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Genetics of irritable bowel syndrome: shifting gear via biobank-scale studies

Michael Camilleri1, Alexandra Zhernakova2, Isotta Bozzarelli3 and Mauro D’Amato5,4, 5✉

Abstract | The pathophysiology of irritable bowel syndrome (IBS) is multifactorial and probably involves genetic predisposition and the effect of environmental factors. Unlike other gastrointestinal diseases with a heritable component, genetic research in IBS has been scarce and mostly characterized by small underpowered studies, leading to inconclusive results. The availability of genomic and health-related data from large international cohorts and population-based biobanks offers unprecedented opportunities for long-awaited, well-powered genetic studies in IBS. This Review focuses on the latest advances that provide compelling evidence for the importance of genes involved in the digestion of carbohydrates, ion channel function, neurotransmitters and their receptors, neuronal pathways and the control of gut motility. These discoveries have generated novel information that might be further refined for the identification of predisposed individuals and selection of management strategies for patients. This Review presents a conceptual framework, the advantages and potential limitations of modern genetic research in IBS, and a summary of available evidence.

Irritable bowel syndrome (IBS) is a disorder of gut–brain interaction (DGBI) that affects between 5% and 15% of the general population. IBS is characterized by recurrent abdominal pain in association with abnormal stool form or frequency. According to consensus criteria (the Rome criteria for IBS; BOX 1), subtypes of IBS are based on the presence of constipation (IBS-C), diarrhoea (IBS-D) or mixed constipation and diarrhoea (IBS-M)2. IBS affects more women than men, and it has a substantial effect on the quality of life and social functioning3,4. Treatment aims to improve both abdominal pain and bowel habit, and is often targeted towards the most troublesome symptom. First-line therapies include dietary changes, soluble fibre and antispasmodic drugs; treatment for more severe symptoms includes central neuromodulators, such as low-dose tricyclic antidepressants, intestinal secretagogues, drugs acting on peripheral opioid or 5-hydroxytryptamine (5-HT) receptors, antibiotics that are predominantly active in the gut lumen and cognitive approaches such as mindfulness and psychotherapy4,5. Reported associations of IBS with anxiety, depression and other psychiatric conditions led to its consideration as a psychosomatic disorder6,7. However, research has identified risk factors and central and peripheral mechanisms that might have important roles in the generation of IBS symptoms8,9. The subset with prior gastroenteritis (post-infection IBS), and associated mucosal immune activation, inflammation or elevated inflammatory markers point to an immune-mediated component10. Subtle quantitative and qualitative changes in the intestinal microbiota have been reported, possibly indicating dysbiosis in the gut of patients with IBS11.

Dietary factors might explain postprandial aggravation of symptoms in some patients with IBS. Avoidance of carbohydrates owing to malabsorption is recommended as a first-line approach by the UK England and Wales National Institute for Health and Care Excellence (NICE) guidelines for IBS, and diets low in fermentable oligosaccharides, disaccharides, monosaccharides and polyols (FODMAPs), which are generally poorly absorbed, are effective in reducing symptoms in some, but not all patients12.

The biopsychosocial model to explain symptoms of abdominal pain and disordered bowel habits in individuals with IBS conceptualized a genetic predisposition, in which adverse events in early life, psychological factors or gastrointestinal infections triggered alterations in the enteric nervous system (ENS) that controlled gastrointestinal motor, sensory, mucosal barrier and secretory responses, thereby leading to the generation of bowel symptoms13 (Fig. 1). Indeed, a heritable component of IBS has been consistently demonstrated in twin and family studies, although with varying degrees of heritability estimates, possibly owing to the different disease definitions adopted in these surveys14.

The strongest available evidence of a genetic component
The pathophysiological mechanisms associated with irritable bowel syndrome (IBS) are only partially understood; this hampers the development of targeted therapeutic strategies.

Genetic research can reveal actionable pathways, but findings have generally been scarce in IBS because genetic investigations have been small scale and based on single-gene approaches that have highlighted only a few putative risk genes related to serotoninergic mechanisms, carbohydrate digestion or ion channels.

Large-scale, biobank-wide genome-wide association studies (GWAS) that focused on IBS and endophenotypic proxies of gut motility have been reported, and have led to the identification of the first set of unequivocal risk loci.

These loci contain several genes relevant to pathways and cell types implicated in the central and enteric nervous system activities, neurotransmitter signalling and enteric motor neuron function; these findings offer avenues for testing novel therapeutic strategies.

The genetic architecture of IBS identified to date through GWAS often seems to be shared with comorbid mood disorders and anxiety, which is consistent with the reported efficacy of psychotropic and cognitive therapies in IBS.

Polygenic scores derived from GWAS data enabled the identification of individuals at increased risk of IBS in the studied cohorts and could be further refined and validated in independent translational studies.

Dysbiosis

Disruption of the intestinal microbiota homeostasis, determining a reduction in microbial diversity, alterations in the composition and distribution of the autochthon commensals, and changes in metabolic activities.

Genetic architecture

Broadly, the genetic landscape that underlies a phenotypic trait and its variation, characterized by the number of genetic variants that contribute to that phenotype, their effect size and the patterns of interactions. It summarizes the ‘genetics’ behind a trait.

Biobanks

Collections of extensive data from biomedical research linked to study participants, including biospecimens, electronic health-related records, genetic data, imaging, biomarkers and information regarding environment and lifestyle.

Prevalence

The proportion of individuals in a population who have a specific characteristic (typically a disease) at a specific time.

SCN5A and intestinal channelopathy

Transient receptor potential (TRP) and voltage-gated ion channels expressed in the gut have important roles in secretion, absorption, motility and sensation, as they mediate the actions of gastrointestinal neurotransmitters.

The genetic variations in the serotonergic signalling pathway might be relevant to the mechanisms for example, sensation, motility and secretion and clinical manifestations (for example, altered bowel function and pain) of IBS, particularly in the IBS-D subgroup of patients. Further investigation is needed to understand whether these findings have clinical utility.
GWAS Catalog
A free online curated collection of human genome-wide association study (GWAS) results representing a collaboration between the European Bioinformatics Institute of the European Molecular Biology Laboratory and the National Human Genome Research Institute; it summarizes significant single-nucleotide polymorphism—trait associations, and sample metadata from each publication (as of 9 July 2022, the catalogue contains 5,848 publications and 598,542 associations).

