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Genetics of Human Gastrointestinal Sensation

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Abstract

The objective is to review the genetics of human visceral pain with particular emphasis on pain associated with irritable bowel syndrome. The biomarkers most commonly employed in identifying visceral hypersensitivity are sensation ratings and thresholds or brain imaging during viscus (e.g. rectal) distension. Genetic studies suggest that variation in the control of candidate genes involved in ion channel function, neurotransmitter synthesis, reuptake or receptor functions, and inflammatory disease susceptibility loci may impact variations in prevalence of the symptom phenotype of abdominal pain or IBS, or quantitative traits (intermediate phenotypes) of rectal sensation. The candidate genes include *SLC6A4*, *CNR1*, and *TNFSF15* reflecting serotonin reuptake, cannabinoid receptors and inflammatory-barrier functions. However, other than *TNFSF15*, the other candidate genes are only univariately associated with pain, IBS symptom complex or quantitative traits of sensation. These data have generated hypotheses and present opportunities for study of mechanisms and treatment of visceral pain in humans, which remains an unmet clinical need in patients with IBS and functional abdominal pain.

Keywords

abdominal pain; irritable bowel syndrome; neurotransmitters; ion channels

Introduction

The objective is to review the genetics of human visceral pain with particular emphasis on pain associated with irritable bowel syndrome. Some of the studies in the recent literature pertaining to mutations have provided insights on the mechanisms of visceral pain. Thus, for example, NaV1.7 mutations in familial rectal pain provide an example of point mutations radically affecting ion channels leading to gain of function and the experience of pain. Study of genetics may provide insights on mechanisms of visceral pain. However, other than the NaV1.7 mutation, there is only one gene that has been demonstrated to be significantly associated (beyond false detection rates) with IBS (*TNFSF15* which was separately evaluated in Scandinavian, American and British patients). The other genetic associations are generally regarded as univariate associations.

In order to understand the studies that have explored the effects of genetic variations on gastrointestinal sensations, introductory sections are provided to briefly describe the transmitters and ion channels involved in visceral sensation and biomarkers for rectal and colonic sensations in humans.

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Neurotransmitters and Ion Channels Involved in Visceral Sensation

Traditionally, the mediators involved in the pathways of visceral sensation were: serotonin (5-HT), nitric oxide (NO), substance P, calcitonin gene related peptide (CGRP), norepinephrine, opiates such as endorphins, enkephalin and dynorphin, proteases (e.g. tryptase) acting on protease-activated receptors-2 (PAR-2) and others. These transmitters classically mediate sensation, pain or relief of pain through specific receptors. Different neurotransmitters or ion channels are involved in certain functions: For example, functional reflexes are mediated by 5-HT and VIP, basal nociception by substance P, and CGRP, sensitization of nerve terminals by PAR-2, nerve growth factor, and inflammatory and non-inflammatory nociception by transient receptor potential [TRP] channels, acid sensing ion channels [ASIC]. Sodium channels are involved in sensory nerve function. *SCN5A*-encoded Na(v)1.5 Na(+) channel is expressed in interstitial cells of Cajal (ICCs) (which may transduce some aspects of mechanical stimuli to extrinsic afferents) and circular layer smooth muscle in human intestine.

Dysfunction of several ion channels in association with pain syndromes have been identified in transgenic mice and heritable human disorders. While these are predominantly understood in somatic sensation, Nav 1.5, Nav 1.8, and Nav 1.9 channels are associated with visceral pain. In addition channels contributing to mechanosensation in the gut have been described (1). TRP channels, which include receptors for compounds found in common herbs and spices, comprises five subfamilies (TRPA, TRPC, TRPM, TRPP and TRPV) in mammals that share several key properties: they are non-selective cation channels and most have six transmembrane domains (2).

Damaged tissue is associated with lower extracellular pH. Protons are known to activate TRPV1 and cause pain. Proton-gated cation channels named ASIC (acid sensing ion channel) are expressed throughout the nervous system and comprise a two transmembrane subunit that place them in the family of amiloride sensitive ENaC (epithelial sodium channel) channels (3).

