Published in final edited form as:

Dig Dis Sci. 2009 November ; 54(11): 2318–2324. doi:10.1007/s10620-009-0903-4.

Genetics and Irritable Bowel Syndrome: From Genomics to Intermediate Phenotype and Pharmacogenetics

Michael Camilleri, M.D.

Clinical Enteric Neuroscience Translational and Epidemiological Research (CENTER) College of Medicine, Mayo Clinic, Rochester, MN 55905

Abstract

Purpose—Familial aggregation and sibling pair studies suggest there is a genetic contribution to development of irritable bowel syndrome (IBS). The aim of this study was to review the evidence of genetics in IBS based on genetic epidemiology, studies of association with intermediate phenotypes and pharmacogenetics.

Results—Genetic association studies with IBS symptom phenotype have generally provided inconsistent results for many candidate genes investigated, such as *SLC6A4*, *GNB3* and *IL-10*. There have been no genome wide association studies in IBS to date. Studies of associations of candidate genes with intermediate phenotypes suggest associations with pathophysiological mechanisms of motor and sensory functions; however, these results also require replication. Pharmacogenetics studies illustrate the potential of genetics to impact on response to therapy, as observed with *SLC6A4* and responses to the 5-HT₃ antagonist alosetron and the 5-HT₄ agonist, tegaserod.

Conclusions—While the heritable component and genetics in the complex disorder of IBS are still poorly understood, studies of the associations of spontaneous genetic variations and altered functions may provide novel insights of the mechanisms contributing to the disease.

Keywords

epidemiology; DNA; *SLC6A4*; *ADRA2A*; *COMT*; *IL-10*; *GNB3*; mitochondrial; cannabinoid; interleukin-10; alosetron; tegaserod

Introduction

Familial aggregation (1,2) and twin studies (3–7) suggest that there is a genetic component of irritable bowel syndrome (IBS) based on epidemiological studies; however, the data are conflicting, and the contribution of common environment to the association of IBS within studies has not been completely resolved. Nevertheless, the data are consistent with the concept that IBS may be a complex genetic disorder. There are no published genome wide association studies in functional GI or motility disorders.

In the absence of consistent findings in familial aggregation and twin studies to support a specific genetic mechanism in IBS based on symptom phenotypes, a different approach has examined associations of candidate genes with gastrointestinal (GI) and colonic physiology, that is, interactions between genotype and intermediate phenotypes. The influence of genetics

on pharmacological response in IBS also demonstrates the potential importance of genetics in IBS.

Candidate Genes and Epidemiology

Studies of genetic epidemiology of IBS have used the candidate gene approach. Examples of this approach include reports of the association of IBS or FGID with IL-10 (an anti-inflammatory cytokine), the serotonin transporter (SERT or SLC6A4), α -2 adrenergic receptors, and G protein functions. In these studies, investigation seeks to explore the association between the genotype and the clinical endpoint. However, there are two major conceptual black boxes in the link between genotype and phenotype: the biological factors linking the genotype to the clinical manifestations, and the environmental or other factors that lead from disease onset to the clinical disease or disorder (Figure 1, upper panel) are unknown. Moreover, because the disease phenotype is quite variable, the sample size to demonstrate association with genotype, even in a hypothesis generating study, has to be quite large.

IL-10

Two studies (8,9) have addressed the association of *IL-10* gene polymorphisms in IBS: this would be consistent with the hypothesis that some patients are genetically predisposed to produce lower amounts of the anti-inflammatory cytokine IL-10, and potentially develop the mild inflammatory manifestations of IBS. The initial positive result (8) was not confirmed by a second study (9).

