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ARE FUNCTIONAL GI DISORDERS GASTROENTEROLOGICAL OR NEUROLOGICAL DISEASES?

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[I disturbi funzionali gastrointestinali sono patologie gastroenterologiche o neurologiche?]

SUMMARY

Functional Gastrointestinal Disorders (FGIDs) are chronic ailments that interest million of subjects on a daily basis.

FGIDs are characterized by recurrent symptoms that indicate a dysfunctional GI tract despite that an organic etiopathogenesis cannot be identified on diagnostic studies.

Although the precise etiology of these disorders remains unknown, there is a growing body of evidence to support genetic, infectious, psychosocial and neurologic factors which contribute to the developing of these pathologies.

Family studies provide strong evidence for a clustering of FGIDs in families and this point towards the role of some hereditary factors and polymorphism of adrenergic, opioidergic and serotonergic receptors as well as G-protein-beta3 and serotonin receptors are suitable mechanisms.

Acute GI infection and mucosal inflammation appear trigger a cascade of events that ultimately results in the manifestation of FGIDs, and is reasonable to assume that functionally relevant polymorphisms of genes with immunomodulating and neuromodulating features might play a role.

The disturbed central processing of abnormal sensory afferent signals and visceral hypersensitivity play a major role, while stress and psychiatric disturbs could have a remarkable comorbidity role, but they cannot be the only cause of symptoms. FGIDs have been relegated to the "prison" of the psychosomatic diseases and identified exclusively by symptoms.

A growing body of evidence seems demonstrate that FGIDs could be organic, multifactorial diseases and that the nervous system and its components play a fundamental role in their pathophysiology.

Key words: Functional Gastrointestinal Disorders (FGIDs), Central Nervous System (CNS), Enteric Nervous System (ENS), Genetics, Infectious GI diseases, Inflammation, Neuroimmunomodulation

RIASSUNTO

I Disturbi Funzionali Gastrointestinali (DFGI) sono patologie croniche che coinvolgono quotidianamente milioni di persone.

Essi sono caratterizzati dalla ricorrenza dei sintomi, indice di una alterata funzionalità del tratto gastrointestinale anche se non ne è ancora nota una etiologia organica.

A tutt'oggi non è ancora conosciuta una precisa eziologia di questi disturbi tuttavia è sempre più evidente il ruolo svolto da fattori genetici, infettivi, psicosociali e neurologici.

Studi su gruppi familiari, hanno identificato un clustering per le malattie funzionale dell'apparato digerente, per le quali sono importanti sia i fattori ereditari che il polimorfismo di recettori adrenergici, oppioidi e serotoninergici.

Si ritiene che infezioni acute del tratto gastrointestinale e la flogosi della mucosa possano essere fattori scatenanti di una cascata di eventi che determinano le manifestazioni cliniche dei DFGI per i quali potrebbe svolgere un ruolo fondamentale il polimorfismo di geni immunomodulanti e neuromodulanti.

L'anormale riconoscimento di segnali sensitivi afferenti e l'ipersensibilità viscerale potrebbero essere fattori determinanti per i DFGI, mentre lo stress ed i disturbi psichiatrici anche se possono avere un importante ruolo nella comorbidità, non possono essere l'unica causa dei sintomi. Attualmente i DFGI sono classificati come una patologia psicosomatica e come tale identificata esclusivamente dai sintomi.

Tuttavia crescenti evidenze scientifiche sembrano dimostrare che i DFGI potrebbero essere delle patologie organiche, multifattoriali nelle quali il sistema nervoso e le sue differenti componenti potrebbero svolgere un ruolo fisiopatologico fondamentale.

Parole chiave: *Disturbi Funzionali Gastrointestinali (DFGI), Sistema Nervoso Centrale (SNC), Sistema Nervoso Enterico (SNE), Genetica, Infezioni Gastrointestinali, Infiammazione, Neuroimmunomodulazione*

Introduction

Despite a worldwide and continuous increasing interest in Functional Gastrointestinal Disorders (FGIDs) by researchers, clinicians as well pharmaceutical industry, the precise etiology of these disorders remain substantially unknown. On the other hand, it is very important to focus on FGIDs because they are chronic disorders that result in a great deal of disability and in a significant burden for patients, physicians, healthcare providers and employers.

