Genetics and genomics in IBS: A primer for the neurogastroenterologist

Genética y genómica en el SII: Una introducción para el neurogastroenterólogo

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ABSTRACT

Irritable bowel syndrome (IBS) is a gut-brain interaction disorder characterized by recurrent or chronic abdominal pain associated with an alteration in the normal pattern of stool consistency. In more than 20% of patients with IBS, there is comorbidity with depression and anxiety impacting on quality of life. The pathophysiology of IBS is not fully understood, but it involves the neural, endocrine, and immune systems as dysfunctional signaling processes of the gut-brain axis and genetic factors. The goal of this review is to prime neurogastroenterologist into genetic or genomic studies. We will describe the different strategies used to discover the genes involved in the development of IBS. We will briefly explain general considerations for genetic studies, the different cohorts used for genetic studies (familial, case-control, general population), and approaches used for these studies (candidate genes or genome wide- association studies). Moreover, we will describe some of the genes associated with IBS. Genetic and genomic studies have been crucial to identify several gene variants associated with IBS.

Keywords: Irritable bowel syndrome. Genetics. Genomics. Candidate genes. Genome-wide association studies.

RESUMEN

El síndrome de intestino irritable (SII) es un trastorno de la interacción intestino-cerebro, que se caracteriza por el dolor abdominal recurrente o crónico y está asociado a la alteración en el patrón normal de la consistencia de las heces. En más del 20% de los pacientes con SII existe comorbilidad con depresión y ansiedad, lo que repercute de forma importante en la calidad de vida. La fisiopatología del SII no se comprende completamente, pero involucra al sistema neural, endocrino e inmunológico en procesos de señalización disfuncional del eje intestino-cerebro y factores genéticos. El objetivo de esta revisión es acercar a los neurogastroenterólogos a los estudios genéticos y genómicos. Se describirán diferentes estrategias utilizadas en el descubrimiento de genes involucrados en el desarrollo del SII. También, se explicarán brevemente las consideraciones generales para los estudios genéticos, las diferentes cohortes que se utilizan (familiar, caso-control y población general), así como los abordajes manejados para estos estudios (genes candidato o estudios de asociación al genoma completo). Además, se describirán algunos

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de los genes candidatos asociados a SII, así como variantes descubiertas por un abordaje sin hipótesis, los estudios de asociación al genoma completo. Los estudios genéticos y genómicos han sido cruciales para identificar diversas variantes génicas asociadas al SII.

Palabras clave: Síndrome de intestino irritable. Genética, genómica. Genes candidatos. Estudios de asociación del genoma completo.

INTRODUCTION

Irritable bowel syndrome (IBS) is a gut-brain interaction disorder (GBID), formerly considered a functional gastrointestinal disorder (FGID)¹. GBIDs are defined as a group of disorders classified by gastrointestinal (GI) symptoms related to any combination of: motility disturbance, visceral hypersensitivity, altered mucosal and immune function, altered gut microbiota and altered central nervous system (CNS) processing². The Rome IV criteria define IBS as recurrent abdominal pain for at least 1 day per week in the past 3 months, associated with 2 or more of the following criteria: abdominal pain related to defecation, a change in stool frequency and/or in stool form³. These defecation problems are typically present in three types: constipation, diarrhea, or a mix of constipation and diarrhea, presenting symptoms of abdominal bloating and/or distention. Therefore, IBS is classified into four subtypes: IBS with diarrhea (IBS-D), IBS with constipation (IBS-C), IBS with mixed symptoms of constipation and diarrhea (IBS-M), or untyped IBS (IBS-U)⁴.

A systematic review reported a prevalence that varies between countries, from 1.1% in France and Iran to 35.5% in Mexico. The average regional prevalence was reported on 17.5% in Latin America, 9.6% in Asia, 7.1% in North America/Europe/Australia/New Zealand, and 5.8% in the Middle East and Africa⁵.

Recently, in 2021, a study described the prevalence, the global distribution, and associated factors of FGIDs in 33 countries. Of the 73,076 adults surveyed, 49.5% were women. Most of the countries revealed a prevalence of IBS between 3 and 5%, affecting 1.8 times more women than in men⁶.

IBS is often associated with psychiatric comorbidities. A recent meta-analysis showed a prevalence of more than 20% of both depression and anxiety, in people with IBS⁷.

Although it is true that IBS does not modify mortality rates, it does have a significant impact on the quality of life of patients. In addition, IBS has a general economic repercussion, since it generates high costs for the patient and the health systems during the diagnosis and treatment process. Moreover, it also causes absenteeism from work in adults and loss of productivity in parents; in children IBS causes absenteeism from school⁸. Therefore, there is a clear need to better understand this disease from the clinical and molecular domains to help the patient cope with it.

IBS pathophysiology is incompletely understood. However, it is hypothesized that the symptoms originate from dysfunctional signaling of the brain-gut axis, including three main systems: neural, endocrine, and immune.

IBS is considered a multifactorial disease, where environmental and genetic factors can influence the development of the disease. Some of the environmental factors are diet, stress, microbiome, which in turn conditions anxiety and depression⁷. On the other hand, genetics and genomics have been crucial to identify several genes associated with IBS. Accordingly, in the current review, we describe the basis of genetic and genomic studies of IBS, as well as some of the gene variants that have been associated with this GBID.

