

Irritable Bowel Syndrome: Methods, Mechanisms, and Pathophysiology. Genetic epidemiology and pharmacogenetics in irritable bowel syndrome

Michael Camilleri and David A. Katzka

Clinical Enteric Neuroscience Translational and Epidemiological Research, Division of Gastroenterology and Hepatology, College of Medicine, Mayo Clinic, Rochester, Minnesota

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Camilleri M, Katzka DA. Genetic epidemiology and pharmacogenetics in irritable bowel syndrome. *Am J Physiol Gastrointest Liver Physiol* 302: G1075–G1084, 2012. First published March 8, 2012; doi:10.1152/ajpgi.00537.2011.—The objectives of this review are twofold. Our first objective is to evaluate the evidence supporting a role for genetics in irritable bowel syndrome (IBS). Specific examples of the associations of genetic variation and symptoms, syndromes, and intermediate phenotypes, including neurotransmitter (serotonergic, α_2 -adrenergic, and cannabinoid) mechanisms, inflammatory pathways (IL-10, TNF α , GN β 3, and susceptibility loci involved in Crohn's disease), and bile acid metabolism, are explored. The second objective is to review pharmacogenetics in IBS, with the focus on cytochrome P-450 metabolism of drugs used in IBS, modulation of motor and sensory responses to serotonergic agents based on the 5-hydroxytryptamine (5-HT) transporter-linked polymorphic region (5-HTTLPR) and 5-HT₃ genetic variants, responses to a nonselective cannabinoid agonist (dronabinol) based on cannabinoid receptor (CNRI) and fatty acid amide hydrolase (FAAH) variation, and responses to a bile acid (sodium chenodeoxycholate) and bile acid binding (colesevelam) based on klotho β (KLB) and fibroblast growth factor receptor 4 (FGFR4) variation. Overall, there is limited evidence of a genetic association with IBS; the most frequently studied association is with 5-HTTLPR, and the most replicated association is with TNF superfamily member 15. Most of the pharmacogenetic associations are reported with intermediate phenotypes in relatively small trials, and confirmation in large clinical trials using validated clinical end points is still required. No published genome-wide association studies in functional gastrointestinal or motility disorders have been published.

adrenergic; serotonergic; solute carrier 6A4; 5-hydroxytryptamine transporter-linked polymorphic region; inflammation; susceptibility; klotho β ; bile acid malabsorption

EPIDEMIOLOGICAL STUDIES of familial aggregation (57, 99) and twins (7, 70, 72, 82, 83) suggest a genetic contribution to irritable bowel syndrome (IBS). While the data are conflicting, they are generally consistent with the hypothesis that IBS may be a complex genetic disorder. Understanding of genetic variation and its influence on gut motility, secretion, sensation, and inflammation, as it is applied to IBS, has the potential to help elucidate mechanisms of control of a poorly understood syndrome. Similarly, the impact of genetic variation on the targets of therapy in IBS may enhance the efficacy of pharmacological therapies. These targets include receptors and regulators of gut function and variations in drug metabolism.

This review addresses the available literature on the role of genetics in IBS. There are no published genome-wide association

studies in functional gastrointestinal (GI) or motility disorders specifically focusing on genetic epidemiology and pharmacogenetics.

Genetic Epidemiology of IBS: Candidate Gene Approach

As the general approach followed in genotype association studies (Fig. 1), investigators have sought the relationship between genotype and clinical phenotype, while appreciating that intermediaries, such as the effect of the environment and other unknown biological factors, are largely unexplored. With this degree of imprecise control or knowledge of potential contributing factors, the identification of significant associations requires large numbers of participants and, importantly, replication of findings in different cohorts or ethnic groups. A second approach explores genotype-endophenotype association studies, that is, the relationship between candidate genes and quantitative traits (biomarkers, intermediate phenotypes, or endophenotypes) of interest. Endophenotypes are defined here as phenotypes that bear a closer relationship to the biological processes that give rise to the illness, have a hereditary component and cosegregate with illness within families, and are independent of whether or not the illness is active (46). Such biomarkers or quantitative traits typically are measurable, have a defined coefficient of variation, usually have a known relationship with the main manifestations of the clinical phenotype, and provide opportunities to assess genetic associations with much smaller sample sizes.

By studying the genetic associations between candidate genes and intermediate phenotypes that are associated with manifestations of the clinical phenotype, one can also evaluate the role of the candidate mechanism in IBS (see Fig. 1 adapted from Ref. 53). The intermediate phenotypes most commonly used in IBS are colonic transit, colonic motility and compliance, and sensation thresholds and ratings. Details regarding the published gene polymorphisms associated with IBS, the magnitude and significance of the disease association, the sample size, and the replication of findings are summarized in an extensive review by Saito et al. (97).