Polygenic scores (PGS). Values summarizing the estimated (cumulative) effects of multiple DNA variants in determining the probability of an individual manifesting specific phenotypes or traits. The score is useful for evaluating an individual’s genetic predisposition for a particular trait and can also be used as a predictor relative to the general population (increased or decreased risk of disease, expressed as a polygenic risk score for that disease).

(an enzyme in the small intestine). Sucrase–isomaltase is key to the degradation of starch and sucrose, and its functional impairment leads to colonic accumulation of undigested carbohydrates, osmotic diarrhoea and gas production due to bacterial fermentation.

In congenital sucrose–isomaltase deficiency (CSID), patients carry two defective copies of the SI gene owing to recessive homozygous or compound heterozygous mutations that abolish or dramatically reduce enzymatic activity. CSID usually manifests early in life, but the phenotype and severity of symptoms can vary depending on the specific nature and position (sucrase or isomaltase domain) of different SI mutations and their homozygous or heterozygous combinations. Cases of adult CSID misdiagnosed as IBS have been reported in the literature, and retrospective analyses of multiple case–control cohorts from tertiary centres have demonstrated that SI functional polymorphisms predispose to IBS.

Several rare and one common (Phe15Val) SI variant with reduced disaccharidase activity (hypo- morphic variants, demonstrated in vitro) confer an increased risk of IBS. Preliminary analyses from the same studies have also suggested that the interaction between SI genotype, gut microbiota and dietary carbohydrates might be important in determining IBS risk in exposed individuals. In 2021, in a large-scale study including more than 70,000 individuals from the UK Biobank (see below for more information on this resource), the prevalence of SI hypomorph carriers was found to be much higher (12.5%) in 248 participants with a diagnosis of IBS in their medical records (based on the tenth version of the International Classification of Diseases (ICD10)) compared with (8.0%) in 31,218 asymptomatic controls (BOX 1). SI carrier status might also influence the therapeutic efficacy of dietary interventions, as individuals carrying SI hypomorph variants were three to four times less likely to benefit from a low-FODMAP regime than non-carriers. These studies support the search for rare (and possibly common) genetic defects in the SI gene in patients with IBS and postprandial manifestations and provide a rationale for personalizing starch-restricted and sucrose-restricted diets.

IBS genetics and large-scale biobanks
Since the first GWAS of age-related macular degeneration in 2005 (REF 1), more than 150,000 genetic variants have been associated with diverse human diseases and traits (GWAS Catalog, https://www.ebi.ac.uk/gwas/). Gastrointestinal diseases, such as inflammatory bowel disease (IBD) and coeliac disease, are examples of diseases in which the GWAS approach has been successfully applied, with more than 200 risk loci identified in IBD and dozens in coeliac disease. Although direct application of this information in clinical practice has been difficult, it led to the identification of key pathogenetic pathways (some of which, such as the IL-23 pathway, have been exploited for therapeutic purposes) and laid the foundation for the development of polygenic scores (PGS), which hold great promise for improving prevention and diagnostic and/or prognostic precision in IBD (PGS are discussed further below). Original GWAS were achieved by establishing international consortia and analysing very large samples (thousands of patients and controls), which is essential to studying the genetics of complex human diseases and detecting small individual risk effects from multiple genes and variants with sufficient statistical power.

However, the conduct of such studies in IBS is compromised by the heterogeneity of the phenotype, possible low heritability relative to environmental factors, and limited availability of established IBS cohorts of suitable size at expert neurogastroenterology centres worldwide.

These limitations in the IBS field are being overcome with novel large-scale approaches, using health-related and lifestyle-related data and DNA samples from several large, mainly population-based international cohorts and biobanks (TABLE 1). These resources often include biobanked specimens that are associated with a wealth of data collected for general and/or specific research purposes, which could be linked to anonymized data from electronic medical records (EMRs) and prescriptions of the participants with their prior informed consent for this type of research. For example, UK Biobank, which is the largest research biobank in Europe, includes comprehensive lifestyle-related and health-related information from questionnaires and EMRs, biological measurements, biomarkers and omics data from urine, blood, serum and plasma, as well as body and brain imaging data, from approximately 500,000 individuals (40–69 years of age at the time of recruitment). Genotype and next-generation sequencing (whole-exome and whole-genome) data are also available for most participants, allowing human genetic studies with unprecedented statistical power. IBS cases can be identified in these cohorts via questionnaires and/or EMR data, based

Box 1 | Different definitions of IBS adopted in genetic studies

| Rome III diagnostic criteria for IBS (gold standard) | ICD10 |
| IBS | K58.0—IBS with diarrhoea | K58.0—IBS with diarrhoea |
| Recurrent abdominal pain or discomfort at least 3 days/month in the past | K58.1—IBS with constipation | K58.1—IBS with constipation |
| 3 months, associated with at least two of the following criteria. | K58.2—mixed IBS | K58.2—mixed IBS |
| • Improvement with defecation | K58.8—other IBS | K58.8—other IBS |
| • Onset associated with a change in frequency of stool | K58.9—IBS without diarrhoea | K58.9—IBS without diarrhoea |

Self-reported doctor’s diagnosis of IBS
From questionnaire data in relation to various question formulations; for example, “Have you ever been diagnosed with IBS” (UK Biobank Digestive Health Questionnaire).

ICD, International Classification of Diseases; IBS, irritable bowel syndrome.

* A more recent version of the Rome criteria (Rome IV) is currently adopted, which does not include the word discomfort in its definitions, requiring abdominal pain at least 1 day/week, and defining IBS subtypes based on the Bristol Stool Form Scale. *Criteria fulfilled for the past 3 months with symptom onset at least 6 months before diagnosis. *Adapted with permission from REF 120, American Gastroenterological Association Institute/Elsevier. *Codes introduced in 2017.
Pathomechanisms

Intermediate phenotypes, called intermediate phenotypes (or endophenotypes), such as colonic motility, mucosal immune activation, bile acid malabsorption and food intolerance. These peripheral pathogenic mechanisms, such as perturbation of the gut–brain axis, dysbiosis, described as contributing to irritable bowel syndrome (IBS), leading to central and contributing to IBS.