METHODOLOGY

Relevant papers about genetics and/or genomics of IBS published from 2005 to 2021 were searched using platforms such as PubMed, Cochrane Reviews, SciELO and ScienceDirect. The words used for this search were IBS, genes, genetics, genomics, genome wide association studies, meta-analysis, ethnicities, and pathophysiology.

RESULTS

IBS is a multifactorial disease, where environmental and genetic factors play an important role in the development of this disease.

Several risk factors have been associated with IBS pathogenesis, including: visceral hypersensitivity, dysbiosis of the intestinal microbiota, immune activation, GI infections, dietary factors, perinatal factors, psychological stressors, depression, and traumatic experiences such as child abuse, punishment, or parental rejection⁹. Then, IBS is a multifactorial disease

where the environment plays an important role in this disease. However, in this review, we will only focus in the genetic factors that give susceptibility to develop IBS.

Genetic factors leading to IBS

In this section, we want to introduce our readers to the tactics used to perform genetic or genomic studies. We will describe the different strategies used to discover the genes involved in the development of IBS. We will briefly explain general considerations for genetic studies, the different cohorts used for genetic studies (familial, case-control, and general population), and approaches used for these studies (candidate genes or genome wide- association studies [GWAS]).

Genetic considerations for genetic studies

Monogenic diseases are caused by rare mutations (present in less than 1% of the population) of a single gene and their effect provokes a malfunction of the gene, leading to the development of the disease. On the other hand, several genes cause polygenic diseases, such as IBS. In the polygenic diseases, the gene variants are commonly present among the individuals (more than 1-5% of the population) and the addition of the effect of multiple variants (from different genes) confer the susceptibility to develop a disease.

In this section, we will describe the steps followed to perform a genetic study of a polygenic disease, such as IBS.

Discovery

In genetic studies, the first step is the discovery of genetic variants associated with a disease. This is normally performed with a case-control cohort, where several polymorphisms could be genotyped and analyzed to be associated with the disease¹⁰.

Replication

Once we have discovered a variant associated with the disease, the second step is the replication in several populations. The replication in different populations indicates that a gene is a plausible physiological actor on the disease¹⁰. One limitation to find association between the gene variant(s) and the disease is the small sample used in the cohorts. This leads to lack of statistical power (lower than 80%), given by the minor allele frequency, the odd ratio (OR) or risk, and the sample size. In polygenic diseases, the variant's OR is usually low, and the allele frequency is common (30-50%), so it all depends on the sample size to be able to achieve a statistical power over 80% and analyze the association between the gene variants and the disease. Therefore, replication and the sample size are important factors to take into consideration when performing genetic studies¹¹.

Ethnicity and meta-analysis

Ethnicity is another factor to consider while analyzing genetic data. It is possible that a gene variant could be associated with IBS in a Caucasian population, but not in an

Asiatic one, or vice versa. To cope with this problem, meta-analysis studies are performed¹².

A meta-analysis study is part of a systematic review. The meta-analysis consists of gathering all the existing data of previous studies, where association of genetic variants with a disease was reported in different populations. Then, statistical tests have to be performed, considering all those previous studies and calculate a new OR for the variant. Therefore, having the overall analysis, we can conclude if a gene variant is associated with a specific disease, considering the ethnicity^{13,14}.

Cohorts used for genetic studies

FAMILIAL STUDIES

Twin and familial studies were the main strategies used to understand the genetic implication behind any disease. Monozygotic twins share 100% of their genome and dizygotic twins only share 50%.

A study involving 281 American twin pairs showed that monozygotic twins have 17% concordance for IBS, while 8% for dizygotic twins¹⁵. Another study with 3334 Norwegian twin pairs also showed a higher concordance rate for IBS (22% monozygotic vs. 9% dizygotic) (Fig. 1A). Moreover, the IBS heritability was 48%¹⁶, showing that genetics is a key factor in the development of IBS.

Another strategy to study the impact of genes on a disease is the adoption studies. Then, we can analyze the influence of the genetic background versus the environment where they

are raised. Interestingly, adoptees whose biological parents suffer IBS present an OR or risk for IBS of 1.67 (95% CI: 1.06-2.62). Whereas, for adoptees whose adoptive parents present IBS was 0.88 (95% CI: 0.48-1.63)¹⁷ (Fig. 1A). Therefore, twin and the adoptive studies add another proof that genetics play an important role in the development of IBS over the environment.

CASE-CONTROL STUDIES

IBS has been described to be a polygenic disease, meaning that several genes are involved in the development of the disease. The discovery of the single nucleotide polymorphism or variants (SNVs), by the human genome sequence, opened a new way of studying genetics. SNVs are a variation of a single base pair or nucleotide in the genome, representing 1% of our genome and conferring the difference among individuals. A SNV (catalogued as rs#) is a common change in the genome (more than 5% of the population has a SNV) and confers susceptibility to develop the disease. However, a mutation is a rare change of a nucleotide in the genome, its frequency is low (less 1% of the population has the variant), and generally leads to the development of a disease.

The SNVs are used to do association studies that consist on comparing the allele frequency of the variant between case and control subjects. These studies are called case-control studies, based on cohorts of people presenting the disease (case) and without the disease (controls) (Fig. 1B). To do this comparison, we can use a Chi-square to obtain the OR. OR values above 1 represent a risk to develop the

disease; and OR values below 1 represent a protection to get the disease¹⁸.

