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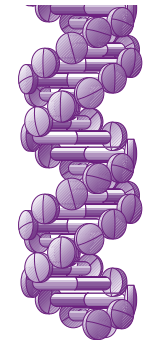

Genetics and pharmacogenetics of aminergic transmitter pathways in functional gastrointestinal disorders

Functional gastrointestinal disorders (FGIDs) are highly prevalent syndromes, without evident underlying organic causes. Their pathogenesis is multifactorial in nature, with a combination of environmental and genetic factors contributing to their clinical manifestations, for which most of current treatments are not satisfactory. It is acknowledged that amine mediators (noradrenaline, dopamine and serotonin) play pivotal regulatory actions on gut functions and visceral sensation. In addition, drugs of therapeutic interest for FGIDs act on these transmitter pathways. The present article reviews current knowledge on the impact of genetics and pharmacogenetics of aminergic pathways on FGID pathophysiology, clinical presentations, symptom severity and medical management, in an attempt of highlighting the most relevant evidence and point out issues that should be addressed in future investigations.

Keywords: dopamine • functional constipation • functional dyspepsia • functional gastrointestinal disorders • irritable bowel syndrome • noradrenaline • polymorphism • serotonin

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Functional gastrointestinal (GI) disorders (FGIDs) comprises a number of heterogeneous syndromes, for which clinical investigations do not reveal any evident organic cause [1]. FGIDs affect a large part of the general population with irritable bowel syndrome (IBS), functional constipation (FC) and functional dyspepsia (FD) representing the most prevalent conditions [2,3]. According to Rome III criteria, IBS is characterized by abdominal pain or discomfort and changes in bowel habit (constipation and/or diarrhea) [4]. FC presents as chronic difficult, infrequent or seemingly incomplete defecation, which does not meet IBS criteria [4]. FD is defined by the presence of gastroduodenal symptoms, such as bothersome postprandial fullness, early satiation and epigastric pain, and its current diagnostic categories comprise: postprandial distress syndrome (PDS) and epigastric pain syndrome (EPS) [5].

From a pathogenic standpoint, FGIDs are considered as multifactorial in nature with alterations of GI motility, visceral hypersensitivity and psychopathological comorbidities playing a pivotal role [5,6]. However, the pathophysiological mechanisms underlying FGIDs remain poorly understood and, in parallel, there is a substantial lack of effective medical therapies, with available drugs often resulting in unsatisfactory outcomes or adverse effects. Consequently, owing to their chronic or recurrent course, FGIDs result in a significant social burden, with a relevant negative impact on both quality of life and healthcare costs [7,8].

Based on current evidence, the predisposing factors to FGID symptoms include a combination of environmental (e.g., diet, infections, stressful events) and genetic factors [9]. Studies on families and twins have given support to the influence of hereditary factors in both IBS and FD [10,11], and, consequently, efforts are being made to evaluate whether polymorphisms of a number of candidate genes are linked to FGIDs or their phenotypes (i.e., intermediate phenotypes or clinical presentation/severity) [9,12,13]. Nev-

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ertheless, even though currently a large body of data substantiate the contribution of genetics to FGIDs, at present it is not possible to define precisely which specific variants are actually associated with FGIDs (or their clinical phenotypes), that likely represent heterogeneous, multifactorial, polygenic complex disorders. Indeed, as better discussed below, several limitations of association studies still oppose relevant barriers against the assessment of the exact roles played by genetic polymorphisms in FGIDs and prevent reliable quantitative estimations of the genetic polymorphisms that are encountered in the population of patients with FGIDs. Of note, the role of genetics has been scarcely investigated in patients with FC.

The homeostasis of digestive tract is ensured by networks of intrinsic and extrinsic regulatory pathways, and their dysfunctions may contribute to the GI symptoms that characterize FGIDs [14,15]. In this context, great attention has been paid to aminergic mediators (i.e., noradrenaline or NA; serotonin, 5-hydroxytryptamine or 5-HT; dopamine or DA), owing to their pivotal roles in the central or peripheral control of GI motility/secretion/sensation [14,15]. It is also noteworthy that prokinetics and other drugs, such as antidepressants, commonly employed in the management of FGIDs, act on these transmitter pathways [16–18]. Stemming from these considerations, gene polymorphisms in aminergic pathways are being evaluated in an attempt of highlighting not only their role in the pathogenesis of FGIDs, but also their potential impact on the therapeutic efficacy and/or safety of drug therapies [9,19].

Based on this background, the present review article intends to appraise current evidence on genetics and pharmacogenetics of aminergic pathways in FGIDs. For this purpose, we identified relevant literature by electronic search in three databases (PubMed, EMBASE, Cochrane Library). The search was performed by a combination of key words regarding FGIDs, aminergic transmitters and drugs employed in the management of FGID symptoms.

Role of aminergic transmitter systems in gut physiology & FGID pathophysiology

A large body of evidence support the notion that NA, 5-HT and DA play pivotal regulatory actions on gut functions and visceral sensation, both at central and peripheral level. This matter has been reviewed extensively in previous articles [20,21], and will be addressed concisely in this section.

Noradrenaline

NA acts as a neurotransmitter in the CNS and mediates also the regulatory actions of sympathetic nerves on GI tract. NA exerts its biological actions through interaction with two families of membrane G-protein coupled receptors (GPCRs) (adrenoceptors), designated as α and β. Each family includes two (α_1, α_2) and three (β_1, β_2, β_3) receptor types, respectively, and each α-adrenoceptor type comprises three subtypes, designated as α_{1A}, α_{1B}, α_{1C} and α_{2A}, α_{2B}, α_{2C}, respectively [20]. Once synthesized, NA is stored within vesicles in the synaptic endings of noradrenergic neurons, and exerts its action upon release and activation of adrenoceptors. Noradrenergic signaling is then terminated by NA reuptake via norepinephrine transporter (NET) and subsequent inactivation by monoamino oxidases (MAOs) and catechol-O-methyltransferases (COMTs) [22].

Noradrenergic nerves regulate various digestive functions, including mucosal secretions, bowel propulsion and gut sensations mainly through α_2-adrenoceptors [15]. The inhibition of fluid/electrolyte secretion into the intestinal lumen by sympathetic nerves occurs through a predominant activation of α-adrenoceptors located on intrinsic secretomotor neurons and epithelial cells [23]. Sympathetic neurons can also slow the transit of intestinal contents mainly in three ways: inhibition of enteric neurons via α_2-adrenoceptors; inhibition of smooth muscle via β_2/β_3-adrenoceptors; regulation of sphincter smooth muscle contractility via α-adrenoceptors [23]. Of note, β_3-adrenoceptors, initially identified as regulators of lipolytic and thermic responses in brown and white adipose tissue, are also expressed throughout the digestive system, where they inhibit colonic motility in humans [24].

Several studies have underscored a role for alterations of noradrenergic pathways in the pathophysiology of FGIDs. For instance, using a neuroendocrine challenge test, abnormal functioning of central α_2-adrenoceptors was suggested in IBS patients [25]. Moreover, in a study on IBS patients, Aggarwal *et al.* [26] found that vagal dysfunction was associated with constipation-predominant IBS (C-IBS) symptoms, whereas adrenergic sympathetic dysfunction was associated especially with a diarrhea-predominant (D-IBS) pattern. In addition, in a pilot clinical trial, the administration of clonidine in patients with D-IBS allowed a satisfactory relief of IBS symptoms, as compared with the placebo group, without any change in GI transit time [27].