Female sex, genetic and environmental risk factors have been organs or other systems.

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Reviews

Risk factors

Pathomechanisms

Intermediate phenotypes

Bile acid malabsorption

Food intolerance

Dysbiosis

Colonic motility and compliance

Visceral sensation

Gut–brain axis dysfunction

• Female sex
• Prior gastroenteritis
• Stress, anxiety and depression
• Abuse, trauma
• Genetics

Risk factors, pathophysiological mechanisms and intermediate phenotypes contributing to IBS. Female sex, genetic and environmental risk factors have been described as contributing to irritable bowel syndrome (IBS), leading to central and peripheral pathogenic mechanisms, such as perturbation of the gut–brain axis, dysbiosis, mucosal immune activation, bile acid malabsorption and food intolerance. These alterations result, at least in part, from dysregulation of measurable intestinal functions and traits, called intermediate phenotypes (or endophenotypes), such as colonic motility, visceral sensation and compliance.

Biomarkers

Characteristics that can be objectively measured and evaluated as indicators of normal biological processes, pathogenic processes or pharmacological responses to a therapeutic intervention (adapted from the definition of the Biomarkers Definitions Working Group).

Omics

A set of scientific disciplines (such as genomics, metabonomics, metabolomics, proteomics and transcriptomics) that exploit large-scale data to characterize and quantify molecular interactions at a global level, whether biochemical, molecular or cellular data, or data from organs or other systems.

on self-reported diagnoses, the Rome criteria and ICD (ICD9 and ICD10) diagnostic codes (Box 1), with the caveat that only partial concordance is observed across different case definitions8.

Pilot IBS GWAS. The use of population-based cohorts for IBS genetics studies was proposed a decade ago16, and a couple of initial proof-of-principle studies were performed16,17 in a few hundred cases and controls. These initial studies were underpowered to detect weak individual association signals at a genome-wide significance level ($P < 5 \times 10^{-8}$). Although still short of genome-wide significant findings, a later GWAS meta-analysis performed in 1,335 patients with IBS and 9,768 asymptomatic controls from five independent European cohorts highlighted ion channel function among the most plausible pathways involved22, based on gene set enrichment analysis of suggestive association signals ($P < 5 \times 10^{-8}$). This finding is notable as it is consistent with the potential of ion channels highlighted in previous SCN5A and TRPM8 studies.

Female-specific risk locus. The first genome-wide significant association for IBS was identified in 2018, in a GWAS performed using UK Biobank data extracted from touchscreen questionnaire information collected at enrolment. By comparing 9,576 patients with a self-reported doctor’s diagnosis of IBS (Box 1) with 336,499 individuals without IBS from the rest of the population (Box 1), Bonfiglio et al. detected an increased risk of IBS associated with a relatively rare SNP variant from chromosome 9q31.2 (rs10512344C, minor allele frequency 0.04), a region previously also linked to age at menarche23–25 (Table 2). Together with the known higher prevalence in women (female to male ratio of 2–5:1)26,27, and the postulated role of sex hormones such as oestrogen and progesterone in IBS8, this evidence led to the hypothesis of sex-specific genetic risk effects for this locus. This hypothesis was confirmed in sex-stratified analyses of UK Biobank data and the observation of an (even stronger) association only in women (7,130 with and 178,076 without IBS)28. Follow-up analyses from the same study also detected female-specific associations with IBS-C in a multicentre cohort from tertiary clinics (2,045 patients with IBS and 7,955 healthy controls) and with harder stools in a small population-based cohort from Sweden (249 individuals)29. Although no specific causative gene was identified, IKBKAP (also known as ELPI1, which is expressed mainly in the adrenal and pituitary glands, cerebellum and ovary) was proposed as the best candidate to mediate IBS risk effects because of its known role in recessive forms of familial dysautonomia, a neurodegenerative disease affecting the development of sensory (afferent) neurons70,71 and other autonomic defects including gastrointestinal dysmotility. The association with the 9q31.2 locus is, therefore, particularly interesting, as it links important sex-specific differences observed in IBS, including higher female prevalence and autonomic dysfunction30. Moreover, hormonal effects on gastrointestinal transit, symptom severity and response to treatment have been observed: IBS symptoms, such as abdominal pain and bloating, tend to be exacerbated during menses in female patients; the effect of alosetron, a serotonin receptor antagonist, in treating IBS-D was shown to be substantially greater in women than in men76,82–87. Notably, however, the 9q31.2 association signal is tagged by a relatively rare variant (rs10512344C, allele frequency 2–5% in individuals of European descent, eventually affecting IBS risk only in a minority of individuals). The fact that it was not detected when different definitions of IBS were used in later UK Biobank studies30,31 (discussed below) suggests that survey-specific factors had a role in the detection of specific associations.

The link to mood and anxiety disorders. Two GWAS of UK Biobank obtained similar evidence of shared genetic risk factors affecting the susceptibility to IBS and mood disorders14,15, although based on alternative definitions and classification of patients with IBS (Box 1).

In a GWAS of IBS (and three other gastrointestinal diseases), Wu et al. included 28,518 patients with IBS identified by pooling diagnoses from multiple data sources: death registers, questionnaires and EMRs from hospital admissions and/or primary care visits65. Comparing these patients with 426,803 individuals without IBS as controls from the rest of the population led to the identification of two genome-wide significant association signals: one mapping within the HLA region densely populated by immune-related genes on chromosome 6 (in proximity to HLA-C), and the other in proximity to the neuronal cell
adhesion molecule (NCAM1) gene on chromosome 11 (REF. [29]) (TABLE 2). Despite the lack of replication in an independent cohort of 3,359 patients and 58,488 controls (possibly owing to insufficient power) [30], both these signals probably represented valid associations, as they were also detected in later independent GWAS meta-analyses (discussed below) [31]. The female-specific 9q31.2 locus discussed above was just short of genome-wide significance in these analyses, in which data on self-reported conditions (as in Bonfiglio et al. [29]) were combined with ICD10 diagnoses from EMRs [30]. In the same study, strong genetic correlations (range 0.20–0.60; indicative of similar genetic architecture, and therefore probably common genetic predisposing mechanisms) were found between IBS and mood and anxiety disorders, including major depression, attention deficit hyperactivity disorder, neurotism and insomnia. In particular, for depression, preliminary evidence of a bidirectional causal genetic relationship with IBS was detected using relaxed statistical constraints in Mendelian randomization analyses [31].