Several genes have been discovered using this approach. For example, the serotonin re-uptake transporter (*SERT* or *SLC6A4* deletion/deletion) was associated with IBS using a case-control cohort of 150 IBS patients and 252 controls, reporting an OR of 2.6 (1.4-4.9) and a $p = 0.003$ ¹⁹. Another example is the association of the serotonin receptor type 3 (HTR3E 3'UTR variant c. *76G_A, rs62625044) with IBS-D by analyzing 100 patients and 100 healthy controls of an English population, showing an OR of 8.53 (1.04-70.28), $p = 0.033$. In addition, in this same study, they analyzed a German population, where they were able to replicate the association with IBS shown before (OR of 4.92 and $p = 0.0046$)²⁰.

GENERAL POPULATION

General population studies are based on recruiting people without knowing the state of the disease, meaning that a subject could have the disease or not, or he/she could be on the stage of developing it. These types of studies are very useful to identify intermediate phenotypes and genes that could be important for the development of the disease (Fig. 1C). Prospective studies based on the general population are used to obtain biomarkers that could predict the progress of a disease in the future¹⁰.

For example, COMT Val158Met polymorphism was evaluated in 867 subjects representative of a general population. Val/Val COMT variant was associated with IBS (OR 3.98, $p = 0.046$) and carriers of this polymorphism

presented increased bowel frequency (stools per day; OR 5.3, $p = 0.03$)²¹.

An interesting example summarizing all what has been explained up to this point is a paper published by Ek et al., where they analyzed a general population sample of 11,326 Swedish twins, selecting 534 cases and 4932 controls for the discovery study. Over one million SNVs were analyzed, finding 42 independent genomic regions with suggestive association. Secondly, they performed a replication study using 6 different population case-control cohorts. Then, they completed a meta-analysis of all those cohorts of different populations and reported that rs12702514 (T/C) have an OR of 1.21 (1.13-1.30) with a p-value of meta-analysis of 5.46×10^{-06} . This variant is close to the gene *KDELR2* that will be further discussed in GWAS section¹⁰.

Therefore, different types of cohorts (familial, case-control or general population) are used to identify gene variants associated with a disease, such as IBS. After selecting the type of genetic study and the cohort, the next step is to decide the approach to analyze the genes: (1) candidate genes or (2) GWAS, the most commonly used in the last decades.

Approaches used for genetic studies

CANDIDATE GENES “FROM PHYSIOLOGY TO GENETICS”

As mentioned above, IBS has a genetic component already established by familial studies. The next step is to identify the exact genes that could be provoking the disease or that confer susceptibility to develop it.

A candidate gene approach consists of knowing the cellular or physiological function of a gene, and then, look for its association with a disease in a cohort. Reason why we commonly say that we go “from physiology to genetics” (Fig. 1D). However, the main limitation of a candidate gene approach is that the function of the gene has to be known beforehand. In addition to this limitation, we can just study a few genes at the same time, by a trial and error approach.

Several reviews about IBS genetics or genomics have been published in the last decade, describing more than 65 polymorphisms of different genes associated with IBS. Some of the functions of these candidate genes are related to serotonergic, adrenergic, or opioidergic systems, as well as neuromodulatory, immune activation and inflammation, intestinal secretion and motility, among others²²⁻²⁴. In addition, meta-analysis studying the immunogenetics of IBS²⁵ or the serotonin receptor's association with IBS has been reported¹³. A recent systematic review and meta-analysis described the candidate genes associated with IBS. In this study, 66 variants were selected to be considered for the meta-analysis, from 3810 publications of IBS related polymorphisms published between 2000 and 2018. After applying their inclusion criteria, only 10 polymorphisms, from 28 studies, were evaluated for their association with IBS¹⁴.

In the next section, we will briefly describe some of the candidate genes implicated in the pathophysiologic mechanisms involved in IBS: neural, endocrine and immune pathways. In Table 1, genes that are associated and have been confirmed by meta-analysis are

FIGURE 1. Different cohorts and approaches used in genetic studies. 1) Cohorts used for genetic studies. **A:** familial: twin or adoption cohorts. **B:** case-control cohorts. **C:** general population cohort. 2) Approaches used for genetic studies. **D:** candidate genes. **E:** genome-wide association studies (genome wide- association studies).

indicated; the other genes have been associated in a few studies, but the replication has to be confirmed in more populations (Table 1).

Neural pathway

CNS can modulate visceral afferent signals, resulting in the facilitation or inhibition of endogenous pain. Functions of the GI tract are modulated by intrinsic and extrinsic innervation, mediated by the autonomic nervous system: parasympathetic and sympathetic. The parasympathetic nervous system stimulates smooth muscle and secretory actions, while the sympathetic inhibits the motor and secretory activity of the

GI tract²⁶. The intrinsic or enteric nervous system provides local reflexes, such as migrating motor complex and peristaltic reflexes, and yet also receives input from the CNS through the autonomic nervous system²⁷.

The dysfunctional gut-brain axis is observed in the pathogenesis of IBS. The existence of autonomic dysfunction in IBS has been hypothesized. Low vagal activity can lead to a reduction in bowel contractions, reduced motility, and constipation, while high vagal activity can result in increased contractions and diarrhea²⁸.