5-HT

The single genetic variation most intensely studied in search of an association with IBS is 5-HTTLPR. This polymorphism is located in the promoter region of the gene *SLC6A4* responsible for the synthesis of 5-HT transporter, SERT. The latter is central to fine-tuning brain 5HT neurotransmission, and is abundant in cortical and limbic areas, and affects emotional aspects of behavior (10). This polymorphism (5-HTTLPR) results in short (s) and long (l) allele, based on the presence or absence of a 44 bp insertion (10,11). The *SLC6A4* gene is located on chromosome 17q11.1–17q12 and organized into 14 exons spanning ~ 38 kb (11). A study from Mayo Clinic (12) did not demonstrate a significant association of any 5-HTTLPR allele and IBS; this contrasted with the results of the small study by Pata et al (13). However studies from Western countries and Asia provided contradictory results (14). A meta-analysis of most of the studies currently in the literature suggests that among Caucasians and Asians, odds ratio for IBS in subjects for SS or LS vs LL (odds ratio [OR] 1.0, 95% CI 0.8–1.2) and for SS vs LL or LS (OR 1.0, 95% CI 0.7–1.4) are not significant (14).

Another genetic variation related to 5-HT control is STin2 VNTR, which is located in intron 2. It consists of variable number (e.g., 9,10, or 12) identical 17-bp segments (15); the 10/12 genotype has been associated with IBS in one study (16). However, other studies found no association between STin2 VNTR and IBS (13,17,18).

A SNP, rs25531, located immediately upstream of 5-HTTLPR is strongly linked, yet has opposing effects on SERT expression. The rs25531 G allele lowers SERT transcription compared with the A-allele and occurs most frequently with 5-HTTLPR – L.. Carriers of the G allele (minor allele frequency 10%) of rs25531 had increased odds [OR 3.3 (95% CI 1.1–9.6)] of having IBS compared with healthy controls (15). The A:G allele frequency (percent) in controls in this study was 96:4, in contrast to the IBS patients (89:11). Given the relatively small numbers of patients (186 with IBS) in the study, this result requires confirmation, particularly because G allele frequencies of 10% and 18% are reported in two studies (that included 20 and 190 patients respectively) conducted in the United States and reported in NCBI

(19), and these G allele frequencies are closer to the 11% observed in the IBS cohort studied in the Northwest region of the United States.

In a study of 54 patients with Rome I positive IBS and 107 healthy individuals, Pata et al reported that increased risk of IBS in patients with homozygous C allele of the 102 T/C polymorphisms (OR: 7.89; CI: 1.079–57.76) or homozygous A allele of the -1438 G/A polymorphism (OR: 11.14; CI: 1.59–78.06) of the 5-HT_{2A} receptor gene. In addition, the T/T genotype of 102 T/C polymorphism may be associated with more severe pain in patients with IBS (20). The wide confidence intervals suggest the need for replication in larger samples.

In a collaborative study between centers in England and Germany, investigating the potential role of genetic variation in 5-HT₃ receptors, an initial study was conducted in 200 IBS patients and 100 healthy controls from the UK. The novel *HTR3E* 3'-UTR variant c.*76G>A (rs62625044) was associated with female IBS-D (OR=8.53, 95% CI = 1.04–70.28). This association was confirmed in a replication study, including 119 IBS-D patients and 195 controls from Germany (OR = 4.92, 95% CI = 1.49–16.30 [21]). Using a reporter assay, Kapeller et al showed that c.*76G>A affected the binding of microRNA-510 (miR-510) to the *HTR3E* 3'-UTR and caused elevated protein expression in 2 different cell lines. In addition, *HTR3E* and miR-510 co-localize in enterocytes as shown by in situ hybridization and RT-PCR (21).

α2-Adrenergic Receptors

Adrenergic receptor and 5-HTTLPR genotypes were evaluated in an original cohort (12) consisting of 274 IBS (of which 90 had IBS-C) and 120 controls. Odds ratio (95% CI) were calculated in IBS-C phenotype versus controls in polymorphic relative to respective wild-type genotypes. There was significant association between IBS-C and the α_{2C} Del 322–325 deletion, which alters the coding region of the gene. This variation results in a receptor that has markedly decreased agonist-mediated responses *in vitro* (22). There is also evidence that the same variation results in altered cold pain perception without affecting cognition (23).

There was a non-significant (p=0.08) association of IBS-C with the α_{2A} -1291 C>G (rs1800544) genotype. These provocative associations demonstrated between genetic variations in α_{2A} and α_{2C} adrenergic receptors and IBS-C have not been assessed by other groups.