Serotonin

5-HT is mainly produced in the GI tract: about 90% in enterochromaffin cells (EC) and 10% in myenteric serotonergic neurons. Only a small fraction (about 5%) is synthesized in serotonergic neurons of CNS [21]. The

rate-limiting step in 5-HT biosynthesis depends on tryptophan hydroxylase (TPH). This enzyme exists in two isoforms, TPH1 and TPH2, which are responsible for the non-neuronal and neuronal biosynthesis of serotonin, respectively [28]. 5-HT exerts its biological actions through distinct membrane receptors, designated as 5-$HT_{1–7}$. With the exception of the 5-HT_3 receptors, which are ligand-gated ion channels, all the remaining 5-HT receptors belong to the family of GPCRs. 5-HT_1 includes five subtypes (A, B, D, E, F), 5-HT_2 three subtypes (A, B, C) and 5-HT_5 two subtypes (A, B) [29]. Serotonergic signaling is terminated by intracellular 5-HT reuptake, operated by a specific 'serotonin transporter' (SERT), followed by enzymatic catabolic inactivation [30]. Fractions of the 5-HT released in the gut from EC cells, mostly during the post-prandial period, fail to be taken up into enteric cells and leak into the blood stream, where 5-HT is taken up by SERT into circulating platelets and stored into their dense granules [30].

5-HT regulates several functions in the CNS, cardiovascular system and GI tract [31]. In the digestive system, 5-HT acts mainly through 5-HT_1, 5-HT_2, 5-HT_3, 5-HT_4 and 5-HT_7 receptors [32]. At this level, a pivotal role in the stimulation of intestinal propulsive motility is played by 5-HT released from EC cells [32]. According to current evidence, EC cells function as transducers, which are able to respond to luminal and/or intramural chemico-physical stimuli with the release of 5-HT, which then initiates local secretory and motor (peristaltic) reflexes as well as the activation of extrinsic sensory nerves [32].

Several lines of evidence support the notion that serotonergic pathways are involved in the pathophysiology of FGIDs, with particular regard for IBS [33,34], as discussed in detail in previous reviews [35–37].

Dopamine

Mesenteric organs (GI tract, spleen and pancreas) represent major sites of DA biosynthesis, accounting for about 45% of all DA produced in the body [38]. DA exerts its biological actions through two types of membrane GPCRs, designated as D_1 and D_2. The D_1 type comprises two subtypes (D_1, D_5), while the D_2 typeincludes three subtypes (D_2, D_3, D_4) [39]. The DA transporter (DAT) plays a key role in modulating dopaminergic signaling by a rapid clearance of DA from synaptic clefts. Once taken up into the presynaptic terminal, DA can be re-stored into synaptic vesicles or be inactivated by MAOs and COMTs [40].

DA receptors have been detected both on gut smooth muscle and enteric nervous system (ENS). In the GI tract, DA inhibits motility through type D1 receptor located on smooth muscle [41], and via type D_2 receptors, which modulate acetylcholine release from myenteric neurons [42].

While DA plays significant roles in the pathophysiology of CNS disorders [43], information on its possible role in the pathophysiology of FGIDs are very scarce. Nevertheless, DA receptors are regarded as viable targets for the pharmacological modulation of GI motility and management of GI motor symptoms associated with FGIDs [44].

Genetics of aminergic system in FGIDs

A number of candidate genes and polymorphisms pertaining to aminergic pathways have been investigated. Some of these genetic variations have been found to be pathogenically associated with FGIDs (particularly FD and IBS), their intermediate phenotype or clinical presentation/severity. Currently, the best studied polymorphisms are those related to the adrenergic and serotonergic systems, COMT and G proteins, while information on DA pathways are quite scarce.

Noradrenaline

Studies on FD have yielded quite heterogeneous results (Table 1). In a US community, 50 Caucasian patients with FD were subdivided according to the presence (n = 20) or absence (n = 30) of meal-related dyspepsia, and Rome II criteria. Regrettably, there was no clear correspondence between the two subcategories of dyspepsia and Rome II criteria. The results showed a lack of association of the -1291C>G polymorphism (currently -1252G>C) in the *ADRA2A* gene promoter and the Del322–325 polymorphism (currently 964_975Del GGGGCGGGGCCG) in the *ADRA2C* gene with FD [45]. Recently, Kushnir *et al.* [46] evaluated whether polymorphisms in *ADRB2* confer a risk for FGIDs. The ADRB2 extracellular domain contains two missense polymorphisms, Arg16Gly (46A>G) and Gln27Glu (79C>G). Gln27Glu leads to a decreased receptor degradation and downregulation, with a consequent enhancement of adrenergic responses. Patients of unspecified ethnicity (n = 170), who met Rome III criteria for at least one FGID (136 FD, 25 functional chest pain and 139 IBS), were included in this study. The status of Gln27Glu G allele carrier was associated with FD (odds ratio [OR]: 2.1, 95% CI: 1.3–3.3). Furthermore, in G allele carriers there was a trend toward functional chest pain (OR: 2.6, 95% CI: 0.9–7.1). By contrast, when analyzing Arg16Gly, the A allele was associated with an increased rate of overall FGIDs [46].

With regard for IBS (Table 1), both *ADRA2A* -1291C>G and *ADRA2C* Del322–325 were found to be associated with C-IBS (mainly Caucasians; 128 D-IBS; 90 C-IBS; 38 mixed diarrhea and constipation, M-IBS; 20 chronic abdominal pain; Rome II) [47].

Table 1. List of candidate genes and related polymorphisms of the adrenergic pathway investigated in patients with functional dyspepsia and/or irritable bowel syndrome.

Gene	SNP rs ID #	Polymorphism	FGID	Finding	Ref.
ADRA2A	rs1800544	-1291C>G (-1252G>C)	FD	No association	[45]
			IBS	Association (C-IBS)	[47]
			IBS	No association	[48,49]
			IBS	Association (D-IBS)	[50,51]
	rs1800035	753 C>G (798C>G)	IBS	No association	[47]
ADRA2C	rs61767072	Del322–325 (964_975Del GGGGCGGGGCCG) [Gly324_Ala327del]	FD	No association	[45]
			IBS	Association (C-IBS)	[47]
			IBS	No association	[48]
ADRB2	rs1042713	46A>G [Arg16Gly]	FD	No association	[46]
			IBS	No association	[46]
	rs1042714	79C>G [Gln27Glu]	FD	Association	[46]
			IBS	No association	[46]
ADRB3	rs4994	190T>C [Trp64Arg]	IBS	Association	[52]
NET	rs121918126	237G>C (1369G>C) [Ala457Pro]	IBS	No association	[47]

Polymorphisms are reported as quoted in the original papers. When applicable, current designations, as reported in the NCBI SNP database [53], are given within round brackets and changes in the amino acid composition of protein products within square brackets.
C-IBS: Constipation-predominant IBS; D-IBS: Diarrhea-predominant IBS; FD: Functional dyspepsia; FGID: Functional gastrointestinal disorder; IBS: Irritable bowel syndrome.