A subset of IBS patients develop symptoms after an acute GI infection, as a transient process that returns to normal after resolution of

TABLE 1. Genes associated with IBS discovered by gene candidate approach and some are confirmed by meta-analysis

Closest gene	Gene function	Variants	Possible associated effect	Type of IBS	Reference
Neural pathway					
<i>SLC6A4</i> (Solute Carrier Family 6 Member 4)	Encodes for SERT, transporter in serotonin reuptake	5-HTTLPR (Short variation)	Decreases the activity of SERT and accelerates intestinal peristalsis	IBS-D (in Mongoloid ethnicity)	Meta-analysis 14,31,32
<i>COMT</i> (Catechol O-Methyltransferase)	Degradation of catecholamines	rs4680 (G/A) (Val158Met)	Decreases its enzyme activity and pain threshold	IBS	Meta-analysis 14,21
<i>NPSR1</i> (Neuropeptide S Receptor)	Involved in stress, fear and anxiety. Increases the expression of <i>CCK</i> , <i>somatostatin</i> and <i>peptide Y</i> .	rs1379928 A/G	Increased intestinal motility, gas, abdominal pain and urgency to defecate	IBS-D	Neural and endocrine pathway 33
Endocrine pathway					
<i>CRF1</i> and <i>CRF2</i> (<i>CRHR1</i> and <i>CRHR2</i>) (Corticotropin Releasing Hormone Receptor 1 and 2)	Modulates the HPA axis and inflammation of the intestinal mucosa.	TT rs7209436 rs4722999 C/T rs3779250 C/G	Increased in stress and anxiety	IBS	Meta-analysis 14,37,63
<i>CNR1</i> (Cannabinoid Receptor 1)	Mediates many cannabinoid-induced effects, including GI motility, anxiety, chronic pain	AAT triplet in 3' flanking region rs806378 C/T	Severity of abdominal pain Increases colonic transit	IBS-D	38
<i>FAAH</i> (Fatty Acid Amide Hydrolase)	Inactivates anandamide, the main endocannabinoid	FAAH C385A	It is inactivated and releases acetylcholine; rapid colon transit.	IBS-D	38
<i>CCK1-R</i> (Cholecystokinin-1 Receptor)	Modulates colon motility and gastric emptying	intron 1, 779 T/C	Delay in gastric emptying	IBS-C and IBS-M	40
Immune pathway					
<i>IL6</i> (Interleukin 6)	Powerful inducer of the acute inflammatory response	rs1800795 C/G	Probably inflammation of the bowel mucosa	IBS-U (in Caucasians subgroup)	Meta-analysis 14
<i>IL23R</i> (Interleukin 23 Receptor)	Binds IL23 and mediates T-cells, NK cells and macrophages	rs11465804 T/A	Probably inflammation of the bowel mucosa	IBS-C	Meta-analysis 14
<i>IL10</i> (Interleukin 10)	Limits and may ultimately cease inflammatory responses	rs1800896 T/C	Anti-inflammatory protector	Lower risk of IBS	Meta-analysis 14,48
<i>IL8</i> (Interleukin 8)	Involved in the initiation and amplification of acute inflammatory responses and in chronic inflammation	IL8 + 396 G/G	Transient mucosal inflammation	IBS (in Mexicans)	49

(Continues)

TABLE 1. Genes associated with IBS discovered by gene candidate approach and some are confirmed by meta-analysis (*Continued*)

Closest gene	Gene function	Variants	Possible associated effect	Type of IBS	Reference
<i>TNFSF15</i> (TL1A) (TNF Superfamily Member 15)	Encodes for TL1A, which is a member of the tumor necrosis factor superfamily. It binds to death receptor 3 (DR3), triggering secretion of interleukin 17	rs4263839 A/G rs6478108 C/T	Probably inflammation of the bowel mucosa	IBS-C IBS	Meta-analysis 14,25
Other mechanisms					
<i>SCN5A</i> (Sodium Voltage-Gated Channel Alpha Subunit 5)	Expressed in the intestinal smooth muscle cells and interstitial cells of Cajal that mediate the GI contractions	p. A997T-Nav1.5	Reduction of the Na ⁺ current impairing its function and leading to a channelopathy	IBS-C and IBS-D 2% IBS patients carry mutations in <i>SCN5A</i> .	11,57
<i>SI</i> (Sucrase-Isomaltase)	Disaccharidase enzyme present in the small intestine, degrades starch or digested sugars	Variant Val15Phe	Carbohydrates malabsorption. This variant reduced 35% the enzyme activity. Correlated with increased stool frequency and abundance of <i>parabacterium</i> fecal microbiota	IBS-D	58

*Meta-analysis: genes that are associated and have been confirmed by meta-analysis are indicated; the other genes have been associated in a few studies, but the replication must be confirmed in more populations. IBS: irritable bowel syndrome; IBS-D: IBS with diarrhea, IBS-C: IBS with constipation; IBS-M: IBS with mixed symptoms; IBS-U: IBS unsubtyped.

inflammation. Evidence from human mucosal biopsies suggests the existence of neuroplastic remodeling in the intestinal epithelium and afferent nerve terminals that could interfere with the release of neuropeptides from these terminals, resulting in neurogenic inflammation²⁹.

The 5-hydroxytryptamine (5-HT), also known as serotonin, is a mediator in the gut-brain interaction. Serotonin plays an important role in intestinal motility and the pathophysiology of IBS, as it is one of the main neurotransmitters involved in peristalsis, intestinal motility, and visceral sensitivity. Ninety-five percent of the serotonin that is formed in the intestine is

for endocrine, autocrine, paracrine, and hormonal functions. The inactivation of serotonin occurs by the serotonin reuptake transporter (SERT) to maintain proper levels. Patients with IBS have an altered transcription of SERT. It is elevated in IBS-C, which causes a decrease in the level of serotonin, and it is low in IBS-D, causing an increase in serotonin, which accelerates motility and causes diarrhea and pain³⁰.