The largest GWAS of IBS reported by Eijsbouts et al. evaluated 53,400 patients with IBS and 433,201 individuals as controls without a gastrointestinal disease [32], based on UK Biobank participants’ data from a digestive health questionnaire (inclusive of Rome III criteria for IBS), combined with data from the Bellygenes Initiative, an international collaboration focused on the genetics of IBS. Patients with IBS were identified based on UK Biobank participants’ data from a digestive health questionnaire (inclusive of Rome III criteria for IBS), combined with data from the Bellygenes Initiative, an international collaboration focused on the genetics of IBS. Patients with IBS were identified based on various criteria and, to maximize power, pooled case definitions to include self-reported doctor’s diagnoses from digestive health questionnaires and other questionnaires, primary and secondary ICD10 diagnoses from EMRs on hospital admission, Rome criteria for IBS (or compatible definitions from similar questionnaires), and diagnoses made at tertiary IBS clinics and neurogastroenterology centres [33,34]. Six genome-wide significant associations were detected through several GWAS in individual cohorts (UK Biobank and multiple cohorts from the Bellygenes Initiative) and their global meta-analysis. The identified risk loci harbour the NCAM1 gene on chromosome 11, CAD2M on chromosome 3, PHEF2/FAM120A on chromosome 9, DOCK9 and CKA2/ITPTE2P3 on chromosome 13 and RAG6 in the HLA region on chromosome 6 (REF. [35]) (TABLE 2). In contrast to previous GWAS, these findings were replicated using data from customers of the genomics company 23andMe, comparing 205,252 people who self-reported as having been diagnosed or treated for IBS with 1,384,055 individuals as controls who did not [36]. The association with the female-specific 9q31.2 gene mutation was not replicated in these analyses.
Although the HLA signal (BAG6 gene) was not the same as the one identified by Wu et al. (HLA-C gene), and no obvious candidate gene could be derived for the CKA2/TPTEP3 locus, the other associations are notable in that all four implicate genes (NCAM1, CADM2, PHF2/FAM120A and DOCK9) previously associated with mood and anxiety disorders, and/or expression in the central nervous system (CNS) and ENS (Table 2). In particular, cell adhesion molecules 2 (CADM2) and NCAM1 (also detected in the UK Biobank GWAS by Wu et al.) are two cell adhesion molecules expressed in the brain and associated with mood disorders, neuroticism, anorexia nervosa, depression, smoking, cannabis use and other traits, possibly via regulation of neural and synaptic plasticity and neurogenesis (Table 2). Although two other loci contain genes of poorly characterized functions, they demonstrated similar patterns, with both PHD finger protein 2 (PHF2) and dedicator of cytokinesis 9 (DOCK9) showing expression and a developmental role in the brain and, the PHF2/FAM120A locus having been previously linked also to neuroticism, depression and autism.

Although NCAM1, PHF2 and DOCK9 (but not CADM2) are also expressed in the ENS, variants affecting the function or expression of IBS candidate genes might potentially exert genetic risk effects in IBS via the modulation of CNS or ENS activities and/or neuronal communication along the gut–brain axis. In addition to single association signals, broader evidence of similar genetic architecture for IBS and mood and anxiety disorders came from genetic correlation analyses, also reported by Eijisbouts et al. Thus, when compared with 751 other diseases and traits with GWAS data available, the most pronounced overlap with IBS susceptibility was detected again (as in Wu et al.) for anxiety, depression, neuroticism and insomnia, among others. Overall, most GWAS provided compelling evidence that IBS and mood and anxiety disorders have shared genetic origins, possibly owing to common risk genes having a role in neuronal pathways and the communication between the gut and the brain, as highlighted by specific findings at individual risk loci. As comorbidity and increased prevalence of mental health conditions...
| Lead SNP  | Chromosome | Prioritized candidate gene(s)* | Function                                                                 | Potential involvement in IBS                                                                                                                                                                                                 | Refs. b |
|-----------|------------|--------------------------------|--------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------|
| rs1248825 | 3          | CADM2                          | Neuronal cell adhesion molecule expressed in the CNS[^24^][^25^]            | Important for synapse organization, it is also associated with comorbid traits including neuroticism and anxiety                                                                                                                     | 09      |
| rs2523599 | 6          | HLA-C, BAG6                     | Genes from the HLA region (in high linkage disequilibrium) coding for immune-related molecules, receptors and cytokines[^26^][^27^] | Low-grade inflammation and immune dysfunction (possibly mediated via gastrointestinal mast cells)                                                                                                                                   | 49,88   |
| rs2736155 |            |                                 |                                                                           |                                                                                                                                                                                                                               |         |
| rs10512344(female-specific) | 9       | ELP1                           | Part of the Pol II complex transcribing RNA, involved in the control of autonomic functions (mutated in familial dysautonomia[^28^][^29^]) | Autonomic response to visceral stresses dysregulated in IBS (more so in women); also associated with age at menarche, and a role for sex hormones is established in IBS                                                                                                                                 | 71      |
| rs10156602| 9          | PHF2                           | Lysine demethylase expressed in the brain and the ENS[^30^]                | Has a role in neurogenesis, previously associated also with comorbidities such as neuroticism and depression, and with autism                                                                                                       | 09      |
| rs7947502 | 11         | NCAM1                          | Neuronal cell adhesion molecule expressed in the CNS and ENS[^31^][^32^]    | Has a role in the development of neurons, including enteric neurons, also associated with comorbidities such as anxiety and depression                                                                                                                                                  | 49,88   |
| rs710643  |            |                                 |                                                                           |                                                                                                                                                                                                                               |         |
| rs9513519 | 13         | DOCK9                          | Guanine nucleotide-exchange factor expressed in the brain and the ENS[^33^][^34^][^35^] | Might have a role in brain development, in particular in dendrite formation                                                                                                                                                    | 09      |
| rs5803650 | 13         | CKAP2                          | Cytoskeleton-associated protein Unknown[^36^]                              |                                                                                                                                                                                                                               | 09      |