Neurotransmitter Mechanisms

Serotonin. Serotonin [5-hydroxytryptamine (5-HT)] is involved in controlling GI secretion, motility, and visceral perception (41, 63). It is, therefore, not surprising that, in patients with IBS, studies have documented alterations in 5-HT levels (6) and 5-HT signaling (4); serotonin and serotonin receptor-modulating drugs are vigorously pursued in IBS, despite poor consensus on the optimal end points for trials and the regulatory agency requirement of rigorous safety standards for serotonergic agents because of potential vascular effects (14, 15, 24). As a result of the widespread interest in serotonin, genetic

Address for reprint requests and other correspondence: M. Camilleri, Mayo Clinic, Charlton 8-110, 200 First St. SW, Rochester, MN 55905 (e-mail: camilleri.michael@mayo.edu).

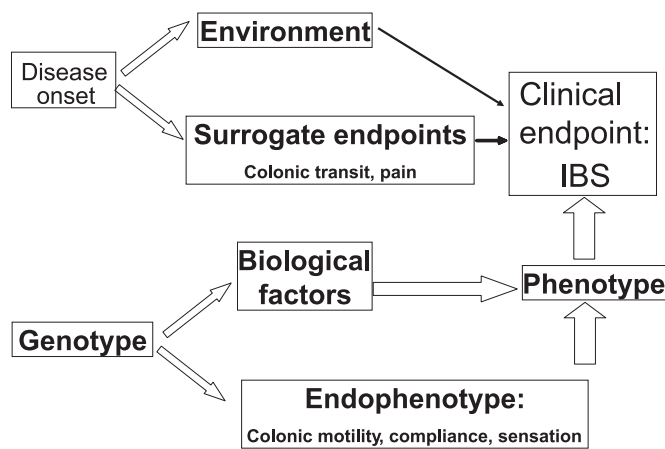


Fig. 1. Genetic associations between candidate genes and intermediate phenotypes that are associated with manifestations of the clinical phenotype. One can also evaluate the role of the candidate mechanism. IBS, irritable bowel syndrome. [Adapted from Huang et al. (53).]

alterations in serotonergic mechanisms are the most extensively studied genetic associations with IBS.

SEROTONIN TRANSPORTER PROMOTER VARIANTS. Serotonin transporter, or solute carrier 6A4 (SLC6A4), is the protein involved in the reuptake of 5-HT after it has interacted with the downstream receptor. Associations have been explored between IBS and two polymorphic regions within the promoter, as well as an intronic region of the gene near exon 2, with a variable number of tandem repeats (VNTR). The main polymorphic region in the promoter region is the 5-HT transporter long polymorphic region (5HTTLPR), which is located on chromosome 17q11.1–17q12 and organized into 14 exons spanning ~38 and ~1.4 kb upstream from the first exon (84). The long polymorphic region consists of a 44-bp insertion (71, 84), although this insertion is 43 bp in certain ethnic groups. The long and short variants of the polymorphic region (71) impact the function of SLC6A4, and the short allele results in a protein that is associated with reduced reuptake of 5-HT.

SLC6A4 is central to fine-tuning brain 5-HT neurotransmission; it is abundant in cortical and limbic areas, affects emotional aspects of behavior, and is associated with anxiety, psychiatric disease, and response to treatment. However, SLC6A4 is also expressed in the GI tract, and there is controversy as to whether it is underexpressed in mucosal biopsies from patients with IBS (17, 28).

Several studies have explored the association of any 5-HTTLPR allele and IBS, including one study from our center (64). Studies from Western countries and Asia on the association of the 5-HTTLPR genotype and IBS provide somewhat contradictory results (110), and a meta-analysis in 2007 suggests that, among Caucasians and Asians, odds ratios (OR) for IBS in subjects for SS or LS vs. LL [OR = 1.0, 95% confidence interval (CI) = 0.8–1.2] and for SS vs. LL or LS (OR = 1.0, 95% CI = 0.7–1.4) are not significant (110). Data from IBS patients from India (74% men) suggest an association of the homozygous S genotype (SS) with constipation-predominant IBS (C-IBS) (101). While the prevalence of IBS in India appears to be higher in men than in women (43), it is unclear why sex would explain the association of the SS genotype (for a gene located on chromosome 17, rather than an X or a Y

chromosome) with C-IBS. A study that included patients with IBS from Germany and the United Kingdom [where prevalence is ~1.3–1.5:1 in women compared with men (38, 47)] showed a lower prevalence of the SS genotype in IBS and, particularly, in diarrhea-predominant IBS (D-IBS), but this was only observed in male patients (34 male IBS patients and 30 male controls) (88).

The biological basis of these genotype-phenotype associations of the 5-HTTLPR SS genotype and C-IBS in India and protection from D-IBS in Germany and the United Kingdom is unclear. Thus, on the basis of the observations of Lesch et al. (71), the SS genotype would be expected to result in increased 5-HT (e.g., in a synaptic cleft), as it is associated with reduced 5-HT reuptake. It is unclear how increased 5-HT (which promotes secretion and motility in the gut) would be associated with C-IBS in patients from India. Similarly, the “protection” from D-IBS in male patients from the United Kingdom and Germany is difficult to understand. These observations emphasize the importance of balancing ethnicity among disease and control conditions, especially in increasingly multiethnic countries. The frequency of the short allele is higher among people of Asian ancestry, and ethnic imbalance in the disease and control groups may account for some of these associations. In summary, the association of 5-HTTLPR and IBS symptoms remains unproven.