The motility and sensitivity of the GI tract is modulated in a 5-HT receptor-dependent manner through various receptors through enteric and submucosal neurons. For this reason, different genes involved in the serotonin

neuronal pathway have been explored as candidate genes for their association with IBS.

A meta-analysis suggested that *HTR3A* and *HTR3E* are associated with a decreased risk of IBS-D¹³. However, a review compiled all the genetic information about these receptors and its association with IBS, concluded that there exist ethnic disparities in the association¹².

The SERT or solute carrier 6A4 (*SLC6A4*) is a transmembrane transport protein that regulates the reuptake of 5-HT after its interaction with its receptor. This gene contains a 44-base pair deletion or insertion, generating the short or long allele, named 5-HT transporter length polymorphic region (*5-HTTLPR*). The short variant reduces the expression of *SLC6A4*, inhibiting the reuptake of serotonin. Several studies had reported association to this gene and has been reviewed elsewhere^{31,32}. A recent meta-analysis showed that *5-HTTLPR* is associated with IBS-D in Mongoloid, but not in Caucasian population¹⁴, remarking the importance of the ethnicity in genetic studies¹².

Another candidate gene is the catechol-O methyltransferase (*COMT*), implicated in the degradation of catecholamine, modulating dopaminergic, and adrenergic/noradrenergic neurotransmitters. Its variant rs4680 provokes a change of amino acids (Val158Met), diminishing the enzyme activity and lowering the pain sensitivity threshold; in addition, rs4680 is associated with IBS²¹. The α 2-adrenergic receptors were also evaluated, due to its implication on modifying sensory and motor functions on IBS patients. Discrepancy on the association of this gene with IBS exists among

different studies; therefore, more research has to be performed to assess the plausible genetic role of these receptors on IBS³².

The β 3 subunit of G protein (*GN β 3*), which modifies the activity of the adrenergic receptors, has been proposed to be associated with IBS. However, a meta-analysis of all the studies analyzing this variant confirmed no evidence of this association¹⁴ and the allele frequency is different among ethnicities¹².

Another candidate gene that has been associated with IBS is the neuropeptide S receptor (*NPSR1*). *NPSR1* is a neuropeptide involved in stress, fear, anxiety and it is expressed in the intestinal epithelium and is expressed upon inflammation²⁴. *NPSR1* induces the expression of CCK, peptide Y, and somatostatin³³. This gene was associated with GI sensory symptoms as gas, pain and urgency, as well as with colonic transit time³³. However, more populations have to be analyzed to confirm its association with IBS.

Endocrine pathway

The intensity of symptoms in patients with IBS is related to chronic stress. The key stress-initiated signaling factor is the hormone corticotropin-releasing factor or hormone (CRF or CRH). CRF is a vital hormone in the body's response to stress, activating through the hypothalamic-pituitary-adrenal (HPA) axis in reaction to a variety of physical and psychological stressors. IBS patients have enhanced levels of adrenocorticotrophic hormone and cortisol compared to healthy subjects. CRF exerts its biological effects by activating the transmembrane G protein-coupled

CRFR1 and CRFR2 receptors. CRFR1 is expressed in enteric neurons and in the intestinal mucosa, so stress induces mechanisms that affect GI function, such as delayed gastric emptying or accelerated intestinal and colonic motility³⁴. The use of knock-out mice CRFR1 -/- has revealed the importance of the CRFR1 subtype in IBS like-symptoms, as these mice exhibit decreased visceral sensitivity, as well as decreased anxiety and an impaired stress response³⁵.

Therefore, a plausible candidate gene for IBS is *CRF*, which modulates the HPA axis and intestinal mucosa inflammation³⁶. Polymorphisms on *CRFR1* and *CRFR2* were shown to be associated with IBS³⁷.

Other candidate genes are the cannabinoid receptors. Anandamide is the main endocannabinoid that binds to the cannabinoid receptors (CB1 and CB2, encoded by the genes *CNR1* and *CNR2*) have also been associated with IBS. CB1 receptors are located in the GI tract, CNS, muscle, fat, and liver; while CB2 are found in immune cells. Anandamide will be synthesized on demand and will inhibit presynaptic neurotransmitters, such as acetylcholine. Then, anandamide is inactivated by the fatty acid amide hydrolase (*FAAH*), upon inactivity leads to acetylcholine release. Genetic studies were performed, finding that a triplet on *CNR1* (AAT)n on 3' flanking region is associated with severity of abdominal pain in IBS and rs806378 with colonic transit in IBS-D³⁸. Moreover, the *FAAH* C385A variant was associated with IBS-D and rapid colon transit³⁸. In fact, a recent review summarizes the clinical research about the potential therapeutic benefits of the cannabinoids over the regulation of GI disorders based on gut-brain

interactions³⁹. However, more genetic studies have to be performed in other populations to assure their implication in IBS.

Cholecystokinin is a neuropeptide that is secreted within the duodenum and the jejunal mucosa. It acts through two receptors CCK1 and CCK2 that are distributed in the CNS and in the GI tract, modulating the colon motility and gastric emptying. *CCK1* was associated with IBS-C and IBS-M patients and with a lower GI motility⁴⁰. Further investigation has to confirm its association with IBS.

Immune pathway and microbiota

In recent years, the role of the gut microbiota in the bidirectional communication of the gut-brain axis has been described, including visceral sensation and nociception⁴¹.