Acid-sensing ion channels (e.g. ASIC3) mediate pain associated with tissue acidosis after inflammation or injury, and are also involved in colonic mechanical transduction (4,5). The reader is referred to authoritative reviews on these channels (5,6); in summary, ASIC1, ASIC2 and TRPA1 contribute to mucosal mechanoreceptor function, whereas, ASIC1, ASIC2 and ASIC3 contribute to tension or mechano-receptor function and TRPV1, TRPV4 and TRPA1 to mechanoreceptor function in different regions or layers of the gut wall (data summarized in reference 1).

The relative importance of these diverse channels is the subject of ongoing research in inflammatory and non-inflammatory nociception. Human studies will likely require the development of pharmacological agents directed to the individual channels. At present, there are genetic studies that suggest these ion channels are relevant in rectal or colonic pain disorders. Thus, based on mouse knockout models, TRPV1 and ASIC3 appear to be important candidates for mediating afferent responses to colonic phasic distensions (5). Human studies complement these observations. Thus, fecal urgency and rectal hypersensitivity were associated with increased numbers of polymodal sensory nerve fibers expressing TRPV1 (7), and the voltage-gated ion channel Nav1.7 innervation is increased in patients with idiopathic familial rectal pain (8). Yiangou et al. (8) conducted full-thickness rectal biopsies from 7 patients with physiologically characterized rectal hypersensitivity, and compared these with 10 control tissues. In rectal hypersensitivity, Nav1.7 immunoreactive nerve fibers were significantly increased in mucosal, sub-mucosal, and muscle layers, while non-specific immunoreactivity of nerve fibers was increased only in the mucosa, suggesting increased expression of Nav1.7 in all layers, and nerve sprouting in the mucosa. This

suggests a gain of function whose cause is uncertain, but it was suggested that it may be due to increase of nerve growth factor (NGF) which regulates the expression of both Na(v)1.7 and TRPV1 which was also reported to be increased in this condition. Two genetic mutations associated with this familial syndrome are discussed below.

Biomarkers for Rectal and Colonic Sensations in Humans

The commonly used methods are applied during barostatically-controlled balloon distensions in the rectum or colon. The readouts for sensation or biomarkers are: sensation thresholds during ascending increases in pressure, sensation ratings in response to random order phasic distensions, and brain imaging during distensions. These have been used in the study of genetics of colorectal sensation.

Consensus methods (9), normal values and validation (such as performance characteristics and responsiveness to acute perturbations or medications) are available for barostat sensation studies in single centers or a few collaborating centers (10,11). These studies show that inter-patient coefficients of variation are large and may be relatively insensitive to identify effects of genetic variation, just as large sample sizes per treatment arm are required (generally ~30 per group to identify a 25% difference in sensation thresholds or ratings), and intra-patient reproducibility is limited, so that cross over within crossover studies is not much smaller than parallel-design studies (10).

In brain imaging studies, the magnitude of effect demonstrable is unclear and clinical correlates are uncertain. However, a recent report (12) shows that brain responses to distension are similar in normosensitive patients and controls, whereas hypersensitive patients with IBS had greater activation of insula and reduced deactivation in pregenual anterior cingulate cortex during noxious rectal distensions, compared to controls and normosensitive patients with IBS. During expectation of rectal distension, normosensitive patients with IBS had more activation in the right hippocampus compared to controls (12).

Genetics of Gastrointestinal Pain

There are a number of mechanisms involved in gut sensation that have been associated with abdominal complaints or with increased or decreased sensation in IBS patients as a result of genetic variations or mutations. The literature is summarized (Table I) based on different visceral sensory mechanisms that are potentially affected in patients with abdominal symptoms such as irritable bowel syndrome, and other conditions associated with abdominal pain such as pancreatitis. Studying these disorders and the associated alterations in sensory mechanisms has the potential to enhance understanding the disease and, potentially, identify targets for therapy. However, it is important to recognize that such extrapolations are difficult for several reasons: first, IBS and abdominal pain are complex syndromes in which environmental factors such as stress and inflammation contribute to symptom generation; second, there is considerable heterogeneity in the spectrum of patients with IBS including the phenotype (e.g. bowel dysfunction), nature of pain (e.g. dull constant pain versus colicky or pre-defecation discomfort) and mechanism (e.g. central hypervigilance versus afferent hypersensitivity). In contrast to the GWAS studies in inflammatory bowel diseases, the literature on visceral pain has used a candidate gene approach based on mechanisms already associated with visceral afferent functions described above. In order to appraise the strength of the genetic associations with symptom phenotypes, Table I includes the odds ratio and 95% confidence intervals of the genetic variations or, where unavailable, the number of patients and controls included in studies that report significance. Table I also includes associations with quantitative traits or endophenotypes.