Epidemiologic data on FGIDs report that they are characterized by recurrent symptoms (i.e. heartburn, abdominal pain or discomfort, bloating, nausea, vomiting, early satiety, constipation or diarrhea) that indicate one or more dysfunctions in the GI tract despite that an organic reason for the symptom generation could not be demonstrated by diagnostic studies.

It is reputed that about 40% of all gastroenterology clinic visits are for FGIDs[1] and a more recent survey of generalists and gastroenterologists demonstrated that nearly one third of their patient's population had symptoms referred to Irritable Bowel Syndrome (IBS).

Characteristically, many patients with FGIDs have overlapping symptoms like dyspepsia, pain, IBS symptoms, heartburn or bloating, suggesting common pathophysiologic mechanisms.

However, the symptoms are typically chronic in nature, but they wax and wane, are worsened by meals and are often induced or aggravated by psychosocial stressors or by total life event stress.

Symptoms of FGIDs can vary over time and a subset of people appear to cycle in and out of symptoms, probably depending on changing of environmental exposure and psychological distress, and on central or peripheral neuroplasticity.

The complexity of neural mechanisms involved in regulating and organizing gut functions is reflected in very articulated and complex symptomatology associated with multifactorial disorders.

As neurobiology and neurophysiology will continue to provide new mechanistic insights, there is a need for improved diagnostic tools in clinical neurogastroenterology in order to advance our knowledge on pathogenesis and to realize rationally targeted therapy.

This article reviews some qualifying aspects of etiology and pathophysiology of FGIDs in the light of most interesting and newest observations concerning the connections and interactions between the different parts of nervous system, the gut, the inflammation and related neuroimmune response, psychological factors, motility and visceral hypersensitivity.

Putative aetiologic and pathophysiologic factors

Traditionally FGIDs have been considered as disorders typified by disturbed gastrointestinal motility, visceral hypersensitivity and psychosocial factors leading to various and often coexisting symptoms. Our understanding about the connections existing between the different pathophysiological alterations and various symptoms referred by patients is very lacking.

In the last years many studies have provided clear evidence that in FGIDs an altered interplay exists between the different parts of the nervous system along the so-called "brain-gut axis".

Besides, immunological changes, post-infection low grade inflammation, abnormal levels of GI neuropeptides and hormones, as well as genetic factors might play a fundamental role in the genesis of FGIDs. The researchers and clinicians turn their effort to understanding how classical pathophysiological factors, such visceral hypersensitivity, altered motility and psychosocial factors could be correlate and integrate with the most recent scientific findings and progresses.

Genetics

Observational and clinical studies seem to suggest a genetic contribution to the development of FGIDs, because they run more frequently in families where the parents suffer for dyspepsia, IBS or heartburn. Twin studies demonstrated concordance rates for FGIDs of 33% for monozygotic twins and 13% for dizygotic twins[2], but the concordance rate was higher between mother and child when compared with monozygotic twins suggesting a social learning effect on the development of IBS[3].

Other researchers[4] observed that the presence of dyspepsia and IBS in a patient was significantly associated with reporting of the same symptoms in first-degree relatives, suggesting a familial link.

A recent study found that the polymorphisms in the promoter region of the serotonin reuptake transporter gene are significantly associated with IBS with diarrhea[5]. Moreover, in a case-control study was observed that individuals homozygous for a G protein beta-3 subunit gene polymorphism were more likely to have unexplained upper abdominal symptoms and FGIDs[6] and various preliminary findings also suggest that FGIDs may be associated with other selected gene polymorphism, including those in IL-10, alpha adrenoceptor, and serotonin reuptake transport (SERT)[7,8,9].