The microbiota plays an important role in the pathogenesis of IBS, and it is characterized by intestinal dysbiosis. Acute and chronic stress have been shown to affect intestinal secretory IgA levels, which affects intestinal homeostasis, the inflammatory response, and ultimately leads to dysbiosis⁴².

Stress changes the internal environment of the GI tract through immune, neurochemical, and physical mechanisms to make it a less habitable space for certain beneficial bacteria, provoking the growth of pathological species. This can alter the pain signaling mechanisms of the GI tract that contribute to visceral hypersensitivity⁴³.

Gut bacteria can modulate the epithelial barrier and the immune response, which ultimately

increases the expression of tight junction proteins of epithelial cells. This translates into lower intestinal permeability to intraluminal xenobiotics that can cause an inflammatory response⁴¹.

The influence of the microbiota on the gut-brain axis is suggested through its interaction with the immune system in the gut mucosa. Dysbiosis is related to low-grade intestinal inflammation and triggering the immune system, contributing to GI symptoms and psychological symptoms⁴³. Intestinal dysbiosis has been associated with the pathogenesis of IBS⁴².

A systematic review and meta-analysis of case-control studies compared the gut microbiota of people with IBS and healthy controls. In people with IBS, there was a decrease in the number of *Lactobacillus* and *Bifidobacterium*; on the other hand, they had elevation of *Escherichia coli* and *Enterobacter*. No differences were found in the number of *Bacteroides* and *Enterococcus*⁴⁴. However, a specific dysbiotic signature has not yet been established through the different studies.

Cytokines regulate immune and inflammatory responses and their production is genetically regulated. Therefore, the cytokines are candidate genes for IBS. Some of the cytokines that have been studied are: tumor necrosis factor-alpha (TNF- α), transforming growth factor beta 1, interleukin (IL)-6 (IL-6), IL-8, IL-10, IL-32 receptor (IL-32R) and TNFSF15.

In postinfectious IBS, it is more likely that there is subtle intestinal inflammation with infiltration of mast cells in the colon and small intestine⁴⁵. A study that measured the pro-inflammatory cytokine profile in patients with

IBS vs. healthy controls, demonstrated that IBS patients have higher serum levels of IL-1 β , IL-6, IL-8, TNF- α and IL-10, although it is not clear a causal relationship between the levels of these cytokines and the symptoms of IBS⁴⁶.

Interestingly, in a Mexican population, *Blastocystis* infection (a commensal parasite) was reported to be associated with IBS; however, IL-6 and TNF- α were not associated with IBS⁴⁷. Moreover, in another study IL-10 and TNF- α were not associated with IBS, but IBS-D subjects presented lower producer IL-10 genotype, suggesting predisposition due to a lower anti-inflammatory⁴⁸. Another cytokine, IL-8 has been reported to be associated in Mexican population. The IL8 +396 G allele was overrepresented in Mexicans with IBS compared to controls and it was associated with an increased risk to develop IBS⁴⁹. In addition, IL-8 and IL-10 variants increase the development of IBS in the presence of *Blastocystis*⁵⁰. A recent study analyzed the gene variants and the gut microbiota in a Mestizo IBS cohort, finding 76 polymorphisms associated with IBS and the enrichment of *Bacteroides*, *Alteromonas*, *Neisseria*, *Streptococcus*, and *Microbacterium* in IBS subjects⁵¹. However, these associations have to be confirmed in other populations.

Several genetic studies have tried to demonstrate its association with IBS. However, there is discrepancy about its association and among ethnicities. Therefore, several meta-analysis summarized all the previous data reported^{25,52}. The latest meta-analysis, from 2019, reported that the pro-inflammatory mediator IL6 rs1800795 increased the risk for IBS in Caucasian population and IL23R rs11465804

was associated with an increased risk for IBS-C. Moreover, the inhibitor of pro-inflammatory mediators and TNF synthesis, *IL10* rs1800896 was associated with a decreased risk of IBS and *IL10* rs1800871 was not associated. Moreover, no evidence of association was found for *TNFA* rs1800629¹⁴.

TNFSF15 encodes for TL1A, which is a member of the TNF superfamily. It binds to death receptor 3, triggering secretion of inflammatory cytokines like IL-17 via T-helper 17 cell proliferation and differentiation⁵³. It has been previously published that increased *TNFSF15* levels are present in IBS and in Crohn's disease patients^{54,55}. Several meta-analysis pointed out the association of *TNFSF15* rs4263839 with IBS showing, particularly with IBS-C^{14,25}, and *TNFSF15* rs6478108 T allele increased IBS risk as well, but no association was found in subgroup analysis¹⁴.

As a summary of this section, the most recent meta-analysis showed that *SLC6A4* 5-HTTLPR, *TNFSF15* rs4263839 and rs6478108 conferred risk to develop IBS, and *IL6* rs1800795 increased the risk for Caucasians IBS patients; *IL23R* rs11465804 increased the risk for IBS-C patients; whether, *COMT* rs4680 and *IL10* rs1800896 were protective for IBS; and *GNB3* rs5443, *TNFA* rs1800629, *IL10* rs1800871 did not show association with IBS (Table 1)¹⁴.

Moreover, another recent review analyzed IBS-related variants and their association depending upon the ethnicity. In this paper, they emphasized the importance of considering the ethnicities, to better understand the genes associated with IBS and their function, to prevent and ameliorate the treatment for IBS¹².