Ion Channels

Patients with mutations in *SCN5A* (Nav 1.5Na⁺ channel) are more likely to report GI symptoms, especially abdominal pain (13). Specifically, 50% of subjects with an *SCN5A* mutation reported abdominal pain compared to only 13% of controls (OR 5.7; 95% CI 1.3–24.4). Over 65% of subjects with an *SCN5A* mutation reported a GI symptom complex compared to 28% of controls (OR 5.2; 95% CI 1.5–18.3). However potential confounding by medications used to treat Brugada syndrome (tachyarrhythmias and hypertension) may conceivably have contributed to the occurrence of the functional GI symptoms.

One of 49 IBS patients (2%) had a G298S missense mutation in *SCN5A*. Functional effects of the mutation were supported by the observation that G298S mutation reduces mechanosensitivity (14). The patient was also heterozygous for the H558R common SNP. No difference in frequency of the commonly encountered *H558R* genotype was observed between IBS patients with at least moderately severe abdominal pain and the reference group.

In paroxysmal extreme pain disorder (familial rectal pain), the gene that encodes Nav1.7 was reported to be mutated (8); in one family, there was T1461I mutation in *SCN9A* and, in a second family, there was a point mutation resulting in a radical amino acid change (R996C) in the intracellular loop of Nav1.7 between the sixth transmembrane helix of the second domain (DIIS6) and the first transmembrane domain of the third domain.

No genetic associations reported between ASIC and IBS/visceral pain. However, other papers have documented potential for other pain syndromes including cortical excitability and migraine. Thus, human subjects homozygous for the G allele in rs222747 in *TRPV1* exhibited larger short-interval intra-cortical facilitation (a measure of glutamate transmission) explored through paired-pulse transcranial magnetic stimulation of the primary motor cortex (15). Similarly, SNP variants within *TRPV1* (rs222741) and *TRPV3* (rs7217270) receptor genes were associated with migraine in a Spanish cohort (16). On the other hand, two studies associated with abdominal pain have failed to confirm associations with *TRPV1* gene variations. Thus, polymorphisms in gene encoding *TRPV1*-receptor involved in pain perception are not significantly associated with chronic pancreatitis (17), and homozygous *TRPV1* 315C (rs222747, which changes Met to Ile and alters protein level) actually reduced the susceptibility to functional dyspepsia in a Japanese cohort. This reduction in susceptibility also applied to epigastric pain syndrome, postprandial distress syndrome, the severity of 7 dyspeptic symptoms, and the sensation of a heavy feeling in the stomach during drinking cold carbonated water (18).

Serotonin

A. Synthesis—Associations of tryptophan hydroxylase gene polymorphisms (5-HT synthesis) with IBS have been explored for both *TPH1* and *TPH2*. There were no associations of any SNPs with abdominal pain, but rs4537731 and rs211105 in *TPH1* gene were associated with diarrhea or watery stool (19).

B. Transporter (reuptake)—There are many studies exploring the relationship of 5-*HTTLPR* genotype (which determines expression of the SLC6A4 or SERT protein) and IBS; overall, the evidence of association is equivocal (20). The daily gastrointestinal symptom severity and quality of life are not significantly associated with 5-*HTTLPR* genotype, but there was reduced social functioning in the LL genotype, and higher levels of depression in the SS genotype patients with IBS (21). Rs25531 in the gene for *SLC6A4* was significantly associated with IBS (22).