In another study, an association between clinical IBS phenotypes and *ADRA2A* -1291C>G was not observed (unspecified ethnicity; 44 D-IBS, 49 C-IBS, 29 M-IBS; Rome II) [48]. Likewise, in a prospective case–control study on 100 IBS patients (Turkish; 70 C-IBS, 3 D-IBS, 27 M-IBS; Rome III) there was a lack of association of *ADRA2A* -1291C>G with IBS. In this case, the authors hypothesized that the predominant number of C-IBS patients over the very low number of D-IBS patients might explain the lack of statistical significance, since α2-adrenoceptors mediate enteric antisecretory actions and are expected to be involved in the patophysiology of D-IBS [49]. At variance with the above findings, a strong association was observed between *ADRA2A* -1291C>G and D-IBS (Indians; 92 D-IBS, 44 C-IBS, 15 M-IBS; Rome II) [50]. Analogously, a recent study found an association between *ADRA2A* -1291C>G and the overall IBS population (Koreans; 51 D-IBS, 13 C-IBS, 35 M-IBS; Rome III). In addition, these authors showed that G-allele carriers were at higher risk of D-IBS (OR: 5.64, 95% CI: 1.18–27.01) [51].

When considering the *ADRB2* gene, in G-allele carriers of the Gln27Glu polymorphism there was a trend for association with IBS (unspecified ethnicity; 139 IBS; Rome III) (OR: 1.3, 95% CI: 0.9–2.1), while negative results were obtained for Arg16Gly [46]. In a study on the Tpr64Arg polymorphism (190T>C) of the *ADRB3* gene, Onodera *et al.* [52] found that the frequency of the T/C genotype in IBS patients (Japanese; 39 D-IBS, 25 C-IBS, 17 M-IBS; Rome III) was higher, as compared with healthy controls. However, the distribution of C/C, T/C and T/T genotypes did not differ significantly among subgroups with different IBS clinical variants [52].

A retrospective study on the relationship between genetic variations of adrenergic pathways and GI motor functions showed a significant interaction between the C/G or G/G genotype of *ADRA2A* -1291C>G and a higher percentage of gastric emptying in M-IBS patients (undetermined ethnicity; 49 C-IBS, 67 D-IBS, 20 M-IBS; unspecified Rome criteria) [54].

Of interest, some relationships between genetic variations of adrenergic pathways and severity of IBS symptoms have been reported also. Kim *et al.* [47] observed that *ADRA2C* Del322–325, either alone or combined with other polymorphisms in the adrenergic (*ADRA2A* -1291C>G) or serotonergic (5-HT transporter-gene-linked polymorphic region, 5-HTTLPR, of the SERT gene; see below) pathway, was associ-

ated with high somatic symptom scores in patients with IBS and chronic abdominal pain. In the study by Kushnir *et al.* [46] G allele carriers of *ADRB2* Gln-27Glu displayed higher IBS symptom severity, bother and frequency when compared with CC homozygotes. By contrast, within the FD and functional chest pain subgroups, the G allele did not affect the symptom burden. Moreover, when considering the overall population, Gln27Glu G-allele carriers showed a higher burden of extra-FGIDs (i.e., chronic back pain, migraine headache, fibromyalgia and chronic pelvic pain) and a poorer health-related quality of life [46].

Serotonin

The large majority of studies on serotonergic pathways have evaluated possible associations between FGIDs and polymorphisms in 5-HT receptors or SERT (*SLC6A4*) (Table 2). The human SERT gene is characterized by a 44-bp insertion/deletion in the 5′-flanking promoter region (5-HTTLPR; -1950_-1949insT,-1950_-1949insC), which gives rise to a short (S) and long (L) allele. Notably, the S allele is associated with a lower transcription of SERT gene and, as a consequence, lower SERT protein expression and 5-HT reuptake efficiency [55].

Camilleri *et al.* [45] investigated a series of polymorphisms in 5-HT receptors and SERT, and found a lack of association for most of the candidate genes with FD, including *HTR1A* Pro16Leu (47C>T), *HTR1A* Gly272Asp (818G>A), *HTR2A* -1438G/A (currently -998G>A), *HTR2C* Cys23Ser (68G>C) and 5-HTTLPR [45]. In The Netherlands, FD patients displayed no association with the 178C>T polymorphism (currently -24C>T, and previously designated also as -42C>T) in the A-subunit of *HTR3A* or 5-HTTLPR (n = 112; Rome II) [13]. However, in a Japanese study, a significant association between the L allele of 5-HTTLPR and PDS was reported (42 PDS, 39 EPS; Rome III) [56]. Arisawa *et al.* [60] investigated the associations between FD (Japanese; 114 EPS, 73 PDS; Rome III) and two SERT polymorphisms: -185A>C (currently -185C>A) in the 5′-UTR and *463G>T (*463T>G) in the 3′-UTR. They considered also a binding site for microRNA 325 (miR-325), designated as pri-miR-325, since there are two polymorphisms (rs5938804; rs5981521) within this region. The authors found no association between FD and SERT polymorphisms (-185C>A, *463T>G). However, rs5981521 T allele and T/T genotype were correlated with an increased risk of FD, and, in patients with SERT polymorphisms, the rs5981521 T allele was correlated to a further risk increment. Overall, these findings suggest that the polymorphism of pri-miR-325 region is associated with FD and interacts with other SERT polymorphisms to further increase the susceptibility of Japanese subjects to FD.

Of interest, Mujakovic *et al.* [67] reported a possible relationship between genetic variations of serotonergic pathways and severity of FD symptoms. In particular, they examined whether *HTR3A* -42C>T and 5-HTTLPR are associated with dyspeptic symptom severity in a Caucasian population (n = 592), and found that -42C>T T-allele carriers were more prevalent among patients with severe dyspepsia (OR: 1.50, 95% CI: 1.06–2.20). This association was stronger in females (OR: 2.05, 95% CI: 1.25–3.39) and patients with 5-HTTLPR L/L genotype (OR: 2.00, 95% CI: 1.01–3.94).

A considerable number of studies have investigated the association of IBS with gene polymorphisms in the serotonergic pathways. Pata *et al.* [64] studied two polymorphisms in *HTR2A*, showing a high incidence of the C/C genotype for 102T>C (currently 102C>T; OR: 7.89) and A/A genotype for –1438G>A (currently -998G>A; OR: 11.14) in 54 Turkish IBS patients (Rome I). Likewise, in a Greek study, an association was found between the A allele and A/A genotype of –1438G>A with IBS. However, no significant association was observed with 102C>T (n = 124; Rome III) [65]. Kapeller *et al.* [66] investigated a number of polymorphisms in the UTRs of *HTR3A* [-42C>T; -25C>T (currently -7C>T); *70C>T (currently *70C>A); *503C>T; Caucasians; 99 C-IBS, 217 D-IBS; Rome II/III] and *HTR3E* (*76G>A, *115T>G, *138C>T, *191T>C; Caucasians; 95 C-IBS, 143 D-IBS; Rome II/III). In this study, *76G>A and -42C>T were associated with D-IBS. However, *76G>A was positively associated only in female patients [66].

Several authors have investigated the association of 5-HTTLPR in the SERT gene with IBS [37], and the results have been assessed by three meta-analysis. In 2007, the meta-analysis of Van Kerkhoven *et al.* [57], which included eight studies covering a total of 1034 IBS patients and 1377 healthy controls (Caucasians and Asians; different IBS criteria), found a negative association. Subsequently, the meta-analysis by Areeshi *et al.* [58], comprising 12 studies on 2068 IBS cases and 2076 controls (undetermined Rome criteria), did not demonstrate a risk of IBS for patients with 5-HTTLPR variants. Interestingly, however, in the subgroup population-based analysis, a reduced risk was found in Americans (OR: 0.685, 95% CI: 0.516–0.908) and Asians (OR: 0.116, 95% CI: 0.068–0.197), but not in Europeans [58]. By contrast, the recent meta-analysis by Zhang *et al.* [59], including 25 studies on a total of 3443 IBS cases and 3359 controls (Rome I/II/III), concluded that the 5-HTTLPR L allele and L/L genotype have a significant impact

Table 2. List of candidate genes of the 5-HT pathway investigated in patients with functional dyspepsia and/or irritable bowel syndrome.