Catechol-o-methyl transferase

Catechol-O-methyltransferase (COMT), which catalyzes the transfer of a methyl group from S-adenosyl methionine to catecholamines (such as dopamine, norepinephrine and epinephrine) and their inactivation, is a key regulator of pain perception, cognitive function, and affective mood. Three common haplotypes of the human *COMT* gene, consisting of two synonymous and one non-synonymous SNPs, code for differences in COMT enzymatic activity due to a reduced amount of translated protein and are associated with pain sensitivity (24). A Valine158Methionine (Val158Met) allele has been identified in the *COMT* gene associated with a three-to-four fold decline of the COMT activity compared with that of the non-Val158Met allele (25). In a recent study, interaction of gender, age, *COMT* Val158Met polymorphism was found in dyspepsia (26).

G-proteins

G protein-coupled receptors are present on every excitable cell and every cell that is susceptible to regulation in the body (27). It is estimated that 80% of ligand-receptor interactions are mediated through G-protein coupled receptors; activation of the receptor leads to production of the βγ heterodimer from the heterotrimeric G protein. This is catalyzed by GNβ3. Initial studies suggested an association between *GNβ3* C825T and dyspepsia (28,29); however, we

did not identify an association of IBS with *GNβ3* C825T (30). The latter finding has been replicated in a smaller study (31). G protein coupled receptors are also important potential sites for drug action. A preliminary report suggests that the CC genotype, which results in decreased intracellular signal transduction, may predict response to therapy in patients with functional dyspepsia treated, based on their predominant symptom, with proton pump inhibitors, prokinetics, spasmolytics and tricyclic antidepressants. At 12 months' follow up, logistic regression analysis showed that the CC genotype was associated with response to therapy (32).

Mitochondrial DNA

Mitochondrial (mt) genome is involved in generation of energy in tissues including the brain, nerves and muscles. There are >100 pathogenic point mutations and many rearrangements associated with multi-system or tissue-specific diseases (33), including conditions rarely seen by gastroenterologists such as mitochondrial neurogastrointestinal encephalopathy (which presents with pseudo-obstruction and small bowel diverticulosis) and Kearns-Sayre syndrome (which presents with high dysphagia).

Since mitochondria are almost exclusively maternally inherited and IBS is more commonly encountered in females, it was hypothesized that mtDNA SNPs could confer risk to IBS. Most mtDNA SNPs are found in the hyper-variable region of non-coding control region "D loop" (including 16519 C>T SNP). The 3010 SNP is located in the 16S ribosomal RNA gene. These two SNPs, 16519C>T and 3010G>A, have been associated with migraine and cyclic vomiting syndrome (34), which are commonly encountered in patients with IBS. In addition, 16519T alone is associated with diabetes and with a poorer prognosis in individuals with pancreatic cancer.

Given this background, we explored the association of any FGID (vs health) with H haplogroup, defined by the presence of 7028C polymorphism (35). A non-significant lower odds for any FGID in haplogroup H (relative to all other haplogroups) was observed (OR [95% CI] = 0.8 [0.6, 1.1]). Constipation-predominant IBS and alternating constipation and diarrhea IBS are less prevalent in individuals with the 7028C mtDNA polymorphism than in individuals with 7028T. Among those with 7028C, non-specific abdominal pain (chronic abdominal pain or dyspepsia) was significantly associated with 3010A compared with 3010G (odds ratio 3.3, P=0.02). No significant associations of mtDNA genotypes tested were detected with small bowel or colonic transit, rectal compliance, and motor or sensory functions. The relationship of mtDNA and manifestations of IBS require further study.

SCN5A

The *SCN5A*-encoded $\text{Na}_v1.5$ Na^+ channel is expressed in interstitial cells of Cajal and smooth muscle in the circular layer of the human intestine. Mutational analysis was performed on genomic DNA in 49 subjects with IBS associated with at least moderately severe abdominal pain. One patient had a loss-of-function missense mutation, G298S, that was not observed in 1,500 healthy control subjects. Na^+ currents were recorded from the four common human *SCN5A* transcripts in transfected HEK-293 cells. The G298S-*SCN5A* missense mutation caused a marked reduction of whole cell Na^+ current and loss of function of $\text{Na}_v1.5$, and the authors suggest *SCN5A* as a candidate gene in the pathophysiology of IBS (36). However, the mutation appears to be rare even among a subset with at least moderately severe pain in IBS.