Studies of colonic pain ratings induced by colonic distension were significantly higher in carriers of the LS/SS genotype in *5-HTTLPR* in American controls and IBS patients (in whom the S allele is minor), and this was not related to any change in rectal compliance (23). This information is consistent with brain imaging studies. *5-HTTLPR* genotype is associated with differential activation of brain regions with 0–40mmHg colorectal distention in humans (24). Thus, SS genotype was associated with larger increase in regional cerebral blood flow than those with L allele and, specifically, more activated left ACC and right parahippocampal gyrus. These observations may reflect the increased arousal or hypervigilance that may contribute to the hypersensitivity to pain in IBS patients.

C. 5-HT₃ receptor genes—Kapellar et al. have reported association of a functional variant in the microRNA-510 target site of the *5-HT_{3E}* gene with IBS-D in cohorts from Germany and the United Kingdom (25). The 42C>T polymorphism in *HTR3A* is associated with severity of IBS symptoms (26). The C/C genotype associated with greater anxiety symptoms and symptom ratings in patients with IBS and controls, increased activation of amygdala under emotional and non-emotional conditions, and a subset of IBS patients with C/C genotype had increased amygdala responses to non-emotional stimuli, compared with other subjects with C/C genotype (26).

Adrenergic Receptors

α_{2A} – 1291C → G in the α_{2A} and α_{2C} Del 322–325 in the α_{2C} adrenoceptor genes are associated with the symptom phenotype of IBS-C in a cohort of 274 IBS patients (90 with IBS-C) compared to 120 controls (27); there was no significant association with rectal sensation (23) or colonic transit (28), but the α_{2C} variant was associated with high somatization score (27), which may be suggestive of increased overall sensitivity to all stimuli, not only those arising in the gut.

Neuropeptide S Receptor 1 (NPSR1)

NPSR1, the receptor for neuropeptide S (NPS), is expressed by gastrointestinal enteroendocrine cells and is involved in inflammation, anxiety, and nociception. *NPSR1* polymorphisms are associated with asthma and inflammatory bowel disease. In one study (29), 17 *NPSR1* SNPs were successfully genotyped in 466 patients with functional gastrointestinal disorders (FGID) and 233 racially similar healthy controls. There were no significant associations with FGID symptoms. However, several *NPSR1* SNPs (rs2609234, rs6972158, and rs1379928) were associated with colonic transit rate after false discovery rate correction. In addition, the rs1379928 SNP was univariately associated with pain, gas, and urgency sensory ratings at 36 mmHg rectal distention.

Cannabinoid Mechanisms

The emerging role of cannabinoid mechanisms in gastrointestinal functions is reviewed elsewhere (30). The enzyme, fatty acid amide hydrolase (FAAH), metabolizes the endogenous cannabinoid, anandamide, which is a fatty acid amide produced in the post-junctional membrane and alters the function of pivotal receptors including cholinergic and nitrergic receptors. The cannabinoid 1 receptor modifies neuromuscular functions, and the gene controlling the synthesis of the receptor protein is *CRNI*. *FAAH C385A* SNP is associated with symptom phenotype and quantitative traits in IBS. CA/AA increases the odds (relative to HV) for IBS-D, IBS-alternating and, possibly, chronic abdominal pain. There was a significant association of *FAAH* CA/AA genotype with accelerated colonic transit in IBS-D. There was no association of *FAAH* genotype with rectal sensation thresholds or ratings (31). We have recently observed a significant overall association of

CNR1 rs806378 with sensation rating of gas and gene by phenotype interaction, with strongest associations in IBS-D and IBS-alternating subgroups (32).

Park et al. identified different distribution of allelic frequency of AAT repeats in the *CNR1* gene in healthy controls and IBS patients in Korea, and a significant association between the *CNR1* >10/ >10 genotype and IBS (33). We have also recently reported *CNR1* (AAT)_n, gene by phenotype interactions for sensations of gas ($p=0.046$, highest for IBS-D, $p=0.034$), but not pain sensation (32).

GTP Cyclohydrolase (GCH1) Gene

GCH1 is the rate-limiting first enzyme in the *de novo* synthesis of the enzyme cofactor tetrahydrobiopterin, BH4. The expression and activity of GCH1 are tightly controlled, ensuring BH4 supply for the BH4-dependent enzymes such as nitric oxide synthases and biogenic amine hydroxylases. BH4 overload is normally prevented by metabolism with production of neopterin, a stable metabolic product excreted in urine.