It is unlikely that a single genetic factor causes FGIDs, it is rather more likely that a genetic factor/s modulates the risk of developing the abnormalities that are characteristic of FGIDs after exposure to one or more environmental factors.

Genes of serotonergic system

Serotonin (5-hydroxytriptamine, or 5-HT) modulates sensory and motor function in the gastrointestinal tract. Seven classes of 5-HT receptors have been identified on the basis of differences in structure, molecular action and function and in particular the 5-HT type 3 receptor is involved in mediation of postprandial colonic motor function.

In IBS patients stimulation of 5-HT type 3 receptors induces cramps, urgency, diarrhea and high-amplitude, peristaltic, colonic contractions[10].

Individuals who are homozygous or heterozygous for the 5-HT-transporter-gene-linked polymorphic region (5-HTTLPR) deletion, have reduced transcription of serotonin reuptake transporter gene SLC6A4, reduced expression of its product (the sodium dependent serotonin transporter) and reduced reuptake of serotonin[11]. In patients with diarrhea predominant IBS, genotyping for the 5-HTTLPR polymorphism demonstrated an association between polymorphism and colonic transit time.

Interaction of adrenergic and serotonergic receptors

An interaction between serotonin and norepinephrine in the modulation of gastrointestinal function is evident and an association has been observed between functionally distinct genetic polymorphism in *SLC6A4* or genes that encode α_{2A} and α_{2C} adrenoceptors and specific IBS subtype[9]. The AA. suggested that a del322-325 polymorphism in *ADRA2C*, gene that encodes the α_{2C} adrenoceptor, is associated with an increased likelihood of constipation in patients with functional disorders of the lower intestinal tract and with an increased likelihood of a high frequency and severity of somatic symptoms.

Various combinations of polymorphysms in the genes that encode the α_{2A} adrenoceptor and α_{2C} adrenoceptor, or of the del322-325 polymorphism in *ADRA2C* and polymorphisms in *SLC6A4*, have evidenced a high somatic symptom score[9].

Cholecystokinin

This neuropeptide is released within the duodenal and jejunal mucosa in correlation with the presence of various nutrients like protein and fats.

Cholecystokinin provokes pancreatic enzyme secretion, inhibition of gastric empting and contraction of gallbladder.

The actions of cholecystokinin are mediated via two receptors, the CCK-A and CCK-B which are located in the peripheral and central nervous system[12]. At the gastrointestinal level the effects of cholecystokinin are mediated via the receptors CCK-A located in the smooth muscles and in the vagal afferent nerves of the GI tract.

Blockade of CCK-A receptors could be a new approach to stimulate gut motility and to reduce colonic transit time in patients with constipation-predominant IBS. Variations in the genes encoding the cholecystokinin receptors, could change the response to endogenous cholecystokinin.

Genotypes studies in patients with constipation-predominant IBS have revealed that a polymorphism in the CCKAR gene could be functionally significative, because the 779C variant is associated with slower gastric emptying.

Cytokines

Chaudhary and Truelove[13] first suggested that an infection could lead to the development of IBS.

Both retrospective and prospective studies have shown that approximately 10%-30% of individuals with acute gastroenteritis later develop symptoms of IBS[14, 16]. Subsequent data have firmly established that gastroenteritis have a significant role in the genesis of IBS[17,18] and a recent study confirmed that in patients with bacillary dysentery (due to Shigella species), IBS develops significantly more frequently than in controls[19].

Although the definition of IBS states that no active inflammation is present to cause symptoms, recent studies demonstrated that an increased number of mast cells is present in the terminal ileum of patients with IBS, and that patients whose ulcerative colitis is in remission, report IBS-like symptoms [20].

Moreover, the number of T lymphocites and enteroendocrine cells is persistently elevated in patients with postinfectious IBS. The above mentioned data suggest that in these patients there is a continuing inflammatory stimulus with increased gut permeability[17]. Cytokines are involved in the regulation of the immune and inflammatory responses. We know proinflammatory cytokines such as tumor necrosis factor *(TNF)* and interferon γ, and anti-inflammatory cytokines such as interleukin 10 *(IL 10)* and transforming growth factor β1. Study of polymorphisms in the genes that encodes these cytokines has revealed the presence of alleles associated with high or low cytokines production[21].