Other studies show association with features that are related to IBS, such as psychological distress, rectal sensorimotor functions, and cerebral blood flow during rectal distensions.

Jarrett et al. (56) reported on 5-HTTLPR polymorphisms in 21 men and 117 women with IBS: the 5-HTTLPR genotype was not associated with GI symptom severity score or current levels of depression, anxiety, or general psychological distress. On the other hand, participants with the 5-HTTLPR SS genotype or those carrying a *STin2.9* VNTR allele (see below) were more likely to have a history of depression, and participants with the 5-HTTLPR LL genotype had a reduced level of social functioning (56). It was concluded that the 5-HTTLPR genotype may modify risk for depressive episodes in IBS.

The 5-HTTLPR genotype (S allele) is associated with higher pain sensory ratings during rectal distension studies in health and IBS, and the increased sensation ratings in carriers of the S allele are not caused by lower rectal compliance (19).

The 5-HTTLPR SS genotype is also associated with greater regional cerebral blood flow in response to colorectal distension in patients with IBS. The most pronounced regional increases were in the left anterior cingulate cortex, right parahippocampal gyrus, and left orbitofrontal cortex (40). The anterior cingulate cortex and orbitofrontal region have been shown to be preferentially activated in patients with IBS during or in anticipation of rectosigmoid stimulation (87). Taken together, these two observations converge on the increased pain sensitivity in IBS patients, owing to increased activity in the emotional motor system of the brain.

Another genetic variation related to 5-HTTLPR is *STin2* VNTR, which is located in intron 2; the biological function of this polymorphism is unclear. It consists of a variable number (e.g., 9, 10, or 12) of identical 17-bp segments (65); *STin2.12* has been reported to possess greater transcriptional activity than *STin2.10* (76). The 10/12 genotype has been associated with IBS in one study (114). However, at least five other

studies found no association between *STin2* VNTR and IBS (65, 73, 88, 90, 121).

A single-nucleotide polymorphism (SNP), rs25531, located immediately upstream of 5-*HTTLPR*, is strongly linked with the latter. The rs25531 G allele lowers SLC6A4 transcription compared with the A allele and occurs most frequently with the 5-*HTTLPR* L allele. In a study of 186 patients with IBS and 50 healthy control subjects, the odds of having IBS were increased (OR = 3.3, 95% CI = 1.1–9.6) in carriers of the G allele of rs25531 compared with healthy controls (65). However, the minor allele frequency is only 10–18% (52); therefore, these results require replication.

SEROTONIN RECEPTOR VARIANTS. The 5-*HT_{2A}* receptor gene is expressed in the brain, vagal nuclei, and the gut (11, 74). The –1438 (G/A) polymorphism has been associated with anorexia nervosa, alcohol dependence, obesity, obsessive compulsive disorder, and seasonal affective disorder (33, 86). The AA genotype is associated with higher 5-*HT_{2A}* gene expression in cell lines endogenously expressing 5-*HT_{2A}* (91). The –1438 polymorphism is in linkage disequilibrium with the 102 T/C polymorphism, which appears to be functionally silent (85).

In a study from Turkey of 54 patients with IBS based on Rome I criteria and 107 healthy controls, increased risk of IBS was associated with the homozygous C allele of the 102 T/C polymorphism (OR = 7.89, 95% CI = 1.079–57.76) and the homozygous A allele of the –1438 G/A polymorphism (OR = 11.14, 95% CI = 1.59–78.06) of the 5-*HT_{2A}* receptor gene. In addition, the T/T genotype of the 102 T/C polymorphism may be associated with more severe pain in patients with IBS (92).

More recently, a study from Greece (78) of 124 patients with Rome III-positive IBS criteria and 238 healthy individuals replicated the association with the A allele (OR = 1.59, 95% CI = 1.17–2.18) and AA genotype (OR = 2.41, 95% CI = 1.30–4.44) of the –1438 G/A polymorphism and IBS. However, associations with the –102 C/T polymorphism with IBS and with more severe pain were not replicated (78).

Expression of 5-HT receptors (*HTR_{3A}* and *HTR_{3E}*) and micro-RNA (miRNA)-510 has been detected in human colonic epithelial cells by in situ hybridization (58). miRNAs are short (20- to 24-nucleotide) noncoding RNAs that are involved in posttranscriptional regulation of gene expression. No *HTR_{3E}* expression was detectable within the lamina propria; a weak expression of miRNA-510 was shown within the lamina propria; *HTR_{3E}* and miRNA-510 colocalize in enterocytes (58). 5-*HT_{3C}*, 5-*HT_{3D}*, and 5-*HT_{3E}* subunits are coexpressed with 5-*HT_{3A}* in cell bodies of human colonic myenteric neurons. Furthermore, 5-*HT_{3A}* and 5-*HT_{3D}* are expressed in the submucosal plexus of the human large intestine (59).