If GCH1 is overexpressed and overactive (e.g. with pro-inflammatory stimuli, ischemia, and neuronal injury), excess BH4 is produced. In rodent models, when peripheral dorsal root ganglia neurons upregulate GCH1 upon axonal injury, chronic neuropathic pain is observed. There is a *GCH1* 'pain protective' haplotype (identified in chromosome 14q22.1-q22.2 by: SNP1 – dbSNP rs8007267 (c.-9610G>A) and SNP2 – dbSNP rs3783641 (c.343 + 8900A>T) located in the promoter region and intron 1). This haplotype has been associated with reduced somatic pain in humans and shows additive inheritance, that is, heterozygous individuals have an intermediate phenotype. Moreover, the effect of the haplotype is much more pronounced when nerve damage has augmented the system (34). This GCH1 pain-protective haplotype does not have a significant effect on pain patterns or severity in recurrent abdominal pain or chronic pancreatitis (35).

Catechol-O-Methyl Transferase Gene

COMT metabolizes catechol neurotransmitters dopamine, norepinephrine and epinephrine. Catecholamines are involved in various physiological functions including mood, cognition and stress response. Human pain is closely related to all these functions.

The gene encoding *COMT* enzyme has functional polymorphisms that contribute to the inter-individual variability in human pain phenotypes, such as pain sensitivity, chronicity, severity and response to pain medicine. The exact mechanism of the effect of *COMT* on human pain is unclear. Pain-sensitive group carries the haplotypes ACCG or ATCA for the SNPs rs6269, rs4633, rs4818, and val158met.

The val158met *COMT* polymorphism is associated with pain syndromes such as fibromyalgia or chronic widespread pain, though not with migraine headache or chronic musculoskeletal pain (36). A recent study explored association with IBS; among IBS patients, val/val carriers had increased bowel frequency, smaller proportion of stools with incomplete defecation and a trend for a smaller proportion of hard stools and a higher frequency of postprandial defecation. However, there was no association with pain (37).

Inflammation-Associated Genes

In patients with post-infectious IBS, toll-like receptor 9 (*TLR9*), which is involved in barrier function; pattern-recognition receptors that elicit innate/adaptive immune response and inflammation; gene controlling interleukin-6 (*IL6*, an inflammatory cytokine); and cadherin 1 (*CDH1*, which is involved in bacterial adhesion to mammalian cells and internalization) were significantly associated with IBS (38). The main susceptibility genes for Crohn's

disease affect several etiopathogenetic mechanisms. There was a statistically significant association between a Crohn's disease susceptibility candidate gene, tumor necrosis factor superfamily 15 (*TNFSF15*, which codes for TL1A, a member of the TNF ligands expressed primarily in macrophages and T cells and in immune cells exposed to proinflammatory stimuli or microbes), and IBS symptom phenotype in both U.S. and Swedish IBS cohorts, relative to regional controls (39). The association with *TNFSF15* was confirmed in a U.K. cohort of IBS patients (34); the SNP tested is in linkage disequilibrium with the one tested in Sweden and U.S. In addition, Swan et al. showed a significant relationship between rectal biopsy *TNFSF15* mRNA normalized abundance and *TNFSF15* SNP rs6478108 genotype (40). Association between the Crohn's disease risk loci and intermediate phenotypes or quantitative traits showed *c11orf30* rs7927894, *PRDM1* rs7746082, *ORMDL3* rs2872507, and *TLR9* rs5743836 were univariately associated with colonic transit at 24 or 48 hours (41). *PRDM1*, also known as BLIMP-1 (repressor of β -interferon gene), is involved in plasma cell differentiation, as well as lymphocyte, macrophage, neural crest and sensory neuron development. *ORMDL3* is a member of ORM transmembrane proteins localized in the endoplasmic reticulum (ER) and has a function in ER stress and in control of membrane structure. In the same cohort, there were univariate associations with *CDH1* rs16260 and rectal pain and between *C13orf31* rs3764147 and rectal urgency when the rectal symptoms were evoked by rectal distensions using a barostat balloon (42). *C13ORF31* is a conserved hypothetical protein showing a multi-copper oxidase domain which is typical for members of the lacasse family (43); it is unclear how its alteration may result in changes in rectal sensation.