Gene	SNP rs ID #	Polymorphism	FGID	Finding	Ref.
SLC6A4	rs4795541	5-HTTLPR (-1950 -1949insT, -1950 -1949insC)	FD	No association	[13,45]
			FD	Association (PDS)	[56]
			IBS	No association	[57,58]
			IBS	Association (C-IBS; East Asians)	[59]
	rs6354	-185A>C (-185C>A)	FD	No association	[60]
	rs1042173	*463G>T (*463T>G)	FD	No association	[60]
	VNTR	STin2	IBS	No association	[61–63]
	rs25531	179A>G (-1936A>G)	IBS	Association	[62]
HTR1A	rs1800041	47C>T [Pro16Leu]	FD	No association	[45]
	rs1800042	818G>A [Gly273Asp]	FD	No association	[45]
HTR2A	rs6311	-1438G>A (-998G>A)	FD	No association	[45]
			IBS	Association	[64,65]
	rs6313	102C>T	IBS	Association	[64]
			IBS	No association	[65]
HTR2C	rs6318	68G>C [Cys23Ser]	FD	No association	[45]
HTR3A	rs1062613	-42C>T; 178C>T (-24C>T)	FD	No association	[13]
			IBS	Association (D-IBS)	[66]
	rs62625041	-25C>T; 195C>T (-7C>T)	IBS	No association	[66]
	rs62625042	*70C>T (*70C>A)	IBS	No association	[66]
	rs55917640	*503C>T	IBS	No association	[66]
HTR3E	rs56109847	*76G>A	IBS	Association (D-IBS)	[66]
	rs62625045	*115T>G	IBS	No association	[66]
	rs55646809	*138C>T	IBS	No association	[66]
	rs62621663	*191T>C	IBS	No association	[66]

Polymorphisms are reported as quoted in the original papers. When applicable, current designations, as reported in the NCBI SNP database [53], are given within round brackets and changes in the amino acid composition of protein products within square brackets.
C-IBS: Constipation-predominant IBS; D-IBS: Diarrhea-predominant IBS; FD: Functional dyspepsia; FGID: Functional gastrointestinal disorder; IBS: Irritable bowel syndrome; VNTR: Variable number tandem repeats.

on C-IBS development, and that this effect is evident in the East Asian population, but not in Caucasian, Iranian, Turkish and Indian populations.

Three studies have examined another SERT gene polymorphism, designated as STin2 (located in intron 2 and consisting of a variable number – usually 9, 10 or 12 – of nearly identical 17-bp repeats), for which any association with IBS or its clinical variants was not found (undetermined ethnicity, 26 C-IBS, 18 D-IBS, 10 M-IBS, Rome I; mainly Caucasians, 42 C-IBS, 98 D-IBS, 36 M-IBS, Rome II; Caucasians, 99 C-IBS, 97 D-IBS, Rome II) [61–63]. In addition, Kohen *et al.* [62] extended their search to 179A>G (currently -1936A>G), a SNP located immediately upstream of 5-HTTLPR with opposite effects on SERT expression, showing a positive association of the G allele with IBS.

With regard for FGID intermediate phenotypes, Camilleri *et al.* [48] evaluated whether 5-HTTLPR is associated with motor and sensory GI functions in IBS patients and related clinical subgroups (undetermined ethnicity; 49 C-IBS, 44 D-IBS, 29 M-IBS; Rome II). All participants underwent studies of satiation and rectal sensation as well as measurements of gastric volumes and rectal compliance. They observed that 5-HTTLPR L/S and S/S genotypes are associated with increased pain sensation and rectal compliance; thus,

raising the hypothesis that visceral hypersensitivity in IBS can be related to genetic factors [48]. Exploring the association between genetic variations and GI motor functions, Grudell *et al.* [54] found that small bowel transit or colonic transit in IBS patients or healthy controls did not differ when the subjects were stratified by 5-HTTLPR genotypes (undetermined ethnicity; 20 M-IBS, 49 C-IBS, 67 D-IBS; undetermined Rome criteria). On the other hand, the authors showed that L/S and S/S genotypes were associated with greater postprandial gastric volumes in patients with M-IBS.

Some studies have evaluated the relationship between 5-HT genetics and IBS symptom severity. In the study by Pata *et al.* [64] the T/T genotype of 102C>T in *HTR2A* was associated with more severe pain. In another study, the C/C genotype of -42C>T in *HTR3A* was associated with increased anxiety and enhanced amygdala responsiveness during emotional and nonemotional tasks, both in IBS patients and controls. Moreover, among subjects with IBS, this genotype was associated with increased symptom severity [68]. Recently, a cross-sectional study by our group investigated the association of 5HTTLPR with IBS symptom severity (Caucasians; 106 C-IBS, 98 D-IBS; Rome III). The overall mean symptom severity was higher in L/S and S/S than L/L patients. Moreover, when comparing the mean values obtained for each item of symptom score, 'abdominal pain severity' and 'bowel dissatisfaction' were higher in patients with L/S and S/S genotypes [12].

COMT

Genetic variations of COMT are expected to impact on adrenergic and dopaminergic neurotransmission. A common polymorphism in the *COMT* gene is Val-158Met (1947G>A; currently 472G>A, rs4680). The Val/Val genotype results in a three- to four-fold higher enzymatic activity, as compared with Met/Met genotype, while the Val/Met genotype displays intermediate activity [69]. Of note, the Met/Met genotype has been associated also to chronic pain conditions [70], and the Val/Val genotype to anxiety and depression [71].

A Japanese study on 91 patients with FD showed a lower frequency of Met allele in dyspeptic patients, but the difference versus healthy controls was not significant [72]. In this study, dyspeptic symptoms were also divided into nine categories (epigastric pain, epigastric discomfort, early satiation/postprandial fullness, nausea/vomiting, anorexia, heartburn, belching, hypochondriac pain, others), but there was again no association of Val158Met with any of these items [72].

Karling *et al.* [73] observed an association between the Val/Val genotype and IBS, both overall and with different clinical phenotypes (Swedish; 70 IBS; Rome III). However, upon adjusting for gender and age, there was only a borderline association between IBS and Val/Val genotype (adjusted OR: 2.02; 95% CI: 0.95–4.29) and a trend toward a protective action of the Val/Met genotype (adjusted OR: 0.57; 95% CI: 0.28–1.13). Within this IBS population, Val/Val patients reported prospectively more bowel movements, fewer harder stools and more stools after meals than the combined population of Val/Met and Met/Met patients, suggesting an association between the Val/Val genotype and D-IBS [73]. In a recent Chinese study (7 C-IBS, 46 D-IBS, 13 M-IBS; Rome III), there was a higher frequency of the Val158Met A allele in patients with D-IBS, and such polymorphism was more prevalent in patients experiencing IBS symptoms since over 5 years [74].

G protein

G proteins comprise a family of trimeric signaling proteins linked to the superfamily of GPCRs [75]. The strategic position hold by G proteins, in transducing signals triggered by GPCR activation, supports the hypothesis that genetic variations of G-protein subunits may translate into changes in functions of GPCRs linked to aminergic transmitters, in the CNS and/or the GI tract, with consequent impacts on the pathophysiology or clinical presentation of FGIDs [76]. A common polymorphism in the *GNB3*, 825C>T, has been shown to affect intracellular signal transduction, leading to motor or sensory abnormalities in the GI tract [77]. The T allele gives rise to a splice variant associated with enhanced G-protein activation and, thereby, increased receptor-mediated responses [78]. The possible association of 825C>T with FD or IBS has been examined by a number of studies (Table 3).