In a study of genetic variation of 5-*HT₃* receptors in IBS patients in the United Kingdom and Germany (58), the novel *HTR_{3E}* 3'-untranslated region variant c.*76G>A (rs62625044) was associated with female D-IBS (OR = 8.53, 95% CI = 1.04–70.28) in the United Kingdom and replicated in D-IBS patients in Germany (OR = 4.92, 95% CI = 1.49–16.30). Using a reporter assay, Kapeller et al. (58) showed that c.*76G>A affected the binding of miRNA-510 to the *HTR_{3E}* 3'-untranslated region and caused elevated protein expression in two different cell lines. While these data suggest an association between rs62625044 and IBS, it is worth noting that the minor allele frequency of rs62625044 is only 0.03 (95% CI = 0.011–0.064), and the difference in prevalence of the

c.*76G>A genotype (11% in D-IBS patients and 4% in controls) in the pooled populations may represent a chance finding that requires replication.

The *HTR_{3A}* polymorphism c.–42C>T (C178T; rs1062613), which causes increased 5-*HT_{3A}* subunit expression in vitro (58), is associated with amygdala responsiveness in IBS patients (62); thus the C/C genotype, compared with T carrier status, is associated with increased anxiety and amygdala responsiveness during emotional and nonemotional tasks.

α_2 -Adrenergic receptors. Several studies have also emphasized the role of the autonomic nervous system in GI motility and, consequently, alteration of this part of the nervous system in IBS (32, 48, 106). In general, adrenergic function is integral to regulation of the autonomic nervous system; more specifically, adrenergic function directly modulates gut and colon function and sensation (8, 112). The α_2 -adrenergic receptor has also been proposed as a mechanism whose genetic variation may modify motor and sensory functions in IBS patients. In 274 IBS patients (90 of whom had C-IBS) and 120 controls, there was a significant association between C-IBS and the α_2C Del 322–325 deletion, which alters the coding region of the gene and encodes a receptor with a loss-of-function phenotype (64). This variation results in a receptor that has markedly decreased agonist-mediated responses in vitro (103). There is also evidence that the same variation results in altered cold pain perception without affecting cognition (66).

A nonsignificant ($P = 0.08$) association of C-IBS with the α_2A –1291 C>G genotype (rs1800544, located in the promoter region of the α_2A -adrenoceptor gene) provides further support for association between genetic variations in α_2A - and α_2C -adrenergic receptors and C-IBS. From a study in Northern India, a genotypic association between the α_2A –1291 C>G polymorphism and D-IBS (OR = 2.08, 95% CI = 1.06–4.07, $P < 0.05$) but no significant association with C-IBS or mixed-bowel-function IBS (M-IBS) was observed (100).

In our study, however, we did not detect associations between the α_2A genotype and a wide range of GI sensory or motor functions (19). Further work is required to assess the potential genetic role of α_2 -adrenergic mechanisms in IBS.

Catechol-O-methyltransferase. Catechol-O-methyltransferase (COMT) is one of the enzymes that metabolizes catecholamines, thereby acting as a key modulator of dopaminergic and adrenergic/noradrenergic neurotransmission. The *COMT* Val¹⁵⁸Met polymorphism is associated with a difference in thermostability, leading to a three- to fourfold reduction in the activity of the COMT enzyme (75). The Met/Met genotype results in reduced COMT enzymatic activity (60). The *COMT* Val¹⁵⁸Met polymorphism influences the human experience of pain and may underlie interindividual differences in the adaptation and responses to pain and other stressful stimuli (124). There is evidence of association between *COMT* Val¹⁵⁸Met genetic variation and reduced presynaptic phasic dopamine release in Met carriers that may result in impaired ability of the prefrontal cortex to downregulate startle responses (and amygdala complex activity) during threat and increased attention to a prepulse (108). The authors suggest that altered early response mechanisms to potential and symptom-related threats may play a role in central pain amplification and hypervigilance and may partially explain the greater reported prevalence of the Met allele in chronic pain syndromes (31).

Cannabinoid receptors and metabolism. Cannabinoid receptors modulate a variety of GI functions, including pain modu-

lation, inflammation, and gastric and colonic motility (55), in healthy humans (34, 35). A recent study has further demonstrated that use of a cannabinoid agonist may reduce fasting colonic motility in patients with nonconstipated IBS and in patients with IBS (116) and, therefore, represents a therapeutic target. As a result, a search for genetic variations in cannabinoid receptors and signaling is warranted.