The association with IBS of tumor necrosis factor α (TNF α) and *IL-10* gene polymorphisms has tested the potential of high production of TNF α (proinflammatory cytokine) and *IL-10* (anti-inflammatory). TNF- α G/A (high producer) were more prevalent in IBS compared to controls (41% vs. 26%, $p=0.02$) and significantly more IBS patients than controls had the high TNF- α and low *IL-10* genotype combination (9 vs. 3%, $p=0.035$), particularly in those with IBS-D (20%). However, the relationship with pain was not investigated (44). Two other studies showed that patients in the U.K. with IBS had significantly reduced frequencies of the high producer genotype for *IL-10* than controls [21% v 32% (45)] and, on the other hand, Korean patients showed no significant association with either *IL-10* or TNF α (46).

In summary, genes influencing ion channels, 5-HT receptors and SERT (SLC6A4), inflammation, or immune function appear, at least, univariately associated with visceral pain in IBS. At the present time, the immediate goal of these studies is to provide insights into biology; however, it is conceivable that in the future, such studies might have clinical utility, either in diagnosis or pharmacogenetics. Although only limited data are available, it is reasonable to hypothesize that genetic variants in these mechanisms contribute to development of IBS visceral pain.

CONCLUSION

Biomarkers of visceral sensation in humans present challenges because of large coefficients of variation and limited clinical validity. Nevertheless, genes influencing ion channels, barrier and immune functions, and neurotransmitters or receptors such as neuropeptide S receptor 1, 5-HT₃ receptors, and cannabinoid₁ receptors or reuptake mechanisms such as SLC6A4 are reported to be univariately associated with visceral pain in IBS. These data are consistent with the hypothesis that genetic variants in these mechanisms contribute to development of IBS, possibly in concert with other predisposing factors such as stress, early life events and exposure to pathogens or other environmental factors. This is an area requiring investment and research in gastroenterology, as it is of critical importance to

understand the mechanisms of visceral pain, a prerequisite to enhancing its management. This is undoubtedly the area of greatest unmet need in IBS and functional gastrointestinal disorders.

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Table 1

Summary of Evidence of Genetic Contributions to Visceral Pain in Humans

Class	Subclass	Functional studies	Association with symptom or symptom phenotype	Association with quantitative traits	Reference
Ion Channels	Nav 1.5	<i>SCN5A</i> mutation alter mechanosensitivity	GI symptom complex (OR 5.2; 95% CI 1.5–18.3), abdominal pain (OR 5.7; 95% CI 1.3–24.4)	-	Locke et al 2006; Saito et al 2009
	Nav 1.7	<i>SCN9A</i> mutations	Familial rectal pain	Sensation thresholds in 7 IBS pts. and 10 controls	Yiangou et al 2007
	TRPV1	rs222747 intra-cortical facilitation	None with pain in chronic pancreatitis; reduced functional dyspepsia	None with carbonated water drink test in dyspepsia	Van Esch et al 2009, Tahara et al 2010
Neurotransmitters	5-HT synthesis, TPH1		None with abdominal pain; rs4537731 and rs211105 associated with diarrhea in 199 IBS pts. and 79 healthy controls	-	Jun et al 2011
	5-HT reuptake	<i>5-HTTLPR</i> -S allele reduces 5-HT uptake	Overall none with IBS in meta-analysis; other inconsistent results	LS/SS with higher pain ratings in 122 IBS pts. and 39 controls; increased cerebral RBF with rectal distension in 28 adults without organic diseases	Camilleri et al 2008 Fukudo et al 2009
	5-HT reuptake	rs25531 inherited with <i>5-HTTLPR</i>	G allele with with IBS (OR 3.3, 95% CI 1.1–9.6)	-	Kohen et al 2009
	5-HT receptors	rs62625044 in 3' UTR of <i>5-HT3E</i> gene affects binding of miR-510	Association with IBS-D in female UK (OR = 8.53, 95% CI = 1.04–70.28) and female German (OR = 4.92, 95% CI = 1.49–16.30) pts.; none with IBS-C		Kapeller et al 2008
		<i>5-HT3A</i> C178T; rs1062613	Greater IBS symptom and anxiety ratings in 26 IBS pts. vs. 29 controls	<i>HTR3A</i> with amygdala responsiveness in 26 IBS pts. and 29 controls	Kilpatrick et al 2011
	Adrenergic receptors	-	α_{2C} Del 322–325 with IBS-C (OR 2.48, 95% CI 0.98, 6.28) and high somatic scores (OR 2.2, 95% CI 1.06, 4.64)	None with transit or sensation	Kim et al 2004; Grudell et al 2008
	Cannabinoid receptor, CB1	<i>CNR1</i> (rs806378)	With IBS symptom phenotype in 455 IBS pts. and 228 healthy controls	Gas (not pain) sensation rating in 74 IBS pts. and 24 controls	Camilleri et al 2012
		<i>CNR1</i> (AAT)n repeats	>10/>10 genotype with IBS and abdominal discomfort/pain in 162 IBS pts. and 423 controls in Korea	Gas (not pain) sensation rating in 74 IBS pts. and 24 controls	Park et al 2011; Camilleri et al 2012
	Cannabinoid metabolism	<i>FAAH</i> C385A rs324420	With IBS-D and IBS-A, and chronic pain in 482 FGID pts. and 252 controls	Rapid transit in IBS-D	Camilleri et al 2008