There is a strong association between *IL10* genotypes and IBS.

The low prevalence of the high-producer genotype in patients with IBS suggest that individuals that produce low amounts of *IL10* could be more prone to develop IBS[22].

SERT expression and genetic factors

Enteric nervous system (ENS) represents the most vast and complex component of peripheral nervous system. The most important functions in the ENS are carried on by 5-HT. The most important source of Serotonin (5-HT) is principally located in the human bowel, primarily in the enterochromaffin cells (EC). 5-Ht is released from EC cells in response to luminal stimuli.

Once released, serotonin acts on receptors located on the processes of sensory neurons that pass into the lamina propria. These include branches of intrinsic sensory neurons whose cell bodies are in submucosal and myenteric ganglia, sensory neurons in spinal (dorsal root) ganglia and vagal (nodose) ganglia.

When serotonin is released from EC cells provokes activation of vasodilatory, secretory and motor reflexes, as well as stimulation of afferent signals to the spinal cord and brain.

The nerve terminals that release serotonin, express a serotonin selective reuptake transporter (SERT), very effective in terminating the signals that they initiated. Intestinal mucosa has an enormous capacity to remove the 5-HT from the interstitial space because all the cells in the luminal surface of the gut express SERT. In the postprandial phase, serotonin enters the bloodstream, where it is removed by platelets which also expresses SERT.

The expression of SERT seems to be influenced in relation to the individual genetic makeup[23] and the activation of G-protein-coupled receptors can provoke changes in SERT by modulating its transcription by moving the transporter to or away from the plasma membrane, so genetic factors might modify SERT expression and function in the intestinal epithelium[24]. The variability in SERT expression depends on SERT gene-linked polymorphic region (SERT-LPR)[25].

However, not all studies demonstrated a correlation between SERT-LPR alleles and SERT expression. Moreover, analysis of mRNA levels failed to detect a correlation with SERT-LPR genotype[26], between SERT-LPR alleles and promoter activity[27]; no significant correlation was observed between SERT-LPR and binding of SERT ligands in the brain of healthy volunteers[28].

The relation between SERT-LPR and FGIDs, in particular IBS, is not firmly defined, because the studies on this topic are somewhat contradictory[5, 29-32].

In general these studies demonstrated a link between SERT-LPR and specific subtype of IBS, but not in IBS as a whole, suggesting the idea that an universal etiology for IBS does not exist. To date is not possible to draw a definitive conclusion about the genetic predisposition to develop IBS, but the above preliminary data seem to be promising. Observation that functional dyspepsia (FD) runs in families suggest a genetic basis to the development of this disease[4]. A case-control study showed that individuals homozygous for a G protein beta-3 subunit gene polymorphism were more prone to have unexplained upper abdominal symptoms and FD[6].

In conclusion the previous mentioned studies do not establish firmly causality and the data are limited in number. Positive association between genetic variants and disease have to be considered with caution because false-positive results are frequent.

The complex interaction of genes, environment and phenotype will continue to present challenge to researchers. It is likely that future studies will demonstrate that genetics play a significant role in the etiology and pathogenesis of FGIDs, providing an explanation and theoretical framework for the heterogeneity of these clinical manifestations.

Infection and neuro-immune interactions

The enteric nervous system and immune system are most effective defenders of the body's enteric function. Hypersecretion and motility (accelerated transit) represent the preferred ways to defend the organism. The enteric and immune systems are strictly integrated and receptors for enteric neurotransmitters are located on the Peyer's patches and lymphocites are located in the lamina propria.

Motor and secretory response are sensitized to specific antigens (e.g. food, bacterial toxins, parasites and chemical agents) and intestinal inflammation provokes active and integrated gut motility and enteric nervous system reaction. Strict connections exist between the submucosal plexus and myenteric plexus which cohordinate mucosal secretion with propulsive motor behavior in the states of flogosis.