The main endogenous cannabinoid agonists (endocannabinoids) are anandamide (AEA) and 2-arachidonyl glycerol, and there are two classes of cannabinoid receptors: CB1 receptors (located in the central nervous system, GI tract, enteric nervous system, liver, fat, and muscle) and CB2 receptors (found predominantly in immune cells) encoded by the *CNR1* and *CNR2* genes, respectively. The proteins involved in endocannabinoid metabolism are fatty acid amide hydrolase (FAAH) for AEA and monoacylglycerol lipase for 2-arachidonyl glycerol. The endocannabinoids are synthesized on demand in postsynaptic neurons (unlike classic neurotransmitters, which are presynthesized and stored in synaptic vesicles), and they signal as retrograde messengers binding to presynaptic CB1 receptors on cholinergic neurons, activating intracellular signaling cascades, inhibiting cAMP production, and, thereby, modulating protein kinases and ion channels, leading to inhibition of presynaptic neurotransmitter release (specifically, ACh in the enteric nervous system). AEA is then inactivated by FAAH; reduced activity of this enzyme leads to more AEA reaching the presynaptic membrane and a greater effect on ACh release from the presynaptic neuron. A common SNP is *FAAH* C385A, which causes reduced cellular expression and activity of FAAH enzyme (27). *FAAH* C385A was significantly associated with D-IBS, M-IBS, and rapid colon transit in a Mayo Clinic study (21).

In a study of 162 Rome II-positive IBS patients and 423 healthy controls from Korea, Park et al. (90) explored the association of IBS with the *CNR1* gene. The gene that codes for *CNR1* contains a polymorphic (AAT)_n triplet repeat in the 3'-flanking region of the *CNR1* gene: a higher number of AAT triplets may induce a Z-shaped conformation in the DNA, thereby altering gene transcription. Thus, expression of the gene can be inversely proportional to the number of AAT repeats. This study showed significant associations between the *CNR1* >10/>10 genotype and IBS overall (C-IBS, D-IBS, and M-IBS) and with severity of abdominal pain in IBS (90).

Association of the *CNR1* genotype with colonic motor response to a nonselective cannabinoid agonist, dronabinol, is described in *Pharmacogenetics in IBS*.

SCN5A. The *SCN5A*-encoded Na⁺ channel, Na_v1.5, is expressed in interstitial cells of Cajal and smooth muscles in the circular muscle layer of the human intestine. Among 49 patients with IBS associated with at least moderately severe abdominal pain, 1 had a loss-of-function missense mutation, G298S (98). This mutation was not observed in 1,500 healthy controls. This G298S *SCN5A* missense mutation is functionally relevant, as it resulted in marked reduction of whole cell Na⁺ current and loss of function of Na_v1.5 in transfected human embryonic kidney (HEK-293) cells (98).

Inflammatory Mechanisms

There is mounting evidence suggesting that, in some patients, IBS may be associated with inflammation or immune

activation. The evidence includes the occurrence of IBS as a postinfectious phenomenon (79, 104), an increase in gut inflammatory cells noted on mucosal biopsy (reviewed in Ref. 89) or full-thickness biopsy (107), and experimental data that demonstrate alterations in gut motility and sensation after naturally acquired or experimentally administered infection (54, 69). As with celiac disease and inflammatory bowel disease, the pauci-inflammatory IBS may result from the interaction between environmental influences, such as nutrients, antibiotics, and the microflora, and the host genetics (95). As in inflammatory bowel disease, genetic alterations in inflammatory signaling and regulation may predispose to IBS.

IL-10, TNF α , and G proteins. IL-10 and transforming growth factor- β 1 have anti-inflammatory properties; genotype variation in the production of these cytokines would be consistent with the hypothesis that some patients are genetically predisposed to produce smaller amounts of the anti-inflammatory cytokine (e.g., IL-10) and potentially develop the mild inflammatory manifestations of IBS. In a first study from the United Kingdom (45), frequencies of the high IL-10 producer genotype for IL-10 (*IL-10* G-1082A) were significantly reduced in patients with IBS compared with controls (21% vs. 32%, $P = 0.003$). Polymorphisms in the 5'-flanking region of IL-10 genetically affect interindividual differences in IL-10 production (36). A subsequent study from the Netherlands (109) did not replicate these results for *IL-10* G-1082A. On the other hand, in the study from the Netherlands, *TNF α* G/A (G-308A, high producer) was more prevalent in IBS patients than in controls (41% vs. 26%, $P = 0.02$), and more patients than controls (41% vs. 30%) were positive for the A allele ($P = 0.044$, OR = 1.68, 95% CI = 1.01–2.79).

G protein-coupled receptors are present on excitable cells and cells that are susceptible to regulation (81). Activation of these receptors leads to production (catalyzed by GN β 3) of the $\beta\gamma$ heterodimer from the heterotrimeric G protein. First studies suggested an association between *GN β 3* C825T and dyspepsia (13, 50); however, this finding was not replicated in patients with IBS (3, 96).

A study from Korea replicated none of these three associations (68).