Holtmann *et al.* [79] found that the distribution of *GNB3* 825C>T genotypes differed in 56 Caucasian patients with FD (40 dysmotility-like symptoms, 16 ulcer-like symptoms, 20 concomitant IBS symptoms; Rome II), as compared with asymptomatic blood donors. Overall, the OR for FD association with C/C was 2.2 (95% CI: 1.4–3.3) versus T/C and T/T genotypes. In particular, the C/C genotype was associated with dysmotility-like, but not IBS or ulcer-like, symptoms [79]. In the study by Camilleri *et al.* [45], both T/T and C/C genotype were associated with FD. In The Netherlands, 112 FD patients (Rome II) referred to tertiary care displayed higher prevalence of the T allele versus healthy controls (OR: 1.60, 95% CI: 1.03–2.49) [13]. Tahara *et al.* [81] reported no association between 825C>T and FD in 89 Japanese patients (unspecified classification criteria), either overall or upon stratification of dyspeptic symptoms in 9 categories (see section above). Of note, among *Helico-*

Table 3. List of findings regarding the *GNB3* polymorphism tested in patients with functional dyspepsia and/or irritable bowel syndrome.

Gene	SNP rs ID #	Polymorphism	FGID	Finding	Ref.
GNB3	rs5443	825C>T	FD	Association	[13,45,79,80]
			FD	Association (Hp-)	[81]
			FD	Association (EPS)	[82]
			FD	Association (PDS)	[83]
			FD	No association	[84]
			IBS	No association	[51,74,84–86]
			IBS	Association (C-IBS)	[80,87]
			IBS	Association	[88]
			IBS	Association (D-IBS)	[80]
			IBS	Association (GI infection +)	[89]

Polymorphisms are reported as quoted in the original papers. When applicable, current designations, as reported in the NCBI SNP database [53], are given within round brackets and changes in the amino acid composition of protein products within square brackets.
C-IBS: Constipation-predominant IBS; D-IBS: Diarrhea-predominant IBS; EPS: Epigastric pain syndrome; FD: Functional dyspepsia; IBS: Irritable bowel syndrome; PDS: Postprandial distress syndrome.

bacter pylori-negative patients, T/T genotype increased the risk of FD (T/T vs C/T+C/C: OR: 3.40, 95% CI: 1.16–9.93) [81]. In a subsequent Japanese study, where FD patients (Rome III) were grouped into either PDS (n = 40) or EPS (n = 43), there was an association between T/T and EPS (OR: 2.00, 95% CI: 1.07–3.76) [82]. On the other hand, Shimpuku *et al.* [83] reported that 825C>T genotype distribution did not differ in FD patients and healthy volunteers (Japanese; 24 EPS, 51 PDS; Rome III), and that there was no association of 825C>T with gastric emptying. However, the authors found a significant relationship between C/C genotype and PDS patients with delayed gastric emptying. Moreover, evaluating the impact of 825C>T with symptoms, the C/C genotype was associated with feeling of hunger [83]. Recently, in a Korean population (167 FD, 60 IBS, 85 overlap of FD and IBS; Rome III), there was no apparent association of 825C>T with the susceptibility to FD, or its overlap with IBS [84]. By contrast, in 102 Korean children (72 with IBS symptom; Rome III), the C/C genotype was associated with FD [80].

Heterogeneous results have been reported in investigations on lower FGIDs. In one study, 825C>T was not associated with lower FGIDs, different IBS presentations, functional abdominal pain, or lower FGID–FD overlap (mainly Caucasians; 82 C-IBS, 94 D-IBS, 38 M-IBS, 19 functional abdominal pain, 159 overlap of lower FGID with FD; Rome II) [85]. In a case–control study, Saito *et al.* [86] observed a negative association of 825C>T with overall IBS or its clinical subtypes (mainly Caucasians; 5 C-IBS, 19 D-IBS, 17 M-IBS; Rome II). By contrast, Lee *et al.* [87] showed that the T allele was associated with C-IBS (Koreans; 12 C-IBS, 51 D-IBS, 31 M-IBS; Rome III). Subsequently, T/T genotype and overall T allele frequencies of 825C>T were found to be associated with IBS in 124 Greek patients (Rome III) [88]. In the study by Kim *et al.* [84], there was no association of 825C>T with susceptibility to IBS. Likewise, two recent studies found no association between 825C>T and overall IBS population (Koreans, 13 C-IBS, 51 D-IBS, 35 M-IBS, Rome III; Chinese, 7 C-IBS, 46 D-IBS, 13 M-IBS, Rome III) [51,74]. Conversely, in the study by Park *et al.* [80] the C/C genotype was associated with D-IBS, while T/T was linked to C-IBS (Koreans; 102 FD, 17 C-IBS, 44 D-IBS, 11 M-IBS; Rome III). When gene–environment interactions in IBS were assessed, significant interactions were observed between GI infections and the T allele of 825C>T [89]. In patients with GI infection, the OR for IBS was 3.9 (95% CI: 1.2–12.7), while an OR of 0.86 (95% CI: 0.65–1.13) was estimated for patients without prior infection (mainly Caucasians; 40 C-IBS, 102 D-IBS, 125 M-IBS; Rome III) [89].

In the study by Camilleri *et al.* [48], the authors evaluated also whether 825C>T impacted on motor and sensory GI functions in IBS patients and related clinical subgroups. Overall, this polymorphism affected only the fasting gastric volume, with T/C and T/T genotypes being associated with a lower volume that might partly explain the reported association of 825C>T with dyspepsia.

Pharmacogenetics of drugs acting on aminergic systems in FGIDs

Clonidine

Clonidine is an α_2-adrenoceptor agonist, approved for clinical use in hypertension, which can also inhibit

intestinal fluid/electrolyte secretion, gastric and colonic tone and colorectal motility and sensation [90]. In IBS patients, clonidine enhances satisfactory bowel relief and improve stool scores, with minimal adverse effects [27]. Since the adrenergic and serotonergic pathways are involved in the control of GI functions, and that both systems exert their regulatory actions through GPCRs, Camilleri *et al.* [91] evaluated the influence of *ADR2A* (-1291C>G), *GNB3* (825C>T) and SERT (5-HTTLPR) polymorphisms on the effects of clonidine on GI sensory-motor functions in healthy humans and IBS patients. To pursue this goal, 40 healthy volunteers and 120 IBS patients (48 C-IBS, 43 D-IBS, 29 M-IBS; Rome II) were treated with clonidine (0.1 or 0.15 mg twice daily) for 6 days. Clonidine reduced gastric satiation and postprandial volumes as well as sensation threshold to rectal distension, while increasing rectal compliance. There were also associations between the responses to clonidine and the investigated polymorphisms for postprandial gastric volume (-1291C>G in *ADR2A* and 5-HTTLPR), rectal sensation of gas (-1291C>G in *ADR2A* and 825C>T in *GNB3*) and rectal compliance to distension (5-HTTLPR). Based on these findings, the authors concluded that genetic determinants may impact on the therapeutic potential of low-dose clonidine on IBS. In particular, IBS patients with *ADR2A* C/G or G/G genotype respond to clonidine with greater changes in postprandial gastric volume, which may translate into an increased amelioration of postprandial fullness and bloating. Likewise, patients with *GNB3* T/C or T/T genotype may experience lower sensations of gas and urgency in response to clonidine.