**Stool frequency**

| Lead SNP  | Chromosome | Prioritized candidate gene(s)* | Function                                                                 | Potential involvement in IBS                                                                                                                                                                                                 | Refs. b |
|-----------|------------|--------------------------------|--------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------|
| rs11240503| 1          | CDK18                          | Cyclin-dependent kinase expressed in colonic M cells and BEST4[^37^] + enterocytes[^38^][^39^] | Potential role in enterocytes specialized in electrolyte and pH sensing, might be involved in colonic osmolarity and transit                                                                                                           | 113     |
| rs39819   | 5          | (SNX24)                        | Sorting nexin expressed in several tissues, with a role in endocytosis, protein trafficking[^40^] | Sorting nexins contribute to neuronal function and synaptic plasticity in the CNS (also linked to psychiatric conditions)                                                                                                       | 115     |
| rs13162291| 5          | FAXDC2                         | Fatty acids hydroxylase expressed in enteric motor neurons[^41^]          | The luminal concentration of fatty acids is known to affect gut motility                                                                                                                                                    | 113     |
| rs1268068(female-specific) | 6       | (HEY2)                         | Transcription factor (repressor) downstream of Notch signalling, involved in cardiovascular development and neurogenesis[^42^][^43^][^44^][^45^] | Notch signalling is key to the development and maintenance of the intestinal epithelium, including enterocyte differentiation and, possibly, barrier function                                                                                 | 113     |
| rs12700026| 7          | (LFNG)                         | Lunatic Fringe glycosyltransferase that participates in the Notch signalling pathway[^46^][^47^][^48^] | Notch signalling is key to the development and maintenance of the intestinal epithelium, including enterocyte differentiation and, possibly, barrier function                                                                                 | 113     |
| rs62482222| 7          | (FBXO24)                       | F-box protein, possibly involved in cell proliferation (inhibitor[^49^])   | Unknown                                                                                                                                                                                                                   | 113     |
| rs4556017 | 7          | ACHE                           | Enzyme expressed in the ENS, hydrolyses acetylcholine at neuromuscular junctions (overexpressed in Hirschsprung disease[^50^][^51^]) | Potential role in the regulation of synaptic signalling and neurotransmission in the gut                                                                                                                                       | 113     |
| rs10957534| 8          | None                           | NA                                                                       | NA                                                                                                                                                                                                                       | 113     |
| rs6486216 | 11         | CALCA, CALCB                   | Coding for calcitonin, calcitonin-related peptides and katacalcin, hormones expressed in CNS and ENS[^52^][^53^] | Shown to stimulate colonic propulsion, motility and defecation                                                                                                                                                    | 113     |
| rs12273363| 11         | BDNF                           | A neurotrophin expressed in the CNS and ENS, involved in neuronal growth, differentiation and survival[^54^][^55^][^56^][^57^] | Known to influence gut function, including a prokinetic effect on motility; associated with stool consistency, and previously implicated in psychiatric disorders, such as major depression, bipolar disorder; recombinant BDNF induces accelerated transit | 113     |
| rs11176001| 12         | None                           | NA                                                                       | NA                                                                                                                                                                                                                       | 113     |
| rs10492268| 12         | None                           | NA                                                                       | NA                                                                                                                                                                                                                       | 113     |
have long been recognized in IBS\(^{101}\), these findings provide molecular evidence and a mechanistic rationale for the observed efficacy of psychotropic drugs and behavioural therapies in some patients with IBS. Additional insight might also come from further investigation and follow-up of association signals involving HLA immune-related genes (BAG6 and HLA-C, which require fine mapping of specific signals from different studies) and currently poorly characterized loci and associated genes (PHF2 and/or FAM120A).

**Genetics of IBS endophenotypes**

An alternative strategy for the identification of genes and pathways relevant to complex diseases (including IBS) is based on the analysis of so-called endophenotypes\(^{102}\), which are quantitative traits that bear a closer relationship to the underlying biological processes, have a hereditary component and cosegregate with the disease in families\(^{102}\). Diverse physiological and pathophysiological biomarkers and intermediate phenotypes (as they should be more appropriately called in the absence of clear evidence of heritability) include grey matter volume for a psychotic disorder, blood pressure for coronary artery disease, methacholine airway response for asthma, immunoglobulin E serum levels for atopy, and many others\(^{102}\). Several gastrointestinal traits have been proposed as endophenotypes or intermediate phenotypes relevant to DGBI and IBS, most frequently gas - intestinal sensation and colonic motility or transit, possibly by impacting bile acid synthesis rate and leading to diarrhea\(^{106,108}\). The luminal concentration of fatty acids is known to affect gut motility\(^{111}\). Syntaxins are involved in the synaptic machinery driving exocytosis and release of neuromediators; CFTR controls water and fluid secretion in the lumen\(^{111}\).

**Mendelian randomization**

An analytical method that uses random segregation of genetic variants as an instrument to emulate a randomized controlled trial, to investigate the putative causal effect of an exposure (using genetic variations strongly associated with it) on an outcome of interest, allowing confounders and reverse causation to be reduced; it can be used to test whether genetic predisposition to a disease (outcome) is mediated via DNA variants (instruments) predisposing to another disease (exposure), and vice versa.

| Lead SNP | Chromosome | Prioritized candidate gene(s)* | Function | Potential involvement in IBS | Refs. |
|----------|------------|--------------------------------|----------|----------------------------|-------|
| rs3856848 | 12         | None                           | NA       | NA                         | 113   |
| rs2732706 | 17         | CRHR1                          | G protein-coupled receptor for neuropeptides of the corticotropin-releasing hormone family\(^{104,105}\) | Involved in the control of stress-related colonic motility | 113   |
| rs10407548 (female-specific) | 19 | FFAR3                          | G protein-coupled receptor activated by short-chain fatty acids\(^{105,106}\) | The luminal concentration of fatty acids is known to affect gut motility | 113   |
| rs6123818 (male-specific) | 20 | (STX16)                        | Syntaxin with a reported role in autophagy, also shown to interact with the CFTR chloride channel, influencing its trafficking to the cell surface\(^{105,106}\) | Syntaxins are involved in the synaptic machinery driving exocytosis and release of neuromediators; CFTR controls water and fluid secretion in the lumen | 113   |
| rs5757162 | 22         | (FAM227A)                      | Unknown  | Unknown                     | 113   |

**Locus**

The specific location of a DNA sequence, gene or genetic marker on a chromosome.