Genetics and Intermediate Phenotype in IBS

By studying the genetic associations between candidate genes and in depth physiological functions (intermediate phenotype) that are associated with manifestations of the clinical

phenotype, it is possible to identify genetic susceptibility or, at least, to generate hypotheses that can be tested to evaluate the role of the candidate mechanism (37). This is illustrated in figure 1 lower panel. The intermediate phenotypes such as colonic motility, compliance, sensation thresholds and ratings are predictors of surrogate endpoints such as colonic transit, stool form or pain that constitute importance components of the clinical endpoint, IBS. Three examples pertain to cannabinoid modulation and to serotonergic control.

Cannabinoid Metabolism

The endocannabinoid anandamide is produced, metabolized and released from the postsynaptic membrane. The released anandamide stimulates the cannabinoid (CB) receptor on presynaptic membrane to modulate its function, such as the production of transmitters like acetylcholine. The rate limiting enzyme for metabolism of anandamide is fatty acid amyl transferase (FAAH). If this enzyme does not work well, there is more anandamide that reaches the presynaptic membrane, and greater effect on the transmitter released from the presynaptic neuron. A common SNP in *FAAH* is *FAAH* C385A. We have demonstrated that this SNP is significantly associated with D-IBS and with rapid colon transit (38).

SLC6A4

5-HTTLPR genotype (s allele) is associated with higher pain sensory ratings during rectal distension studies in health and IBS (39). The higher pain sensation is not the result of a decrease in rectal compliance since the same study identified that rectal compliance is increased in patients carrying the s allele of 5-HTTLPR (39). The 5-HTTLPR SS genotype is also associated with greater regional cerebral blood flow in response to colorectal distension in patients with IBS, with regional increases most pronounced in the left anterior cingulate cortex, right parahippocampal gyrus and left orbitofrontal cortex, which may represent increased activity in the emotional motor system of the brain (40).

Neuropeptide S receptor 1

Neuropeptide S receptor 1 (*NPSR1*) gene maps to a region of chromosome 7 and is associated with asthma and inflammatory bowel disease (41). *NPSR1* is expressed on the intestinal epithelium, and is up-regulated in inflammation. It may conceivably be associated with IBS given the increasing evidence of association with minor inflammation or prior infection with IBS. In a study of 18 *NPSR1* polymorphisms (42) that span the gene in 699 participants (~2/3 patients, 1/3 healthy controls), *NPSR1*_RS1419793 was significantly associated with colonic transit ($P < 0.003$ with FDR [false discovery rate] correction). While the mechanisms are unclear, whereby *NPSR1* SNPs might result in altered motor functions, the data suggest that specific *NPSR1* alleles might act as genetic risk factors for colonic diseases that are associated with changes in epithelial barrier function, including IBD and IBS.

Pharmacogenetics

The most informative studies of pharmacogenetics in IBS revolve around *SERT* genetics and the efficacy of alosetron in normalizing colonic transit in IBS-D (43) and efficacy of tegaserod in the treatment of bowel dysfunction IBS-C (18). Thus, the 5-HT₃ antagonist alosetron is more effective when the 5-HTTLPR LL genotype results in greater efficacy of the 5-HT reuptake process, presumably because there is less 5-HT that needs to be competitively inhibited at the 5-HT₃ receptor. Conversely, the 5-HT₄ agonist tegaserod results in lower efficacy in carriers of the LL genotype since there is less endogenous 5-HT to complement the effects of the exogenous tegaserod in activating the 5-HT₄ receptor.

In a recent pharmacodynamic study of low doses of the α_2 adrenergic agonist (44), clonidine, there were significant associations between post-clonidine responses and α_2A 1291C>G SNPs for gastric accommodation, and rectal sensations of gas and urgency.