Other mechanisms for candidate genes

Besides the three typical pathways involved in IBS (neural, endocrine, and immune), there are other mechanisms, such as sodium channels or disaccharidases that could play a role in gut motility that could lead to IBS.

The voltage-gated sodium channel NaV1.5 encoded by the gene *SCN5A* is expressed in the intestinal smooth muscle cells and interstitial cells of Cajal that mediate the GI contractions⁵⁶. *SCN5A* gene sequencing in a case-control cohort revealed that 2% of all IBS cases presented functionally deleterious mutations⁵⁷. Remarkably, *SCN5A* loss of function mutations provoked a reduction of the Na⁺ current impairing its function and leading to a channelopathy¹¹, making it a good biomarker for IBS.

For the next candidate gene, two important concepts have to be introduced: nutrigenetics and nutrigenomics. Nutrigenetics is the science that studies how the genotype of an individual determines the optimal dietary requirements. On the other hand, nutrigenomics studies how the diet influences the DNA structure, hence the gene expression.

When we talk about IBS nutrigenetics, the sucrase-isomaltase gene is a clear example of it. Sucrase-isomaltase is a disaccharidase enzyme present in the small intestine that degrades starch or digested sugars. Sucrase-isomaltase deficiency or intolerance is characterized by carbohydrate malabsorption, having as a consequence diarrhea, bloating, abdominal pain, similar to IBS-D.

Therefore, this sucrase-isomaltase is a good candidate gene for IBS.

The sucrase-isomaltase variant Val15Phe was associated with increased risk of IBS, and it correlated with stool frequency and *Parabacteroide faecal* microbiota abundance. Interestingly, this variant reduced 35% the enzyme activity, predisposing the individual to IBS⁵⁸.

GWAS: variants without hypothesis, “from genomics to physiology”

As mentioned above, the candidate gene approach consists on knowing the gene's function, previously to be genetically analyzed in a case-control cohort. However, for the GWAS approach, the polymorphisms are randomly chosen, covering the entire genome. Therefore, the GWAS is a hypothesis-free approach, because the polymorphisms are not selected in a specific gene, nor the function is known.

The GWAS approach provoked a revolution in the genetic area. When using a candidate approach, we could only analyze one or few genes at the same time and prove its association to a disease, performing genetics. However, the GWAS technique consists on analyzing millions of gene variants (polymorphisms) at the same time, on a case-control cohort. Then, allelic frequency comparison between the individuals presenting the disease vs. the controls is analyzed, obtaining a list of gene variants associated with IBS, and becoming genomics instead of genetics (Fig. 1E).

It is important to clarify that the polymorphisms reported to be associated with

a disease by a GWAS approach, could be many kilobases or megabases far from the gene and they can be in intergenic regions, promoters, introns or exons. These variants are not chosen by the location of the gene or their function. So when reading a GWAS report, the name of the gene reported to be associated with a disease is the closest gene to the variant. That variant not necessarily regulates the gene next to it nor has a known function that could explain the IBS phenotype. Therefore, we then go “from genomics to physiology” to understand the function of that gene and variant.

Several IBS GWAS have been reported until now^{10,59,60}. The first exploratory GWAS was performed in a general population of 11,326 Swedish twins. From this first pilot study, the variants that were associated were replicated in 6 different case-control cohorts from the United States, Greece, Italy, Germany, Belgium, and Sweden and performed a meta-analysis. The locus on chromosome 7p22.1 was associated to higher risk of IBS and it includes two genes: *KDELR2* (KDEL endoplasmic reticulum protein retention receptor 2) and *GRID2IP* (glutamate receptor, ionotropic, delta 2 [Grid2] interacting protein). Moreover, genetic variants on that region modulate *KDLER2* mRNA expression, having a trend for higher rectal mucosal *KDLER2* mRNA expression in IBS cases versus controls. In addition, this study reported a nominal significance for association with IBS for candidate genes, such as *TNFSF15*, *SCN5A*, *COMT*, *NPSR1*, and *SLC6A4*, as mentioned above, Table 2¹⁰.

It is unknown how these variants in this locus could regulate the genes that are close to them (*KDELR2* or *GRID2IP*) or even if those variants can regulate genes that are far

apart and could be the cause of the disease. However, it is proposed that *KDLER2* belongs to a receptor family, which cycles between the Golgi apparatus and the endoplasmic reticulum controlling endoplasmic reticulum quality control and stress response. The researchers suggested that *KDLER2* could mediate effects of bacterial toxins in the gut, hence influencing IBS. On the other hand, *GRID2IP* encodes for a delphinin that is expressed in fiber-Purkinje cell synapses in the brain, where it binds to the monocarboxylate transporter 2, which is present in the enteric nervous system, hence leading to IBS¹⁰.

Later, a GWAS meta-analysis of five population-based cohorts was performed to study the IBS association in 18,659 individuals from Finland, Germany, the Netherlands, Sweden, and the United Kingdom. The results showed 7 genomic regions, harboring 64 gene candidates associated with IBS. Functional annotation through gene set enrichment analysis was obtained with functional class scoring against gene ontology resulting in enrichment in molecular signatures of: channel regulatory activity, ion channel activity, and liver genes- metabolism and xenobiotics. On the biological process, there was enrichment on regulation of sodium ion transmembrane transporter activity and the molecular function was a sodium channel regulator activity. In addition, association of *SCN5A*, *SI*, *NPSR1* genes with IBS was confirmed, as well as *KDLER2/GRID2IP* obtained in the previous GWAS⁶⁰.