**Genetic correlations**

Parameters that quantify the proportion of variance that two traits share owing to genetic causes, estimating the degree of pleiotropy (the phenomenon by which a single gene or locus influences the phenotypic appearance of multiple traits, including different diseases) or causal overlap.

**Table 2 (cont.)**

| Lead SNP | Chromosome | Prioritized candidate gene(s)* | Function | Potential involvement in IBS | Refs. |
|----------|------------|--------------------------------|----------|----------------------------|-------|
| rs3856848 | 12         | None                           | NA       | NA                         | 113   |
| rs2732706 | 17         | CRHR1                          | G protein-coupled receptor for neuropeptides of the corticotropin-releasing hormone family\(^{104,105}\) | Involved in the control of stress-related colonic motility | 113   |
| rs10407548 (female-specific) | 19 | FFAR3                          | G protein-coupled receptor activated by short-chain fatty acids\(^{105,106}\) | The luminal concentration of fatty acids is known to affect gut motility | 113   |
| rs6123818 (male-specific) | 20 | (STX16)                        | Syntaxin with a reported role in autophagy, also shown to interact with the CFTR chloride channel, influencing its trafficking to the cell surface\(^{105,106}\) | Syntaxins are involved in the synaptic machinery driving exocytosis and release of neuromediators; CFTR controls water and fluid secretion in the lumen | 113   |
| rs5757162 | 22         | (FAM227A)                      | Unknown  | Unknown                     | 113   |

BDNF, brain-derived neurotrophic factor; BEST4, bestrophin 4; CFTR, cystic fibrosis transmembrane conductance regulator; CNS, central nervous system; ENs, enteric nervous system; GWAS, genome-wide association studies; IBS, irritable bowel syndrome; NA, not applicable; SNP, single-nucleotide polymorphism. *For genes reported in parentheses the evidence is weaker and they were picked merely as the gene nearest to the association signal (shortest distance from the lead SNP within 100 kb)."
Primary and secondary ICD10 diagnoses

According to the ICD-10-CM Official Guidelines for Coding and Reporting, FY 2021, the principal diagnosis is defined as the condition established to be chiefly responsible for occasioning the admission of the patient to the hospital, whereas other diagnoses are all conditions that coexist at the time of admission, develop subsequently, or affect the treatment received and/or the length of stay.

Linkage disequilibrium

In a given population, the non-random segregation of alleles from different loci, resulting in their combinations (haplotypes) occurring more or less often than expected based merely on their individual allele frequencies (random, independent segregation).

Lead SNP

The marker that gives rise to the strongest association signal at a given genome-wide association study locus.

SNP heritability

The proportion of phenotypic variance of a given trait, which is causally explained by a specific set of single-nucleotide polymorphisms (SNPs).

Functional annotation

The process of collecting and assigning functional information to genomic regions, such as genome-wide association study loci (including gene content, regulatory elements, expression, molecular function, subcellular location, interactions, etc.).

Major allele

The more common allele for a specific single-nucleotide polymorphism in a given population.

Promoter

A regulatory element; a sequence of DNA that allows the binding of RNA polymerase and transcription factors responsible for the transcription of the downstream gene, therefore fundamentally contributing to its expression.

Stool frequency GWAS and IBS relevance

A pilot GWAS (meta-analysis) of stool frequency was carried out by Jankipersadsing et al. in 2016, based on genotype and bowel diary data from 1,281 individuals from two population-based cohorts, LifeLines-Deep and PopCo112. Although no genome-wide significant findings were obtained, GWAS suggestive signals collectively highlighted xenobiotic metabolism and voltage-gated sodium channel activity as the biological pathways most likely involved, therefore linking these to previous findings in IBS. Bonfiglio et al. reported a large GWAS meta-analysis of 163,616 individuals from the UK Biobank and 4,259 from four smaller population-based cohorts (LifeLines-Deep, The Flemish Gut Flora Project, Genes for Good and PopCo) in relation to a simple query about the number of times they open the bowels per day (from the Digestive Health Questionnaire in the UK Biobank), or extrapolated from bowel diaries and similar data (for the other cohorts)113. Hence, although infrequent bowel movements (and therefore constipation) could not be appropriately tested (UK Biobank participants, that is, the vast majority of participants included in the study, could not report bowel movements less frequently than once a day because of the way the question was posed), this survey allowed for the first time the genetic underpinnings of gut motility to be studied via powered large-scale GWAS analyses. The researchers demonstrated that human stool frequency (a proxy for gut motility) is a heritable character, with SNP heritability of 7%, higher than previously reported for IBS (3–5% in different studies, also depending on specific definitions)106,108. Furthermore, the genetic architecture of stool frequency was found to be most similar to that of IBS, diverticular disease, abdominal pain and related conditions, providing further evidence that the genes influencing gut motility are also relevant to the risk of gastrointestinal disease113. The GWAS meta-analysis identified 17 genome-wide significant signals (three showing sex-specific effects, with association detected only in women or men), linking several candidate genes to stool frequency via specific DNA variations influencing their expression in different tissues113. In particular, these genes seemed to be enriched in excitatory and/or inhibitory motor neurons involved in the control of peristalsis and mechanosensation of gut distension (subtypes expressing the mechanosensitive ion channel PIEZO2)114,115. Functional annotation of the biological mechanisms most likely involved highlighted neuropeptide and neurotransmitter signalling, chemical stimuli across synapses and ligand–receptor interactions underlying sensory perception, all of which are relevant to the control of intestinal motility via the ENS116. Several individual genes were identified (TABLE 2) as most likely to exert functional effects, the best example being the brain-derived neurotrophic factor (BDNF) gene on chromosome 11 (REF 113). A variant upstream BDNF gene (major allele T at SNP rs12273363) gave rise to the strongest GWAS signal in the stool frequency meta-analysis (P = 4.8 × 10^-8 for the association in the direction of more frequent stool). This variant showed consistent genetic effects in follow-up analyses of stool consistency (associated with looser stools measured in 2,338 healthy individuals based on BSFS data) and colonic transit time (associated with faster transit in a small cohort of 160 patients with IBS from Sweden)113. The rs12273363 T allele affects both the activity of an alternative BDNF promoter, and the transcription of an antisense RNA molecule (BDNF-AS) that induces BDNF mRNA degradation, therefore ultimately resulting in increased levels of circulating active BDNF (compared with the minor allele C from the same SNP site)117,118. BDNF is a neurotrophin expressed in the CNS and ENS and it is important for the growth and differentiation of cells of the neuronal lineage119. It is implicated in the development of mental health disorders such as major depression and bipolar disorder120, but it is also known to have a key role in the gut, where it affects sensation and motility, among other functions121. In particular, BDNF shows prokinetic effects on gut motility in mice and rats122-123 and reduced colonic expression in patients with slow-transit constipation124. Notably, administration of recombinant BDNF (r-metHuBDNF) was shown to induce accelerated colonic transit in a pilot trial performed in 40 healthy individuals125, therefore suggesting the possibility that the rs12273363 T variant might be of pharmacogenetic relevance in the response to treatments designed to target constipation via manipulation of the BDNF signalling pathway.