Conclusions

The study of genetics in motility and functional GI disorders is still in its infancy and replication is required for all of the observations described above. However, these genetic studies enhance observations of familial aggregation or twin studies. Studies of associations between genotype and intermediate phenotype afford an opportunity to generate hypotheses regarding the mechanisms in IBS. These studies are at an advantage over association studies with symptom phenotypes because the biomarker or intermediate phenotype is more clearly defined and measured, its coefficient of variation is known, and this allows for a robust statistical assessment and planning the sample size for a desirable effect to be demonstrated. In addition, the link between the biomarker (e.g. transit) and symptom phenotype (e.g. diarrhea or constipation) is generally well characterized, and therefore the biological significance of an association can be inferred.

In conclusion, while the heritable component and genetics in the complex disorder of IBS are still poorly understood, studies of the associations of spontaneous genetic variations or SNPs and altered functions may provide novel insights of the mechanisms contributing to the disease. Similarly, Mendelian randomization and natural history studies can identify mechanisms and potential drug targets, as recently reported for the association of lipoprotein a and myocardial infarction (45). Molecular manipulations such as knock-outs, or gene overexpression or RNA silencing cannot be performed in humans; the study of genetic variation provides insight on the potential of molecular alterations to the manifestations of such diseases and the basis for individual differences in response to therapy.

Acknowledgments

Dr. Camilleri is funded in part by grants RO1-DK-54681 and K24-DK-02638 from National Institutes of Health. The assistance of Paula J Carlson BS is gratefully acknowledged.

References

1. Kalantar JS, Locke GR 3rd, Zinsmeister AR, Beighley CM, Talley NJ. Familial aggregation of irritable bowel syndrome: a prospective study. *Gut* 2003;52:1703–1707. [PubMed: 14633946]
2. Saito YA, Zimmerman JM, Harmsen WS, et al. Irritable bowel syndrome aggregates strongly in families: A family-based case-control study. *Neurogastroenterol Motil* 2008;7:790–797. [PubMed: 18221250]
3. Morris-Yates A, Talley NJ, Boyce PM, et al. Evidence of a genetic contribution to functional bowel disorder. *Am J Gastroenterol* 1998;93:1311–1317. [PubMed: 9707057]
4. Levy RL, Jones KR, Whitehead WE, et al. Irritable bowel syndrome in twins: heredity and social learning both contribute to etiology. *Gastroenterology* 2001;121:799–804. [PubMed: 11606493]
5. Mohammed I, Cherkas LF, Riley SA, et al. Genetic influences in irritable bowel syndrome: a twin study. *Am J Gastroenterol* 2005;100:1340–1344. [PubMed: 15929767]
6. Bengtson MB, Ronning T, Vatn MH, et al. Irritable bowel syndrome in twins: genes and environment. *Gut* 2006;55:1754–1759. [PubMed: 17008364]
7. Lembo A, Zaman M, Jones M, et al. Influence of genetics on irritable bowel syndrome, gastro-oesophageal reflux and dyspepsia: a twin study. *Aliment Pharmacol Ther* 2007;25:1343–1350. [PubMed: 17509102]
8. Gonsalkorale WM, Perrey C, Pravica V, Whorwell PJ, Hutchinson IV. Interleukin 10 genotypes in irritable bowel syndrome: evidence for an inflammatory component? *Gut* 2003;52:91–93. [PubMed: 12477767]