For example, during the enteritis caused by Clostridium difficile toxin A, enteric neurons are excited and sympathetic transmission is blocked.

The immune system and its messengers, the inflammation mediators, sensitise the primary afferents fibres and in particular the C fibre nociceptors, which induce a secondary spinal activation.

This is the so-called "neurogenic inflammation". The messengers of inflammation are calcitonin gene-related peptide, tackykinins, 5-HT, histamine, prostaglandins, leucotrienes, cytokines and mast cells which are directly innerved by projections from the central nervous system and degranulate with the release of many substances like 5-HT, histamine and nerve growth factor. Newer classes of receptors involved in neurogenic inflammation are evolving, for example the cell-surface protease-activated receptors 2, which seems to be related to inflammation, but also to hyperalgesia.

Neurogenic inflammation is reduced by some of the receptor antagonists which compete with the released inflammatory mediators (e.g. neurokinin receptor antagonists). The pathophysiology of IBS is partially understood, but there are various evidence to support both host and environmental factors. The role of enteric infection in the pathogenesis of IBS has been recognized and several studies have evaluated the risk factors for its onset. In particular two recent meta-analysis[33, 34] demonstrated an increase of post-infective IBS (PI-IBS) in subjects who suffered of acute gastroenteritis.

In particular, Thabane & Coll (34), observed a strong association between intestinal infection and IBS with an estimated incidence of 10%. The development of symptoms consistent with IBS and their meta-analysis suggested that the odds of developing IBS are increased about sixfold after acute gastrointestinal infection. The AA. confirmed an association between increasing risk of PI- IBS and younger age and in some way this difference is explained. by the age-gender effect, considered that many functional disorders are more common among females and could be both referred to circulating female hormones and to psychological distress frequent among young women. A recent study[35] outlined that behavioral and cognitive profiles and emotion are significant risk factors for the development of PI-IBS, suggesting an interactive model of psychology and biology, considered that infections may trigger symptoms and psychologic factors may maintain and prolong them over time.

In general, seems that more severe are gastrointestinal infection (bacterial infection, prolonged fever, diarrhea, vomiting), more increased will be the risk of PI-IBS[33, 36].

In the literature are present more limited data on the possible correlation between functional dyspepsia (FD) and infectious illnesses.

Tack[37] described a group of patients with dyspeptic symptoms after prior gastrointestinal infectious disease.

A larger proportion of patients with post infectious dyspepsia had complaints of nausea, vomiting, early satiety and weight loss.

The patients manifested an impaired accommodation of the proximal stomach and sumatriptan, an activator of nitrergic neurons, did not cause fundic relaxation in the patients with post infectious dyspepsia. This observation indicates a dysfunction at the level of gastric nitrergic neurons in these patients, leading to impaired accommodation.

Several, well-controlled studies did not demonstrated significant relationship between FD and Helicobacter pylori infection. Sarnelli et al.[38] demonstrated that H pylori infection was not associated with FD with respect to symptoms, rates of gastric empting, degree of gastric relaxation, or sensitivity to gastric distension. Many other studies failed to demonstrate a symptomatic benefit in patients eradicated[39, 40], but a recent Cochrane database meta-analysis concluded that eradication of H pylori led to a small but significant improvement of dyspeptic symptoms[41].

There is a sufficient evidence that prior infectious disease may predispose the patient to develop FGIDs and that exposure to a viral or bacterial infection inducing an inflammation, could provoke an alteration in function of enteric nervous system. Inflammation is very useful for survival but also can induce considerable morbidity and mortality.

The inflammation has profound effects on neuronal function and inflammatory neuropathy is becoming an important issue in neurogastroenterology[42]. Inflammation of enteric ganglia in different tracts of gastrointestinal tube by the infiltration of inflammatory cells produces dysfunction and degeneration which over time can lead to aganglionosis and severe motor disturbances. Another important role is played by glia cells which are much more frequent of neurons (4:1) and have a fundamental role in protecting the integrity of the gut.

