evidence that any therapy was effective for irritable bowel syndrome (IBS). The quality of clinical trials was poor,1,2 and no systematic review3–5 can redeem faulty data.6 Mindful of this, pharmaceutical companies now employ modern clinical trial principles to test IBS drugs. The most of these efforts is an Asia-Pacific randomised controlled trial of tegaserod by Kellow et al, described in this issue of Gut [see page 671].7 To judge how well tegaserod matches the needs of IBS patients we must examine the trial methods, results, and conclusions and divine what is missing from the reports.

The Asia-Pacific study is similar to Western IBS trials of tegaserod.1,2 These represent substantial improvements in trial methodology. The entered subjects had criteria defined IBS and most had a “non-diarrhoea” bowel habit suitable to the drug’s effects. Recruitment was sufficient to show definitive results, and subjects were double blinded and randomly allocated. A primary global outcome was selected with appropriate secondary measures, and the analysis was “intention to treat”. Primary outcome differences were consistently significant at the pre-decided end point, and over 12 weeks. Tegaserod appears safe (sine qua non for IBS), and post-marketing surveillance should detect unexpected adverse effects.

The Kellow study achieved greater therapeutic gain (absolute benefit in- crease) than prior tegaserod studies (21% v 11.8% and 5.7%), but with several methodological differences. Whereas the subjects in the Western trials were 98% Caucasian and mostly English speaking, 84% of the Asia-Pacific subjects were Asian whose language is unreported. The Western trials entered patients with Rome I IBS criteria plus two of three constipation criteria. The Asia-Pacific trial used Rome II criteria excluding only those with predominant diarrhoea.

In the West, the primary outcome measure was the subject’s global assessment (SGA) of relief recorded weekly on a five point scale with predetermined responder definition. The Asia-Pacific measure was “yes” or “no” to “satisfactory relief of symptoms of IBS”. In previous trials, tegaserod produced relief earlier than placebo, but the therapeutic gain lessened over 12 weeks. Therefore, the Asia-Pacific investigators chose the first rather than the last four weeks as the primary end point. Earlier trials compared responders as their primary outcome, while the Asia-Pacific study compared responses (“responders” a secondary outcome). These differences help explain the greater therapeutic gain in the Asia-Pacific trial.

The methods and data described in the tegaserod reports are vastly superior to previous trials of available drugs (alosertinon aside), and the results are consistent. Nevertheless, some facts and interpretations are missing. In the Asia-Pacific trial, the “prokinetic” properties of tegaserod are demonstrated by diarrhoea (10% v 3% for placebo), less laxative consumption (23.2% v 34.1%), and more frequent, and looser stools. In all three trials these effects are immediate. While participants were double blinded initially, no exit tests for blinding are described. Did some subjects suspect they were on tegaserod, because of its prokinetic effects? The outcomes are subjective, so results could be biased if some patients or investigators realised who received tegaserod.

The “SGA of relief” is attractive because it embraces the multifaceted symptoms of IBS and identifies satisfied patients. However, there is a pitfall. Let us suppose that the only effect of tegaserod is prokinetic (without the believed reduction in visceral hypersensitivity). Increased defecation in the mainly constipated patients could provoke a “yes” response to the SGA. The tendency of other secondary outcome measures to improve is reassuring, but in the Kellow study discomfort, pain, and bloating improvements were insignificant. Indeed, diary data indicate “no bowel movements” and “hard or lumpy stools” as the only secondary measures significantly improved in either the first
or last 28 days (see table 3). Could a suitable laxative more cheaply achieve the same result? Could relief of constipation improve discomfort, bloating, or even pain? Without trials comparing tegaserod with laxatives, we cannot know.

Over 80% of subjects are female and one trial omitted men.7 Data are insufficient to evaluate the benefits of tegaserod in men, but the present study shows a non-significant 10% therapeutic gain in the first four weeks and none over 12 weeks. Why should only women respond? Perhaps there are hormonal and psychological explanations, and bloating (part of global outcome) is uncommon in men.20

The authors of all three reports regret the "relatively high placebo response", implying that if it were lower a greater therapeutic gain might be achieved. This is likely fallacious. The effects of a treatment depend on its physiological effects plus natural history of the condition being treated plus "placebo effect" (that is, those benefits of healer-patient interaction).8,9,10 These act in concert to make patients feel better. The placebo response in the tegaserod studies are similar in other IBS, dyspepsia, peptic ulcer, and ulcerative colitis trials.11,12 The increasing placebo response over the 12 week treatment can be attributed to the care, enthusiasm, and education provided by the protocol, and the tendency of IBS symptoms to improve.13 Placebo responses are allies that should be recruited with all treatments.

Missing from the discussion is guidance on how to use this newly available drug (not yet in Europe). It is not a perfect match for IBS. The data establish the efficacy of tegaserod for women with Rome IBS without diarrhoea. However, for many IBS is a fluctuating lifetime experience beginning in the teens. Do they all need tegaserod? If so, should they take it indefinitely? Would tegaserod work best for short troublesome periods or "as needed"? What guides its use through IBS patients' inevitable alterations in bowel habit? What about using tegaserod in other functional disorders such as dyspepsia or functional constipation? Current trial data provide no answers.

While IBS clinical trials may now be state of the art, there is room for improvement. Future designs should ensure blinding or allow for its breach. Designers must improve treatment protocols and outcome measures to more accurately match the needs of IBS patients. Gender differences and IBS subtypes require clarification. Pathogenesis is unknown and therefore cure is unlikely in the short term. Meanwhile, scientists and clinicians should strive to improve IBS palliation. Drugs affecting gut motility should be compared with cheaper antidiarrhoeals and laxatives. Placebo effects should be seen as complimentary, not the enemies of science and drug validation.

What can practising doctors make of this? Recent IBS trials represent a breakthrough in IBS trial methodology, but tegaserod provides palliation not cure. Most women with IBS and constipation do well with good doctor-patient relationships; confident diagnosis, explanation, optimistic yet realistic prognosis, management of psychological comorbidity, and diet and lifestyle advice.14,15 For the unimproved with impaired enjoyment of life, tegaserod offers hope and confirmation. Available information suggests that treatments should be short—for example, 2–4 weeks—continuing only if constipated IBS symptoms persist and improvement justifies the cost. What is yet unjustified is the use of tegaserod indefinitely, in men, in IBS with diarrhoea, or other diagnoses. The tegaserod trials are progress but for IBS no therapy is perfect.

Conflict of interest: Over my 30 year study of IBS I served as an advisor and teacher about IBS for several pharmaceutical companies including in the last year, Novartis, Glaxo Smith Kline, Merck (Germany), Shering Plough, and Procter and Gamble.

Gut 2003;52:621–622

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