In addition to BDNF, other candidate genes were prioritized in the study by Bonfiglio et al.113, based on their known functions and potential mechanistic involvement in the control of gut motility (TABLE 2), including CALCA and CALCB on chromosome 11, coding for calcitonin, the α-calcitonin and β-calcitonin gene-related peptides and katalcalcin (resulting from tissue-specific alternative splicing and post-translational modifications), which are nearly identical forms of small hormones with known roles in gut physiology, including inhibition of gastric emptying and intestinal propulsion, as found in rat models125,126; CRHR1 on a gene-dense region on chromosome 17, coding for the receptor for the corticotropin-releasing hormone, a neurotransmitter involved in the control of stress-related gut motility whose signalling pathway often seems to be overactivated in patients with IBS126,127; and FFAR3 on a female-specific locus on chromosome 19, coding for a free fatty acid receptor expressed on enterochromaffin cells and enteric neurons, in which it can modulate peristalsis in response to short-chain fatty acids, which are known to affect gut motility128-132. In addition, as sex-specific differences in gut microbiome composition have been reported133, these might be relevant to bacterial fermentation and short-chain fatty acid production134, possibly helping to explain the observation of an FFAR3 association signal only in women.

The remaining identified loci do not harbour obvious candidates with a role in the modulation of gut motility, although some genes might be prioritized in follow-up studies, including CDK18, ACHE and FAXDC2 (REF 113) (TABLE 2). Finally, the results of the GWAS meta-analysis of stool frequency were also shown to be directly relevant to IBS because they allowed the calculation of PGS which were used to identify individuals at increased risk of IBS among >450,000 UK Biobank participants113.
Bonfiglio et al. showed that stool frequency PGS are differentially distributed in patients with IBS (based on Rome III criteria or self-reported doctor’s diagnoses) compared with controls (asymptomatic or IBS-free) and that individuals with the highest PGS were up to five times more likely to develop Rome III IBS-D than the rest of the population. At the same time, an opposite pattern was observed in patients with IBS-C, whose prevalence was at its minimum among individuals with the lowest PGS, suggesting that similar genetic mechanisms might contribute (in opposite directions) to both diarrhoea and constipation. This observation is an important finding that might have been suspected but never experimentally demonstrated, with potential implications for the treatment of these conditions by eventually targeting the same biological pathways.

Altogether, stool frequency GWAS findings have highlighted relevant cell types, pathways and individual genes that might be further studied to obtain novel mechanistic insights into multiple gut functions important for the control of intestinal motility and its eventual modulation for therapeutic purposes. They also suggest that personal genetic profiling might contribute to the identification of subgroups of patients at higher risk of developing IBS, particularly when characterized by diarrhoea.

**Host genetics and the microbiome.** The development of culture-independent next-generation sequencing technologies has allowed the investigation of the relationship between the microbiome and human diseases and phenotypes. Multiple studies have analysed the gut microbiome in IBS, although most included relatively small cohorts and showed inconsistent results. Studies in larger cohorts identified changes in the abundance of *Streptococcus, Lactobacillus, Veillonella* and *Prevotella copri*, and confirmed the decrease in beneficial bacteria, including *Faecalibacterium prausnitzii*, in patients with IBS compared with healthy individuals. A role for non-bacterial members of the gut ecosystem, including fungi and viruses, was also suggested in relation to visceral hypersensitivity and IBS. By contrast, the link between the microbiota and stool consistency and frequency is strong and consistent across studies, which suggests that stool patterns might need to be taken into consideration to explore microbial associations with IBS better. Intuitively, host genetic factors affecting gut microbiota composition might ultimately affect IBS risk via modulation of host–microbiota interactions and by effects on the relative abundance of deleterious and/or beneficial taxa, as already shown for other diseases.

**GWAS of human gut microbiota.** Although environmental factors dominate over genetics in regulating human gut microbiota composition, twin and family studies have shown that about 10% of bacteria (that is, *Collinsella, Akkermansia* and *Bifidobacterium* species) have substantial heritability. Several GWAS exploring the genetics of the gut microbiome (mbGWAS) in more than 1,000 individuals have been performed, including a GWAS meta-analysis of more than 18,000 people and 23 cohorts from the MiBioGen consortium. Only three host loci were consistently associated with gut microbiota composition across these studies: functional SNPs in the lactase (*LCT*) gene; genetic variants determining the blood group antigens from the *ABO* locus; and the secretor status (the ability to secrete the H-antigen on mucosal surfaces) determined by genotype at the fucosyltransferase 2 (*FUT2*) locus. Although none of these three loci has been directly associated with IBS so far, the function of both *LCT* and *FUT2* is important for food metabolism and digestion, as also reflected by the fact that both these genes were associated with food preferences in UK Biobank. *LCT* SNPs are known for their direct effect on lactose metabolism, and individuals homozygous for the rs4988235 G allele (which is associated with impaired lactase expression) are not able to digest milk and are called lactose non-persistent (LNP). The association between functional *LCT* variants and human gut microbiota composition is strong and consistent across multiple studies, with individuals who are LNP showing a higher gut abundance of *Bifidobacterium* than individuals who are not LNP. The abundance of *Bifidobacterium* in individuals who are LNP is also positively correlated with the intake of milk products. Interestingly, the study in 959 participants of a Dutch population cohort showed that the abundance of *Bifidobacterium* in individuals who are LNP was also positively correlated ($r = 0.33$) with bowel complaints, in particular abdominal pain, discomfort and bloating, which are hallmarks of IBS. This positive correlation suggests that the results of interactions among *LCT* genotype, diet and the gut microbiome might be relevant to IBS symptoms in individuals who are LNP.