Selective serotonin reuptake inhibitors

Selective serotonin reuptake inhibitors (SSRIs) comprise a class of drugs, which targets SERT and are employed in the management of depression and other psychiatric disorders. Besides the psychiatric setting, there is a great interest for SSRIs in gastroenterology, since clinical investigations have shown that these drugs can exert beneficial effects in patients with IBS [92]. Although SSRIs are generally better tolerated than tricyclic antidepressants, the former can elicit GI adverse effects, including nausea, with an incidence of up to 40%, which can be severe enough to lead to treatment discontinuation [93].

Sugai *et al.* [94] examined the impact of polymorphisms in *HTR3A* and *HTR3B* and the enzyme CYP2D6 on nausea associated with paroxetine. The investigation was carried out on 78 Japanese psychiatric patients on treatment with paroxetine (from 10 to 40 mg/day), who underwent genotyping for: 195C>T (currently -7C>T) and -24C>T for *HTR3A*; Tyr129Ser (386A>C) for *HTR3B*; **5* and **10* alleles of *CYP2D6*. Tyr129Ser in *HTR3B* was found to increase the incidence of nausea, as also confirmed by logistic regression analysis, which showed that patients with Tyr/Tyr genotype had a 3.95-fold higher risk of developing nausea in response to paroxetine, than patients with the Ser allele. Given the role played by 5-HT3 receptors in the pathogenesis of nausea and vomiting [95], and considering that they are distributed in the enteric and sensory nerve pathways, where they regulate digestive functions and visceral pain [35], the findings by Sugai *et al.* [94] encourage the implementation of future studies, designed to assess the impact of serotonergic polymorphisms on the effectiveness and tolerability of SSRIs in patients with IBS.

Tegaserod

Tegaserod is a partial agonist of 5-HT_4 receptors, able to normalize GI functions by stimulating neurotransmitter release from enteric nerves. The resulting effects include increased intestinal secretions and contractility, enhancement of peristaltic and secretory reflexes and inhibition of visceral afferent responses [96]. After approval of tegaserod for use in women with C-IBS [97], it was withdrawn from market upon request by regulatory authorities, due to reports of adverse cardiovascular events [98]. In clinical trials, a proportion of patients was found to respond poorly to tegaserod [99], this variability likely depending on genetic factors. To address this issue, Li *et al.* [100] investigated the relationship between the efficacy of tegaserod in 41 C-IBS Chinese patients and SERT 5HTTLPR. After tegaserod administration (6 mg twice daily) for 4 weeks, responder rates were 85% and 70% in patients with S/S and L/S genotype, respectively. These rates differed significantly from the rate of 36.4% in L/L patients. Consistently with this observation, symptom improvements, increments of bowel movements and decrease in stool consistency were significantly greater in individuals with S/S and L/S than L/L genotype. Moreover, over the third and fourth week of treatment, the scores for abdominal pain/discomfort, bloating and number of complete bowel evacuations were higher in S/S or L/S than L/L. To explain these findings, the authors speculated that a decreased serotonergic neurotransmission (consequent to the enhanced 5-HT uptake in L/L subjects) likely results in an altered 5-HT_4 receptor affinity and sensitivity in response to endogenous 5-HT or exogenous tegaserod. However, besides its partial agonistic activity on 5-HT_4 receptors, tegaserod can also act as a SERT blocker [101], and therefore such pharmacodynamic property might account for the association between tegaserod efficacy and 5HTTLPR variants.

Alosetron

5-HT_3 receptors mediate stimulant effects of 5-HT on colonic motor functions during the postprandial period. However, in patients with IBS this bowel response is often associated with the occurrence of symptoms, such as abdominal cramping, stool urgency and diarrhea [102]. Alosetron, a selective 5-HT_3 receptor antagonist, was developed for the management of these symptoms, and clinical studies showed that this drug was able to relieve abdominal pain and discomfort as well as to improve bowel motor dysfunctions in women with D-IBS, even though similar benefits were not obtained in male patients [103]. In 2000, alosetron was approved in USA for treatment of D-IBS in women. However, it was withdrawn in 2002, due to reports of severe adverse effects, including ischemic colitis and severe constipation, and reintroduced later on under a restricted prescribing program [104].

In clinical studies, alosetron slowed colonic transit to greater extents in females than in males. However, this transit response was inhomogeneous in a gender-independent manner, thus suggesting the involvement of other sources of variability [105]. To address this issue, Camilleri *et al.* [106] investigated a possible association of 5HTTLPR variants with the effects of alosetron on colonic transit in 23 European American men and women with D-IBS (Rome I). Patients were treated with alosetron (1 mg twice daily) for 6 weeks, and their GI and colonic transit were assessed both at baseline and during the last week of therapy. The results showed a greater slowing of colonic transit in L/L than L/S or S/S patients. Likewise, there was a higher frequency of high responders to alosetron among L/L than L/S or S/S patients. A possible explanation for these findings is that L/L individuals, being characterized by higher SERT expression, and thereby by enhanced 5-HT reuptake, would display lower synaptic 5-HT levels, with consequent less competition between endogenous 5-HT and alosetron for binding to 5-HT_3 receptors. Along the same line, the L allele might promote an excess of colonic transit inhibition by alosetron, leading to episodes of severe constipation, even though evidence supporting this hypothesis is lacking.

Domperidone

Domperidone is a DA type D_2 receptor antagonist, acting both as antiemetic and prokinetic, owing to its effects on chemoreceptor trigger zone and upper GI motor functions. Despite its antagonism on D_2 receptors, domperidone is associated with a very low risk of neurological adverse effects, since it has a minimal ability of crossing the blood–brain barrier. Accordingly, it is characterized by a favorable safety profile upon long-term oral administration [107]. Oral domperidone, although available in several Countries, is not approved by US FDA, but can be obtained through an investigational new drug application [108].

Domperidone can be employed for treatment of gastroparesis and the management of gastric symptoms, particularly nausea and vomiting, associated with FGIDs [107]. Parkman *et al.* [109] evaluated 48 patients with gastroparesis (mainly Caucasians), under oral treatment with domperidone (from 10 to 30 mg QID), for an average period ranging from 2 months to 2 years, to identify associations with 14 polymorphisms in seven candidate genes: *ABCB1* (drug transporter); *CYP2D6* (drug metabolism); *DRD2* (DA D2 receptor); *KCNE1*, *KCNE2*, *KCNH2*, *KCNQ1* (voltage-gated potassium channels). These authors examined also associations with 17 polymorphisms in the promoter regions of α_1-adrenoceptors: five in *ADRA1A*, three in *ADRA1B* and nine in *ADRA1D*. Associations were assessed in terms of domperidone effectiveness in controlling gastroparetic symptoms, dose levels required to ensure efficacy and adverse effects. Overall, polymorphisms in *KCNH2* (rs3815459) were associated with domperidone effectiveness, and its efficacious dose was affected by *ABCB1* (rs9282564) variants. The association with rs6277 (957C>T, Pro319Pro) in *DRD2* approached, but did not reach, statistical significance. Moreover, only four polymorphisms in the promoter of *ADRA1D* (rs6107461, rs6084673, rs6052462, rs4813680) were associated with effectiveness, dose level and adverse effects of domperidone. The latter finding was not unexpected, since recent studies have identified α_{1A}-, α_{1B}-, and α_{1D}-adrenoceptors as candidate targets for domperidone, with particular regards for its potential of eliciting adverse cardiovascular effects [110]. Accordingly, a possible explanation could be that functional polymorphisms in the *ADRA1D* promoter can affect the level of encoded receptor protein [109].