Susceptibility genes for Crohn's and inflammatory bowel diseases. The main susceptibility genes for Crohn's disease affect several etiopathogenetic mechanisms, including bacterial recognition, epithelial transport and barrier function, prostaglandins, autophagy, and Th17 differentiation (10). Three genes, Toll-like receptor 9 [*TLR9*, rs5743836 (OR = 1.536, 95% CI = 1.080–2.182)], interleukin-6 [*IL6*, rs206986 (OR = 1.509, 95% CI = 1.031–2.209)], and E-cadherin 1 [*CDH1*, rs16260 (OR = 1.398, 95% CI = 1.069–1.829)], have been reported in association with postinfectious IBS in a Walkerton, Ontario, Canada cohort (111).

In a study involving patients from Sweden and the United States (125), associations between 30 Crohn's disease candidate genes and IBS identified a significant association of TNF superfamily member 15 [*TNFSF15*, rs4263839 (G/a)] with IBS ($P = 2.2 \times 10^{-5}$, OR = 1.37) and C-IBS ($P = 8.7 \times 10^{-7}$, OR = 1.79). Importantly, the association was detected independently in patients in Sweden and in the Upper Midwest in the United States (125), many of whom are of Scandinavian and Northern European ethnicity. More recently, the association of IBS with *TNFSF15* was also reported in a cohort of

patients in England (105). *TNFSF15* is strongly associated with Crohn's disease in Japan and more modestly in European populations. *TNFSF15* is upregulated in Crohn's disease and has a variety of actions, including induction of nuclear factor- κ B activation, potentiation of IL-2 signaling, and secretion of γ -interferon by T lymphocytes (122).

In the same US cohort of IBS patients and controls, we observed univariate associations of IBS with *IL23R* [rs11465804 (T/g), $P = 0.006$], positive regulatory domain I-binding factor [*PRDM1*, rs7746082 (G/c), $P = 0.031$], and immunity-related GTPase family M protein [*IRGM*, rs11747270 (A/g), $P = 0.050$] and D-IBS with Toll-like receptor 9 [*TLR9*, rs5743836 (A/g), $P = 0.02$]; the latter confirmed the observations in postinfectious IBS patients in Ontario, Canada (111). The association with *IL23R* and the other genetic variants was not significant, as $P = 0.0015$ was required by Bonferroni's correction for 34 genes (22). Also, the association with *IL23R* was not observed in the Swedish cohort (125).

Univariate associations (not corrected for multiple comparisons) of inflammation susceptibility loci with the intermediate phenotype (quantitative trait) of scintigraphic colonic transit were also reported in the Mayo Clinic study of 172 IBS patients (22). On the basis of the dominant inheritance model, rs7746082 (*PRDM1*, $P = 0.01$) is associated with colonic transit at 24 and 48 h, rs5743836 (*TLR9*, $P = 0.01$) with colonic transit at 48 h, and rs7927894 [chromosome 11 open reading frame 30 (*c11orf30*), $P = 0.007$] and rs2872507 (*ORMDL3*, $P = 0.014$) with colonic transit at 24 h. The association with *TNFSF15* and colonic transit was not significant, even at the univariate level ($P = 0.174$ and $P = 0.256$ for colonic transit at 24 and 48 h, respectively).

The biological plausibility of these associations is illustrated by the biological functions of the susceptibility genes. *PRDM1* [also called B lymphocyte-induced maturation protein 1 (*BLIMP-1*)] is a zinc finger transcriptional repressor that silences β -interferon gene expression, regulates plasma cell differentiation, and controls gene expression in T lymphocytes, macrophages, sebaceous gland, and skin epidermis (9). However, the equivalent molecule in zebrafish is essential for neural crest and sensory neuron specification (49), suggesting that the *PRDM1* polymorphism may impact colorectal functions by influencing neural function. *TLR9* is involved in barrier function and pattern-recognition receptors that elicit innate/adaptive immune response and inflammation, and *c11orf30* is involved in epithelial immunity, growth, and/or differentiation (42, 77). The orosomucoid (*ORM*) gene family encodes transmembrane proteins localized in the endoplasmic reticulum; *ORMDL3* is involved in control of sphingolipids (12), which are structural components of membranes, including those in neural tissues.

Neuropeptide S receptor 1. The neuropeptide S (NPS) receptor 1 (*NPSR1*) gene on chromosome 7 is associated with asthma and inflammatory bowel disease (30). *NPSR1* is expressed on the intestinal epithelium and is upregulated in inflammation; NPS-*NPSR1* signaling induced increased expression of cholecystokinin, vasoactive intestinal peptide, peptide YY, and somatostatin in HEK-293 cells (23). In a study with 699 participants (approximately two-thirds patients and one-third healthy controls) of 18 *NPSR1* polymorphisms (23) that span the gene, rs1419793 was significantly associated with colonic transit ($P < 0.003$, with false discovery rate correc-

tion). The mechanisms whereby *NPSR1* SNPs might result in altered motor or secretory functions are unclear.