9. van der Veek PP, van den Berg M, de Kroon YE, Verspaget HW, Masclee AA. Role of tumor necrosis factor-alpha and interleukin-10 gene polymorphisms in irritable bowel syndrome. *Am J Gastroenterol* 2005;100:2510–2516. [PubMed: 16279907]
10. Lesch KP, Bengel D, Heils A, Sabol SZ, Greenberg BD, Petri S, Benjamin J, Müller CR, Hamer DH, Murphy DL. Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science* 1996;274:1527–1531. [PubMed: 8929413]
11. Murphy DL, Lerner A, Rudnick G, Lesch KP. Serotonin transporter: gene, genetic disorders, and pharmacogenetics. *Mol Interv* 2004;4:109–123. [PubMed: 15087484]
12. Kim HJ, Camilleri M, Carlson PJ, Cremonini F, Ferber I, Stephens D, McKinzie S, Zinsmeister AR, Urrutia R. Association of distinct $\alpha 2$ adrenoceptor and serotonin-transporter polymorphisms associated with constipation and somatic symptoms in functional gastrointestinal disorders. *Gut* 2004;53:829–837. [PubMed: 15138209]
13. Pata C, Erdal ME, Derici E, Yazar A, Kanik A, Ulu O. Serotonin transporter gene polymorphism in irritable bowel syndrome. *Am J Gastroenterol* 2002;97:1780–1784. [PubMed: 12135035]
14. Van Kerkhoven LA, Laheij RJ, Jansen JB. Meta-analysis: a functional polymorphism in the gene encoding for activity of the serotonin transporter protein is not associated with the irritable bowel syndrome. *Aliment Pharmacol Ther* 2007;26:979–986. [PubMed: 17877505]
15. Kohen R, Jarrett ME, Cain KC, Jun SE, Navaja GP, Symonds S, Heitkemper MM. The serotonin transporter polymorphism rs25531 is associated with irritable bowel syndrome. *Dig Dis Sci*. 2009 Jan 1; [Epub ahead of print].
16. Wang BM, Wang YM, Zhang WM, Zhang QY, Liu WT, Jiang K, Zhang J. Serotonin transporter gene polymorphism in irritable bowel syndrome. *Zhong-Hua Nei Ke Za Zhi Chin J Intern Med* 2004;43:439–441.
17. Yeo A, Boyd P, Lumsden S, Saunders T, Handley A, Stubbs M, Knaggs A, Asquith S, Taylor I, Bahari B, Crocker N, Rallan R, Varsani S, Montgomery D, Alpers DH, Dukes GE, Purvis I, Hicks GA. Association between a functional polymorphism in the serotonin transporter gene and diarrhoea predominant irritable bowel syndrome in women. *Gut* 2004;53:1452–1458. [PubMed: 15361494]
18. Li Y, Nie Y, Xie J, Tang W, Liang P, Sha W, Yang H, Zhou Y. The association of serotonin transporter genetic polymorphisms and irritable bowel syndrome and its influence on tegaserod treatment in Chinese patients. *Dig Dis Sci* 2000;52:2942–2949. [PubMed: 17394071]
19. http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=25531
20. Pata C, Erdal E, Yazc K, Camdeviren H, Ozkaya M, Ulu O. Association of the -1438 G/A and 102 T/C polymorphism of the 5-HT2A receptor gene with irritable bowel syndrome 5-HT2A gene polymorphism in irritable bowel syndrome. *J Clin Gastroenterol* 2004;38:561–566. [PubMed: 15232358]
21. Kapeller J, Houghton LA, Mönnikes H, Walstab J, Möller D, Bönisch H, Burwinkel B, Autschbach F, Funke B, Lasitschka F, Gassler N, Fischer C, Whorwell PJ, Atkinson W, Fell C, Büchner KJ, Schmidtman M, van der Voort I, Wisser AS, Berg T, Rappold G, Niesler B. First evidence for an association of a functional variant in the microRNA-510 target site of the serotonin receptor-type 3E gene with diarrhea predominant irritable bowel syndrome. *Hum Mol Genet* 2008;17:2967–2977. [PubMed: 18614545]
22. Small KM, Wagoner LE, Levin AM, Kardias SL, Liggett SB. Synergistic polymorphisms of beta1- and alpha2C-adrenergic receptors and the risk of congestive heart failure. *N Engl J Med* 2002;347:1135–1142. [PubMed: 12374873]
23. Kohli U, Muszkat M, Sofowora GG, Harris PA, Friedman EA, Dupont WD, Scheinin M, Wood AJ, Stein CM, Kurnik D. Effects of variation in the human alpha(2A)- and alpha(2C)-adrenoceptor genes on cognitive tasks and pain perception. *Eur J Pain*. 2009 May 5; [Epub ahead of print].
24. Nackley AG, Shabalina SA, Tchivileva IE, Satterfield K, Korchynskyi O, Makarov SS, Maixner W, Diatchenko L. Human catechol-O-methyltransferase haplotypes modulate protein expression by altering mRNA secondary structure. *Science* 2006;314:1930–1933. [PubMed: 17185601]
25. Lachman HM, Papolos DF, Saito T, Yu YM, Szumlanski CL, Weinshilboum RM. Human catechol-O-methyl transferase pharmacogenetics: description of a functional polymorphisms and its potential application to neuropsychiatric disorders. *Pharmacogenetics* 1996;6:243–250. [PubMed: 8807664]