In addition to SNPs tested in mbGWAS, DNA structural variation, including gene copy numbers of the *AMY1* gene (coding for the salivary amylase facilitating starch digestion), has been associated with changes in the composition of oral and gut microbiota; however, these studies require methodologically challenging analyses, and the association has not yet been explored in large cohorts of IBS. Although still in their early phases, mbGWAS have demonstrated the potential of host genetic factors to influence the composition of the human gut microbiota, suggesting that the combination of genetic variants, gut microbiota and their interaction might contribute to building a prediction model for IBS.

**Limitations and future directions**

Genomic and health-related data available from large population-based cohorts and biobanks have finally provided unprecedented opportunities for IBS genetic research and are likely to lead to the identification of more and more risk genes and pathways with future analysis of additional data from multiple sources, as genetic surveys of millions of people have now become a reality. Endophenotype studies, which focus on reducing IBS complexity, are probably more suited to identifying individual mechanisms that might be targeted therapeutically via drug repurposing or discovery, although they need to be validated to evaluate their applicability for determining clinical utility. Actionable biomarkers have been identified with potential in IBS and other DGBIs (faecal bile acids, gastric emptying and 

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**Alternative splicing**

The post-transcriptional process resulting in different combinations of exons from the same precursor miRNA producing alternative mRNA transcripts, this allows a single gene to code for multiple proteins, often associated with developmental and tissue-specific expression.

**Post-translational modifications**

All the chemical modifications that take place after the translation of a polypeptide chain (such as the addition of functional groups and/or polypeptides, proteolytic cleavage, glycosylation and phosphorylation), expanding the diversity in structures and functions of proteins.
accommodation, transit time, breath tests and rectal gas volume)\(^{66,67}\), and genetic analysis of some of these might be possible in large populations via surrogate traits from questionnaire data, in an approach similar to that used for investigating stool frequency in relation to gut motility\(^{68,161}\). Abdominal pain and bloating are possibly the best candidates for the most immediate application of these approaches, as suitable data are already available in several large cohorts from questionnaires\(^{68,161}\). This strategy could shift the paradigm from symptom-based definitions and treatments towards targeting pathways based on a better mechanistic understanding. Integrating genetic and other biological information, omics and exposome data (including those collected with personal wearable devices) is likely to be key to the identification of disease subtypes at the molecular level, and the eventual stratification of patients into different treatment groups for improved therapeutic precision\(^{69}\). A scenario can be envisaged where some IBS forms can be identified and reclassified as ‘IBS mimickers’ corresponding to organic disease, as already reported for rare and dysfunctional variants in the SI and SCN5A genes (FIG. 3). For the vast majority of patients with IBS, however, integration of genetic and other data is likely to be required to achieve individual profiling that is sufficiently informative to assign patients to specific treatment modalities. Thus, stool frequency and other endophenotype-related PGS can be particularly useful after being further validated in independent cohorts and in populations of different ancestry (similar to other complex diseases, only individuals of European ancestry have been studied so far, therefore emphasizing the need for increasing population diversity in GWAS\(^{65,162}\)). PGS associated with these quantitative phenotypes might ultimately contribute to the early identification of individuals at increased risk of IBS, therefore allowing preventive approaches (as can be envisaged for individuals with hypomorphic SI variants) and the implementation of dietary intervention strategies. At the same time, the availability of PGS for IBS and other gastrointestinal conditions\(^{69}\) might contribute to clinical decision-making algorithms based, for instance, on the relative (PGS) risk of IBS versus IBD, and the need to screen via invasive and costly procedures (that is, colonoscopy) to reach a diagnosis, with important repercussions both for the patient (avoiding unnecessary examinations) and the health-care system (reducing costs or health-care utilization).

Important challenges for future studies remain, including meaningful identification of underlying pathophysiological mechanisms of IBS in modern large-scale genetic studies. Biobank-based digital genotyping, as described above, has been useful to boost statistical power and detect genome-wide significant associations\(^{69}\). However, whether these are unequivocally relevant across different IBS definitions remains an open question as definition-specific GWAS signals not confirmed in pooled analyses have already been reported\(^{66,73}\). It is also possible that the neuronal emphasis of current results derives from pooling heterogeneous IBS subtypes (for example, constipation and diarrhoea) so that gut-specific effects are lost, and the brain remains the only (detectable) common denominator among these\(^{69}\). Additional challenges will derive from the need to validate eventual associations with specific (surrogate) endophenotypes and proposed mechanisms in patients with well-characterized IBS to translate them into tangible clinical utility, as questionnaire-based traits only partially correlate with endophenotypes directly measured...
in patients (for example, stool frequency and colonic transit time).

Conclusions
The complexity and heterogeneity of the IBS phenotype have hampered genetic research for decades owing to the scarcity of research tools and data suitable to test hypotheses with adequate statistical power. Multicentre case–control studies and large biobank-scale population-based studies have started to emerge, generating valuable and statistically robust findings for the first time. These findings have largely been derived from minimal phenotyping and questionnaire-based data that include bowel function, demonstrating their clinical utility, and more detailed phenotyping or biomarker measurements applicable in large cohorts will enhance future studies. Whereas the number of IBS genes and pathways will grow from the analysis of an ever-increasing number of individuals with IBS, the next challenge will be to translate statistical observations into mechanisms that can be targeted in individual patients based on molecular data beyond their symptom presentations.

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All authors researched data for the article. All authors contributed substantially to discussion of the content. M.D’A., M.C. and A.Z. wrote the article. All authors reviewed and/or edited the manuscript before submission.

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