Metoclopramide

Metoclopramide is a prokinetic drug approved for management of gastroparesis, where it is often employed as first-line agent, although with high levels of variability in the clinical response and susceptibility to develop adverse effects. The prokinetic effect of metoclopramide is ascribed to its antagonism on inhibitory dopamine type D_2 receptors and agonism on excitatory 5-HT_4 receptors in the GI tract. It also exerts antiemetic effects through the blockade of D_2 and 5-HT_3 receptors in the CNS. Owing to these pharmacodynamic properties, metoclopramide is commonly employed also for the management of other conditions associated with nausea and vomiting, such as pregnancy [111]. The interaction of metoclopramide

with central DA receptors accounts for the majority of its neurologic adverse effects, which are mainly extrapyramidal in nature, and the US FDA has issued a black box warning to advice prescribers on the risk of tardive dyskinesia [111].

Parkman *et al.* [112] examined the efficacy and adverse effect patterns of metoclopramide in a cohort of 100 patients (mainly white Caucasians), who underwent genotyping for 20 SNPs in eight candidate genes: drug transporter (*ABCB1*); α_{1D}-adrenoceptor (*ADRA1D*); drug metabolism (*CYP1A2*, *CYP2D6*); DA D2 and D3 receptors (*DRD2*, *DRD3*); 5-HT4 receptor (*HTR4*); voltage-gated potassium channels (*KCNH2*). As remarked by Camilleri and Shin [113], in this study, the overall definition of efficacy did not provide sufficient information on individual symptoms, such as nausea, fullness and early satiety. In addition, adverse events were clustered as neurological, cardiovascular, musculoskeletal and endocrine effects, without information on specific outcomes, such as tardive dyskinesia. A decrease in metoclopramide efficacy was found to be associated with polymorphisms in *KCNH2* (rs1805123) and *ADRA1D* (rs2236554, *129A>T), while adverse effects were associated with polymorphisms in *CYP2D6* (rs1080985, rs16947, rs3892097), *KCNH2* (rs3815459) and *HTR4* (rs9325104, 353+4854T>C) [112]. Although the association of *129A>T in *ADRA1D* with reduced efficacy and 353 + 4854T>C in *HTR4* with adverse effects remain unclear, there was a trend toward an association of the same *HTR4* polymorphism with efficacy in response to metoclopramide. Since 5-HT4 receptors mediate stimulant actions of 5-HT on GI propulsive motility [35], it is conceivable that the 353+4854T>C variant of *HTR4* is associated with a gain of 5-HT4 receptor function, that might enhance the efficacy of metoclopramide in stimulating gastric motility. Of note, D3 receptor polymorphisms predispose to tardive dyskinesia in patients treated with antipsychotics [114]. However, in the study by Parkman *et al.* [112], rs7625282 (271–2813T>C) in *DRD3* was not associated with metoclopramide adverse effects, thus suggesting the need for further investigations on metoclopramide-induced dyskinesia

In a pilot study, Lehmann *et al.* [115] evaluated possible associations between the efficacy of different antiemetic medications, including metoclopramide, in the control of nausea and vomiting in pregnancy with polymorphisms in HTR3A and HTR3B subunits. A group of 28 pregnant women (9 Caucasians, 17 Afro-Americans, 2 Hispanic), receiving metoclopramide, promethazine, prochlorperazine or ondansetron for nausea and vomiting, underwent assessment of symptom severity and quality of life, both at baseline and after 1 week of therapy. They were also genotyped for: *HTR3A* (-42C>T, associated with increased receptor expression); *HTR3B* 386A>C (Tyr129Ser, associated with enhanced receptor response to 5-HT); intronic rs3782025 (696+3792G>A); rs3831455 (-104_-102delAGA, currently designated as -106_-104delGGA, associated with changes in binding of nuclear proteins to promoter). Women with *HTR3B* 386A>C C/C genotype (i.e., enhanced receptor response to 5-HT) were found to require more antiemetic medication, suggesting a condition of scarce drug sensitivity. Patients with *HTR3A* -42C>T C/T and T/T genotypes (i.e., increased receptor subunit expression) displayed a reduced symptom improvement to anti-emetic drugs, while those with *HT*R3B 696+3792G>A variant alleles had less severe symptoms. These findings, although preliminary in nature and generated in the setting of pregnancy, encourage more extensive studies in patients taking metoclopramide to manage nausea and vomiting.

Conclusion & future perspective

Abnormalities in aminergic transmitter pathways, particularly NA and 5-HT, are thought to play a relevant role in the pathophysiology of FGIDs. On this basis, efforts are being made to assess whether a number of polymorphisms in candidate genes pertaining to amine mediators are pathogenically associated with FGIDs (particularly FD and IBS), their intermediate phenotypes, clinical presentations and/or symptom severity. Moreover, since several drugs employed in the management of FGIDs act on these transmitter pathways, the potential impact of pharmacogenomics on therapeutic efficacy and/or safety of medical therapies has raised attention.

According to the present overview, with very few exceptions, conclusive evidence, supporting a role of genetics and pharmacogenetics of aminergic pathways in FGIDs, is lacking. Among the reasons likely accounting for the inconsistencies in current knowledge, it is important to consider: the heterogeneity of pathophysiological mechanisms underlying FGIDs, where a number of different environmental and genetic factors contribute, at different extents, as predisposing conditions; of note, FD and IBS comprise different clinical phenotypes, likely underscoring different pathogenic mechanisms; the clinical classifications of FGIDs, which have been subjected to substantial changes over the years (i.e., Manning, Rome II, Rome III) [1,116,117], thus generating differences in the selection criteria for patient enrolment in genetic and pharmacogenetic investigations; the small size of patient populations included in the majority of studies, in conjunction with ethnic differences of the study populations evaluated for the same polymorphisms in different investigations.

In order to appraise critically the heterogeneous results available for the genetics of aminergic pathways in the setting of FGIDs, one should take into account the gen-

eral pitfalls affecting genetic association studies, particularly in the field of FGIDs, which may contribute to conflicting findings. First, FGIDs likely represent complex 'genetic diseases' resulting from multiple variants of different genes, with relevant influences from environmental factors. Thus, a source of study error involves the relatively small effect of various genetic/environmental factors that contribute to FGID development. On the other hand, such multifactorial etiology underlies very heterogeneous patient groups, including various clinical presentations/severity and intermediate phenotypes. Furthermore, FGIDs are characterized by fluctuation of different phenotypes over time (for instance, IBS-C patients may convert into IBS-D patients or *vice versa*), and overlaps among different FGIDs are known to occur [118]. In addition, there is a lack of gold standard in the diagnostic evaluation of FGIDs, which implies patient selection bias. Genetic associations can be influenced also by other factors, such as the important role of ethnic diversity. Indeed, the evolutionary history of haplotypes and linkage disequilibrium patterns will vary significantly in different ethnic populations. At last, failure in attempting study replications, failure in detecting linkage disequilibrium with adjacent loci and the selection of poorly matched control groups, often retrospectively defined in case–control studies, can represent common errors in association studies [119].