Preliminary data from a study that is still in review, analyzed variants association with the stool frequency to study the genetics of gut motility. They identified 13-loci involved in sensory perception and neurotransmitter/

neuropeptide signaling, enriched expression in enteric motor neurons associated with peristalsis, GI motility and dysmotility syndromes. Surprisingly, the *BDNF* locus (rs12273363) was associated with IBS, lying in the promoter region of *BDNF*, repressing its activity. *BDNF* is recognized to have gut functions as sensation, neuroprotection, neuroplasticity, motility and epithelial barrier⁶¹. Other genes reported to be associated to stool frequency were acetylcholinesterase (*ACHE*), fatty acid hydroxylase (*FAXDC2*), and protein kinase expressed in colonic M cells and BEST4+ enterocytes (*CDK18*)⁶¹. However, all this data has to be peer-reviewed and published, as well as confirmed to have an impact on IBS.

More evidence is accumulating about the role of genes involved in gut motility and neurotransmission of enteric neurons implicated on IBS. In fact, the enteric nervous system coordinates GI homeostasis and digestive processes, establishing a gut-brain axis⁶².

Therefore, after the genomic era, where several variants were reported to be associated with a disease, the challenge now is to discover the function of those gene variants and its implication in the disease. This may lead to changing the mindset from genomics to physiology, understanding the implication of the genes in the etiology of IBS.

CONCLUSION

Genetic and genomic studies have been crucial to identify several genes associated with IBS. With a candidate gene or a hypothesis free approach (GWAS) several variants have been reported to be associated with IBS.

TABLE 2. Genes associated with IBS discovered by GWAS approach

Closest gene	Gene function	Variants	Associated effect	Type of IBS	Reference
<i>KDEL2</i> (KDEL endoplasmic reticulum protein retention receptor 2)	Vesicle trafficking and transport into the endoplasmic reticulum	rs12702514 T/C rs4574760 C/T	Might mediate effects of bacterial toxins	IBS	10,60
<i>GRID2IP</i> (Glutamate receptor, ionotropic, delta 2 [Grid2] interacting protein)	Encodes for a delphilin that is expressed in fiber-Purkinje cell synapses in the brain, where it binds to the monocarboxylate transporter 2	rs13222291 A/G rs4574760 C/T	Might affect host-microbiota interactions in relation to gut motility	IBS	10,60
<i>TNFSF15</i> (TL1A) (TNF Superfamily Member 15)	Encodes for TL1A, which is a member of the tumor necrosis factor superfamily. It binds to death receptor 3 (DR3), triggering secretion of interleukin 17	rs7867918 G/T	Probably inflammation of the bowel mucosa	IBS, IBS-C, IBS-D, IBS-A	10
<i>SCN5A</i> (Sodium Voltage-Gated Channel Alpha Subunit 5)	Expressed in the intestinal smooth muscle cells and interstitial cells of Cajal that mediate the GI contractions	rs7432532 C/A rs7430407 C/T	Reduction of the Na ⁺ current impairing its function and leading to a channelopathy	IBS	10,60
<i>COMT</i> (Catechol O-Methyltransferase)	Degradation of catecholamines	rs9332348 C/A	Decreases its enzyme activity and pain threshold	IBS	10
<i>NPSR1</i> (Neuropeptide S receptor)	Involved in stress, fear and anxiety. Increases the expression of CCK, somatostatin and peptide Y	rs324389 C/A rs324381 G/T	Increased intestinal motility, gas, abdominal pain and urgency to defecate	Colonic transit, sensory ratings IBS	10,60
<i>SLC6A4</i> (Solute Carrier Family 6 Member 4)	Encodes for SERT (Transporter in serotonin reuptake)	rs8073965 G/A	Decreases the activity of SERT and accelerates intestinal peristalsis	IBS, IBS-C, IBS-D, colonic transit	10
<i>SI</i> (Sucrase-isomaltase)	Disaccharidase enzyme present in the small intestine, degrades starch or digested sugars	rs75982521 C/A	Carbohydrates malabsorption	Diarrhea, bloating, abdominal pain, similar to IBS-D	60
<i>BDNF</i> (Brain-Derived Neurotrophic Factor)	Promotes the survival and differentiation of selected neuronal populations of the peripheral and central nervous systems	rs12273363 T/A	Gut functions as sensation, neuroprotection, neuroplasticity, motility, and epithelial barrier	Repressing its activity Stool frequency	under review 61 *eQTLs
<i>ACHE</i> (acetylcholinesterase)	Hydrolyzes acetylcholine at neuromuscular junctions; expressed in enteric and motor neurons	rs4556017 C/T	Gut motility	Stool frequency	under review 61 *eQTL
<i>FAXDC2</i> (fatty acid hydroxylase)	Hydrolyzes fatty acid; expressed in enteric and motor neurons	rs13162291 G/A	Gut motility	Stool frequency	under review 61 *eQTL
<i>CDK18</i> (Cyclin Dependent Kinase 18)	Protein kinase expressed in colonic M cells and BEST4 ⁺ enterocytes	rs11240503 G/A	Senses electrolyte and pH; colonic osmolarity, and transit	Stool frequency	under review 61 *eQTL

*eQTL: expression quantitative trait loci.

However, the challenge now is to understand the implication of those variants in conferring susceptibility to develop the disease and their relation to the underlying pathophysiological mechanism of this GBID.

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CONFLICTS OF INTEREST

The authors have no conflict of interest to declare.

ETHICAL DISCLOSURES

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

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