Genetic Predisposition to Bile Acid Malabsorption in IBS

Bile acids have potent secretory effects on the colonic mucosa. As a result, patients with predispositions to bile acid malabsorption (BAM), such as those who have undergone extensive ileal resection, commonly suffer from diarrhea. More recent data, however, have demonstrated that, in subjects and patients with intact small bowel, alterations in BAM may contribute to gut symptoms. For example, among patients with chronic functional diarrhea (which overlaps with D-IBS), a systematic review estimated that BAM, shown by ^{75}Se -homocholic acid taurine scanning, affects $\sim 30\%$ of patients (115). A surrogate serological test indicating increased bile acid synthesis is serum $7\alpha\text{C}_4$, which was elevated in 20% of D-IBS patients in a small study (25). Elevation of fecal deoxycholate has been documented in patients with IBS (67). Walters et al. (113) provided evidence that impaired feedback inhibition of bile acid synthesis by the ileal hormone FGF-19 plays a role in bile acid-associated diarrhea. Conversely, patients with C-IBS and functional constipation had altered diurnal rhythm with no serum $7\alpha\text{C}_4$ peak at lunchtime (1). These data might suggest that alterations in genes that regulate bile acid metabolism and feedback may contribute to abnormal bowel pattern and symptoms in IBS.

Genetic variation in bile acid metabolism. Wong et al. (117) explored the hypothesis that variants of genes regulating hepatic bile acid synthesis play a role in D-IBS. In 435 IBS patients and 279 healthy subjects, we tested individual associations of 15 common SNPs from 7 genes critical to bile acid homeostasis. S rs17618244 [Arg⁷²⁸Gln in *klotho* (*KLB*)] was associated with colonic transit at 24 h. The G allele (Arg⁷²⁸) compared with the A allele (Gln⁷²⁸) was associated with accelerated colonic transit ($P = 0.0007$) in the overall cohort. This association was restricted to D-IBS ($P = 0.0018$); both were significant after Bonferroni's and false discovery rate corrections. Interaction tests of *KLB* rs17618244 with 3 non-synonymous SNPs of FGF receptor 4 (*FGFR4*) revealed that rs1966265 (Val¹⁰Ile) and rs351855 (Gly³⁸⁸Arg) modulate the association of rs17618244 with colonic transit in D-IBS ($P = 0.0025$ and $P = 0.0023$, respectively). The associated SNP in *KLB* (rs17618244) was functionally significant. *KLB* Arg⁷²⁸ significantly reduced protein stability compared with *KLB* Gln⁷²⁸ (117).

The diminished stability of the *KLB* protein resulting from *KLB* Arg⁷²⁸ (G allele) is expected to weaken the FGFR4-*KLB* receptor complex on the hepatocyte plasma membrane, leading to decreased negative feedback by endogenous FGF-19, increased hepatocyte CYP7A1 expression, and increased hepatic bile acid synthesis. Excess bile acid reaching the small bowel may overwhelm the ileal capacity for bile acid absorption, spill over into the colonic lumen, and accelerate colonic transit by stimulating motility and secretion (5, 80).

Genetic variation in the bile acid receptor TGR5. The membrane-bound bile acid receptor TGR5 is located on myenteric, cholinergic, and nitrergic neurons in colon and proximal small intestine. In humans, TGR5 is colocalized with the CFTR in gallbladder epithelium, and stimulation of TGR5 in gallbladder cells activates CFTR (61). In a study of 230 healthy

controls and 414 patients with lower functional GI disorders, we tested the association between *TGR5* SNP rs11554825 (minor allele frequency of 41%), located in the untranslated *TGR5* exon 1, which is associated with reduced expression of *TGR5* mRNA in a lymphoblastoid cell line (51) with symptom phenotype, and the intermediate phenotypes of small bowel and colonic transit by radioscinigraphy, which was available in 213 people (26). There was no significant association with symptom phenotype. We observed a potential association with overall small bowel (colonic filling at 6 h, $P = 0.061$) and colonic transit at 24 h ($P = 0.083$). The association of the SNP with colonic filling at 6 h in the D-IBS subgroup ($P = 0.017$) indicated an average 50% faster small bowel transit in the TC/CC than the TT subgroup. No association was observed in D-IBS patients for colonic transit at 24 h (26). Further studies are required to characterize the potential role of the bile acid receptor *TGR5* and genetic variation in *TGR5* in the mechanism of D-IBS.

Pharmacogenetics in IBS

In addition to understanding the pathophysiology of IBS, insight into pharmacogenetics and, specifically, the manner in which genetic abnormalities influence drug activity, binding, and metabolism has become central to proposing control mechanisms in IBS and may impact the management of individual IBS patients.

Drug metabolism and pharmacogenetics. Overall effects of medications result from metabolism, disposition, and effects of the agent. The enzymatic metabolism of drugs involves modifications of functional groups (phase I reactions, such as oxidation, dehydrogenation, and esterification) or conjugation with endogenous substituents (phase II reactions) (37). The commonest and most relevant drug modifications in IBS result from cytochrome *P*-450 (*CYP*) 2D6 (*CYP2D6*) metabolism, which is an example of phase I drug metabolism. There are distinct geographic variations of the *CYP* genes, suggesting that population substructure can strongly affect the variation in pharmacogenetic loci (102).