26. Tahara T, Arisawa T, Shibata T, Nakamura M, Wang F, Hirata I. COMT gene val158met polymorphism in patients with dyspeptic symptoms. *Hepatology* 2008;55:979–982. [PubMed: 18705311]
27. Miller LJ. G protein-coupled receptor structures, molecular associations, and modes of regulation. *Ann N Y Acad Sci* 2008 Nov;1144:1–5. [PubMed: 19076357]
28. Holtmann G, Siffert W, Haag S, Mueller N, Langkafel M, Senf W, Zotz R, Talley NJ. G-protein beta 3 subunit 825 CC genotype is associated with unexplained (functional) dyspepsia. *Gastroenterology* 2004;126:971–979. [PubMed: 15057736]
29. Camilleri CE, Carlson PJ, Camilleri M, Castillo EJ, Locke GR 3rd, Geno DM, Stephens DA, Zinsmeister AR, Urrutia R. A study of candidate genotypes associated with dyspepsia in a U.S. community. *Am J Gastroenterol* 2006;101:581–592. [PubMed: 16464220]
30. Andresen V, Camilleri M, Kim HJ, Stephens DA, Carlson PJ, Talley NJ, Saito YA, Urrutia R, Zinsmeister AR. Is there an association between GNBeta3-C825T genotype and lower functional gastrointestinal disorders? *Gastroenterology* 2006;130:1985–1994. [PubMed: 16762621]
31. Saito YA, Locke GR III, Zimmerman JM, et al. A genetic association study of 5-HTT LPR and GNBeta3 C825T polymorphisms with irritable bowel syndrome. *Neurogastroenterol Motil* 2007;19:465–470. [PubMed: 17564628]
32. Holtmann G, Siffert W, Grote E, et al. G-protein mediated receptor-cell-coupling as a predictor for the long term response to treatment in patients with functional dyspepsia. *Gastroenterology* 2003;124:A80.
33. Taylor RW, Turnbull DM. Mitochondrial DNA mutations in human disease. *Nat Rev Genet* 2005;6:389–402. [PubMed: 15861210]
34. Wang Q, Ito M, Adams K, Li BU, Klopstock T, Maslim A, Higashimoto T, Herzog J, Boles RG. Mitochondrial DNA control region sequence variation in migraine headache and cyclic vomiting syndrome. *Am J Med Genet A* 2004;131:50–58. [PubMed: 15368478]
35. Camilleri M, Carlson P, Zinsmeister AR, McKinzie S, Busciglio I, Burton D, Zaki EA, Boles RG. Mitochondrial DNA and gastrointestinal motor and sensory functions in health and functional gastrointestinal disorders. *Am J Physiol* 2009;296:G510–G516.
36. Saito YA, Strega PR, Tester DJ, Locke GR 3rd, Talley NJ, Bernard CE, Rae JL, Makielski JC, Ackerman MJ, Farrugia G. Sodium channel mutation in irritable bowel syndrome: evidence for an ion channelopathy. *Am J Physiol* 2009;296:G211–G218.
37. Huang GH, Hsieh CC, Chen CH, Chen WJ. Statistical validation of endophenotypes using a surrogate endpoint analytic analogue. *Genet Epidemiol.* 2009 Feb 4; [Epub ahead of print].
38. Camilleri M, Carlson P, McKinzie S, Grudell A, Busciglio I, Burton D, Baxter K, Ryks M, Zinsmeister AR. Genetic variation in endocannabinoid metabolism, gastrointestinal motility and sensation. *Am J Physiol* 2008;294:G13–G19.
39. Camilleri M, Busciglio I, Carlson P, McKinzie S, Burton D, Baxter K, Ryks M, Zinsmeister AR. Candidate genes and sensory functions in health and irritable bowel syndrome. *Am J Physiol* 2008;295:G219–G225.
40. Fukudo S, Kanazawa M, Mizuno T, Hamaguchi T, Kano M, Watanabe S, Sagami Y, Shoji T, Endo Y, Hongo M, Itoyama Y, Yanai K, Tashiro M, Aoki M. Impact of serotonin transporter gene polymorphism on brain activation by colorectal distention. *Neuroimage.* 2009 May 6; [Epub ahead of print].
41. D'Amato M, Bruce S, Bresso F, Zucchelli M, Ezer S, Pulkkinen V, Lindgren C, Astegiano M, Rizzetto M, Gionchetti P, Riegler G, Sostegni R, Daperno M, D'Alfonso S, Momigliano-Richiardi P, Torkvist L, Puolakkainen P, Lappalainen M, Paavola-Sakki P, Halme L, Farkkila M, Turunen U, Kontula K, Lofberg R, Pettersson S, Kere J. Neuropeptide s receptor 1 gene polymorphism is associated with susceptibility to inflammatory bowel disease. *Gastroenterology* 2007;133:808–817. [PubMed: 17854592]
42. Carlson P, Camilleri M, Zinsmeister AR, McKinzie S, Busciglio I, Burton D, D'Amato M. Neuropeptide S receptor 1 (NPSR1) gene polymorphism is associated with susceptibility to altered colonic transit and rectal sensitivity in patients with functional gastrointestinal disorders. *Gastroenterology* 2009;136(Suppl 1):402.