Class	Subclass	Functional studies	Association with symptom or symptom phenotype	Association with quantitative traits	Reference
Enzymes	Neuropeptide S receptor	Synthesis of neurotransmitters	None	Colonic transit in 171, and rectal sensation 116 pts. or controls	Camilleri et al 2010
	GCHI	BH4-dependent enzymes; neuropathic pain	None with RAP or chronic pancreatitis	-	Lazarey et al 2008
	COMT	Pain sensitive e.g. vall158met	With IBS overall, and increased bowel frequency but not with pain in 70 IBS pts. and 867 healthy controls	-	Karling et al 2011
Inflammatory Mediators	TNFSF15	<i>TNFSF15</i> rs4263839 associated with inflammation	With IBS (OR 1.37; 95% CI 1.19–1.58); with IBS-C (OR 1.79; 95% CI 1.41–2.26) in Sweden, USA separate cohorts, and UK	-	Zucchelli et al 2011; Swan et al 2012
	TLR9	<i>TLR9</i> rs352139 and rs5743836 associated with inflammation; barrier function	With PI-IBS in 228 pts. and 581 controls	Colonic transit at 48h in total of 171 IBS pts. or controls	Villani et al 2010; Camilleri et al 2011
	CDH1	<i>CDH1</i> rs16260 with tight junction protein	With PI-IBS in 228 pts. and 581 controls	-	Villani et al 2010
	IL6	<i>IL6</i> rs1800795 Inflammation	With PI-IBS in 228 pts. and 581 controls	-	Villani et al 2010
	IL10	Protection from inflammation	Inconsistent with IBS	-	Gonsalkorale et al 2003; Lee et al 2010
	TNFα	Inflammation	Inconsistent with IBS	-	Gonsalkorale et al 2003; Lee et al 2010

5-HT = serotonin; IBS = irritable bowel syndrome; IBS-C = constipation-predominant irritable bowel syndrome; IBS-D = diarrhea-predominant irritable bowel syndrome; PI-IBS = post-infectious irritable bowel syndrome; IL = interleukin; Na = sodium; RBF=regional blood flow; TRP = transient receptor potential channels; FAAH = fatty acid amide hydrolase; *GCHI* = *GTP cyclohydrolase* gene; *COMT* = *catechol-o-methyl transferase* gene; *CDH1* = *cadherin 1* gene; *TNFSF15*= *tumor necrosis factor superfamily 15* gene; *TLR9* = *toll-like receptor 9* gene; *TLR9* = *cadherin 1* gene; tumor necrosis factor α = TNFα.