Despite the above limitations, there is evidence allowing to draw some tentative conclusions. With regard for NA pathways, there is no apparent association of polymorphisms in *ADRA2A*, *ADRA2C*, *ADRB2*, *ADRB3* and *NET* genes with overall FD or IBS. However, few studies suggest an association of *ADRA2A* and *ADRA2C* with IBS clinical phenotypes. Moreover, some interesting relationships between genetic variations of adrenergic pathways and IBS symptom severity have been reported. When considering the serotonergic system, the most updated meta-analysis points out a positive association of 5-HTTLPR with C-IBS in the East Asian population. Of interest, some studies suggest an influence of 5-HTTLPR genotypes on IBS intermediate phenotypes (i.e., motor and sensory GI functions) or symptom severity, with particular regard for abdominal pain. Conversely, most evidence argues against an association of 5-HTTLPR with FD. Moreover, the majority of available data show no association of polymorphisms in 5-HT receptor genes, including *HTR1A*, *HTR2A*, *HTR2C*, *HTR3A* and *HTR3E*, with FD or IBS. Studies examining the *GNB3* 825C>T polymorphism have yielded heterogeneous evidence in the setting of IBS and its clinical phenotypes, with about 50% of investigations not showing any association with this syndrome. Likewise, in the case of FD, the findings reported by different studies were also heterogeneous, since associations were shown with different genotypes of the 825C>T polymorphism and/or with different phenotypes of this disorder.

Current knowledge on the pharmacogenetics of drugs acting on aminergic systems is limited and extremely heterogeneous, with some preliminary evidence suggesting that polymorphisms of aminergic genes may affect the efficacy or tolerability of α2-adrenoceptor agonists, SSRIs and prokinetics in patients with FGIDs.

Based on current data, some issues deserve attention and should be investigated further by means of large-sized, prospective and homogeneous studies on patients conforming to updated classification criteria for FD and IBS. This represents an essential step to achieve a more extensive and solid understanding of the roles that polymorphisms in candidate genes, including those related with aminergic pathways, may play on FGIDs and the activities of drugs employed for their management. Further suggestions to better design future genetic studies include the following: to evaluate associations with FGID clinical phenotypes, symptom severity and intermediate phenotypes, as well as to stratify FGID patients on the basis of comorbidities; to include in the analysis gene–environment interactions and studies of epigenetics (i.e., DNA methylation); to foster the use of newer genome-wide association studies and the newest techniques involving deep sequencing to identify less common variants; replication of allelic associations; and avoidance of population stratification (such as by means of family-based controls) [9,119]. At last, comprehensive meta-analyses of similar studies should be performed whenever possible, as indeed it has been done in the case of the serotonergic system, pointing out a positive association of 5-HTTLPR with C-IBS within the East Asian population. In this regard, future investigations are warranted to verify the actual impact of 5-HTTLPR on IBS intermediate phenotypes and/or clinical symptom severity. Along the same line, specific clinical studies should ascertain whether this SERT polymorphism might influence the responses of IBS patients to antidepressants (particularly SSRIs) or other serotonergic drugs. Likewise, efforts should be made to achieve more consistent evidence on the relevance of *GNB3* gene polymorphism in FD and IBS, as well as to assess whether it may account for variability in the responses of patients to drugs employed for FD and IBS symptom management.

At last, considering the relevance of amine transmitters in the control of gut functions (motility/secretion/sensation) and pathophysiology of FGIDs, the significance of gene polymorphisms pertaining to aminergic pathways should be evaluated also for the therapeutic activity of novel drugs, recently approved or currently under clinical development for the management of FGIDs, including those molecules not directly targeting the aminergic systems.

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Executive summary

Role of aminergic transmitter systems in gut physiology & pathophysiology of functional gastrointestinal disorders

- Noradrenaline (NA), serotonin (5-HT) and dopamine (DA) play pivotal regulatory actions on various gut functions and visceral sensation, both at central and peripheral level.
- NA is a neurotransmitter in the CNS and sympathetic nerves supplying the GI tract. Alterations of NA pathways have been implicated in the pathophysiology of functional gastrointestinal disorders (FGIDs).
- 5-HT regulates a number of functions in the CNS and GI tract. In the latter system, 5-HT is involved in the regulation of motor, secretory and sensory functions, with a predominant role in the control of colonic propulsive motility. Abnormalities of serotonergic pathways can contribute to the pathophysiology of FGIDs, with particular regard for irritable bowel syndrome (IBS).
- DA acts as neurotransmitter in the CNS and mesenteric organs, including the GI tract, where it takes part mainly in the inhibitory regulation of motor functions. Information on the possible role of DA in the pathophysiology of FGIDs are very scarce.

Genetics of aminergic transmitter pathways in FGIDs

- A number of clinical investigations have evaluated the association of polymorphisms in candidate genes pertaining to the pathways of amine transmitters with FGIDs (particularly functional dyspepsia [FD] and IBS) as well as their intermediate phenotypes, clinical presentations or symptom severity.
- Current evidence on the genetics of NA pathways in the setting of FGIDs are quite heterogeneous. Apparently, there is no association of polymorphisms in *ADRA2A*, *ADRA2C*, *ADRB2*, *ADRB3* and *NET* genes with FD or IBS, overall. However, few studies suggest an association of *ADRA2A* and *ADRA2C* with IBS clinical phenotypes, and some relationships between genetic variations of adrenergic pathways and IBS symptom severity have been reported.
- With regard for serotonergic pathways, the most updated meta-analysis points out a significant association of 5-HTTLPR in the gene coding for 5-HT transporter (SERT) with constipation-predominant IBS in the East Asian population. Some studies suggest an influence of 5-HTTLPR on IBS intermediate phenotypes or clinical symptom severity, with particular regard for abdominal pain.
- The majority of available data do not support associations of polymorphisms in 5-HT receptor genes, including *HTR1A*, *HTR2A*, *HTR2C*, *HTR3A* and *HTR3E*, with FD or IBS.
- Studies examining 825C>T polymorphism in the gene coding for the G-protein β_3 subunit (*GNB3*) have generated heterogeneous evidence supporting its significant association with FD and IBS, both overall and within the different clinical phenotypes of these digestive disorders.

Pharmacogenetics of drugs targeting the aminergic pathways in FGIDs

- Current knowledge on the pharmacogenetics of drugs targeting the aminergic systems is limited and extremely heterogeneous, with some preliminary evidence suggesting that polymorphisms of aminergic genes may influence the efficacy or tolerability of α_2-adrenoceptor agonists, selective serotonin reuptake inhibitors (SSRIs), and prokinetics in patients with FGIDs.
- An interesting trend toward an association of *HTR4* 353+4854T>C with metoclopramide efficacy has been reported, suggesting that this polymorphism is associated with a gain of 5-HT_4 receptor function that might enhance the efficacy of metoclopramide in stimulating gastric motility.

Conclusion & future perspective

- Conclusive evidence, supporting a role of genetics and pharmacogenetics of aminergic pathways in FGIDs, is lacking, most likely as a consequence of heterogeneity in the pathophysiological mechanisms and classification of FGIDs as well as small size of study populations and differences in selection criteria and ethnical origins.
- Despite current limitations, some lines of evidence point out a significant association of 5-HTTLPR in the serotonergic pathway with IBS, and the *GNB3* gene polymorphism with both FD and IBS.
- Additional studies are warranted to confirm the significance of some gene polymorphisms in the aminergic transmitter pathways (e.g., 5HTTLPR and *GNB3*) in the pathophysiology and clinical appearance of FGIDs, as well as to assess whether these polymorphisms have a potential impact on the therapeutic efficacy and/or safety of drug therapies employed in FD and IBS.

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