43. Camilleri M, Atanasova E, Carlson PJ, Ahmad U, Kim HJ, Viramontes BE, McKinzie S, Urrutia R. Serotonin transporter polymorphism pharmacogenetics in diarrhea-predominant irritable bowel syndrome. *Gastroenterology* 2002;123:425–432. [PubMed: 12145795]
44. Camilleri M, Busciglio I, Carlson P, McKinzie S, Burton D, Baxter K, Ryks M, Zinsmeister AR. Pharmacogenetics of low dose clonidine in irritable bowel syndrome. *Neurogastroenterol Motil* 2009;21:399–410. [PubMed: 19309415]
45. Thanassoulis G, O'Donnell CJ. Mendelian randomization: Nature's randomized trial in the post-genome era. *JAMA* 2009;301:2386–2388. [PubMed: 19509388]

Figure 1.

Upper panel: In genotype association studies, the relationship between genotype and clinical phenotype includes “black box” intermediaries such as the effect of the environment and other unknown biological factors. Identification of significant association therefore requires large numbers of participants. Lower panel: In genotype-endophenotype association studies, the relationship between candidate genes that affect the biomarkers (endophenotype) of interest is explored with confidence that the biomarkers are associated with a known (often robust) relationship with the main manifestations of the clinical phenotype. Since the biomarker functions are measurable and have a defined coefficient of variation, the size of the function that is detectable can be used to inform the much smaller sample size for the genetic association study.

Figure 2.

Summary of genetic associations that have been explored to date in patients with functional gastrointestinal disorders, predominantly IBS, provide a model of the candidate mechanisms involved in the motor and sensory disorders associated with IBS. Thus, *NPSR1* may enhance barrier permeability, 5-HTTLPR alter the reuptake of 5-HT released from enteroendocrine cells in response to chemical or mechanical stimuli in the lumen, adrenergic receptors alter sympathetic input, and cannabinoid mechanisms (through changes in the function of the rate-limiting enzyme for anandamide, fatty acid amide hydrolase, *FAAH*) alter cholinergic supply to the smooth muscle cell. Moreover, the function of the myocyte is influenced by energy generation that depends on mitochondrial DNA.