The number of functional alleles (>3, 2, 1, and 0) determines whether *CYP2D6* metabolism is ultrarapid, extensive, intermediate, or poor. About 1% of Asians and 5–10% of Caucasians are poor metabolizers (120). Gene multiplication may result in ≥ 3 functional alleles, and ethnic groups with >10 functional alleles (29) are infrequent among Northern Europeans but frequent (as high as 29%) in East African populations (2). The most common nonfunctional alleles are *CYP2D6**3, *CYP2D6**4, *CYP2D6**5, and *CYP2D6**6, which constitute ~98% of nonfunctional alleles in Caucasians (44).

CYP2D6 metabolism impacts the extensively used tricyclic antidepressants and selective serotonin reuptake inhibitors in functional GI disorders and visceral hypersensitivity (39). Although not yet fully appreciated in IBS practice, a multitude of drugs are metabolized by *CYP2D6* and many drugs that inhibit or activate *CYP2D6*, setting up the possibility of significant drug interactions. In some ethnic groups, it may be advisable to use *CYP2D6* testing if antidepressants are being prescribed (123).

The second category of pharmacogenetic modulation of drug effects in IBS reflects variations in receptors, transporters, or function of rate-limiting enzymes.

Serotonergic pharmacogenetics. The most informative studies of pharmacogenetics in IBS revolve around *5-HTTLPR* genetics and the efficacy of alosetron in normalizing colonic transit in D-IBS (18) and the efficacy of tegaserod in the treatment of bowel dysfunction in C-IBS (73). 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 less 5-HT 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 (73), since there is less endogenous 5-HT to complement the effects of the exogenous tegaserod in activating the 5-HT₄ receptor.

Adrenergic pharmacogenetics. In pharmacodynamic studies of low doses of the α_2 -adrenergic agonist clonidine, there were significant associations between post-clonidine responses and $\alpha 2A$ 1291C>G SNPs for gastric accommodation and rectal sensations of gas and urgency (20).

Bile acids and pharmacogenetics in C-IBS and D-IBS. In addition to the observations that genetic variations in *KLB* and, possibly, *FGFR4* are associated with accelerated colonic transit in patients with D-IBS (see Fig. 2 in Ref. 117), variations in the same genes influence the colonic transit response to chenodeoxycholic acid in C-IBS (94) and to colestevlam in D-IBS patients (118).

Dronabinol pharmacogenetics, *CNR1*, and colonic motility in nonconstipated IBS. Wong et al. (116) found that, in 75 IBS patients (35 with C-IBS, 35 with D-IBS, and 5 with M-IBS), dronabinol (a synthetic tetrahydrocannabinol derivative, CB1 agonist) decreased fasting colonic phasic motility and increased colonic compliance, and the effects were greatest in patients with D-IBS or M-IBS. *CNR1* rs806378 (CC vs. CT/TT) appeared to affect fasting proximal colonic motility (prior to dronabinol treatment) in all patients with IBS ($P = 0.075$), and dronabinol affected fasting proximal colonic motility in patients with D-IBS or M-IBS with the variant *FAAH* rs324420 CA/AA ($P = 0.013$).

In a second study of 36 D-IBS patients (119), differential treatment effects of 5 mg (but not 2.5 mg) of dronabinol compared with placebo were observed for *CNR1* rs806378 (CC vs. CT/TT), with the CT/TT group demonstrating a trend to slowing of overall colonic transit ($P = 0.13$); this underpowered study requires replication, preferably with peripherally restricted and CB1-selective cannabinoid agents.

Conclusion

Within the past few decades, the combination of major breakthroughs in genetic analysis and understanding the pathophysiology of IBS has led to a more unified approach to understanding mechanisms and potential therapy in IBS. Specifically, the understanding of genetic variation, its influence on gut motility, secretion, sensation, and inflammation, and its application to IBS have provided further elucidation of a syndrome that is so little understood. The most convincing association of the IBS phenotype is with *TNFSF15*, which has been observed in three independent cohorts in Sweden, the United States, and England. Genes influencing neural, barrier, mast cell, or immune function are univariately associated with colonic transit in IBS, supporting the hypothesis that local immune activation and, possibly, altered barrier function, such

as increased permeability, and reflex motor responses may contribute to development of IBS. Study of the genetic abnormalities associated with IBS may enhance pharmacological therapies by targeting key controlling receptors and regulators of gut function and by understanding the influence of variations in drug metabolism, potentially leading to individualized medicine in IBS.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

M.C. and D.A.K. prepared the figures; M.C. and D.A.K. drafted the manuscript; M.C. and D.A.K. edited and revised the manuscript; M.C. and D.A.K. approved the final version of the manuscript.

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