Enteric glia are strictly associated with neurons in the enteric nervous system (ENS) and display morphologic and molecular similarities to the central nervous system (CNS) astrocytes, because like them are actively participants in neuronal communication by neurotransmitter receptors.

In the ENS the purine adenosine triphosphate (ATP) is co-released with the neurotransmitter acetylcholine and noradrenaline. Enteric glia express purinergic receptors and respond to ATP in vitro suggesting that enteric glia actively participate in functional gastrointestinal response to nerve signaling.

Gulbransen and Sharkey[43] very recently demonstrated that exists a functional purinergic neuron-glia communication in the enteric neuron system, suggesting the possibility that ATP released with neurotransmitters during enteric synaptic transmission functions as a signal to enteric glia.

Aube et al.[44] used a transgenic approach to disrupt glia in order to examine changes in the phenotype of enteric neurons and functional consequences for intestinal motility and permeability.

The study showed that glia disruption induces changes in the neurochemistry of enteric neurons leading to altered motility and increased epithelial permeability as a consequence respectively of decreased of vasoactive intestinal peptide (VIP) and substance P expression in submucosal plexus and a decreased nitric oxide synthesis with concomitant increase of choline acetyltransferase expression in the myenteric plexus[44]. Inflammation activates enteric glia cells and they play a role in antigen presentation and cytokine production and in presence of IL-1b, TFN-α, lipopolysaccaride and proinflammatory cytokines, their number is increased[45].

Anti-inflammatory citokines are useful in controlling the intensity of inflammation, but when pro-inflammatory cytokines are unchecked they can produce dramatic consequences. These studies suggest that the alterations in glia functions impair the gastrointestinal mucosal barrier system and in concert with enteric nerves may be involved also in cancer and inflammatory bowel disease (IBD)[46].

Vagus nerve plays an interesting role in anti-inflammatory mechanisms. In fact, acetylcholine released within the gut wall, following vagal stimulation, block nicotine receptors on resident macrophage to inhibit their production and release of pro-inflammatory TNF-α. The intriguing question is: what links the vagus and macrophage in the gut wall? De Jonge et al.[47] demonstrated that cholinergic neurons come into close proximity with macrophages in the rat ileum. These neurons could be vagal efferent fibres, but probably are of enteric neural origin[48].

De Jonge[47] suggests that surgical manipulation provokes macrophage activation and recruitment that lead to the release of proinflammatory cytokines. Specifically, vagal stimulation suppress the inflammatory response via a Jack2 STAT3 signaling pathway with an action of phosphorilated STAT3 on its DNA response elements. The nicotinic receptor mediating the immune-suppressive effect, was the α7 subtype, distinct from the α3β4 subtype that typifies ganglionic transmission in the ENS.

This could point to a direct action of pre-ganglionic vagal efferents on macrophage function or a neuro-effector response of acetylcholine from post-ganglionic neurons that is not mediated by muscarinic receptors[47].

Carbon monoxide is produced in neurons by heme oxidase 2 (HO2). This enzyme is localized in a sub-population of neurons that also produce NOS and its reduction induces a deficit in neuromuscular transmission and gut transit is slowed.

During tissue injury, like it happens during abdominal surgery, carbon monoxide is produced by another enzyme, heme oxidase 1 (HO1).

The role of carbon monoxide in gastrointestinal tract has been recently reviewed[49]. Nitric oxide (NOS) and carbon monoxide are gaseous mediators found both in the brain and in the gastrointestinal tract.

NOS is present in enteric neurons but it is also generated by components of the gastrointestinal immune system, in particular macrophages and monocytes, as a consequence of intestinal manipulation and this mechanism play an important role in post-surgical smooth-muscle dysfunction tipical of ileus. L-NMMA, an NOS inhibitor, provokes an immediate post-surgical recovery of gastrointestinal motility, inducing the authors to think that NOS is the final common pathway for postoperative inhibition of motility[50].

Hydrogen sulphide (HS) has been recently suggested as the third gaseous mediator[51]. It is a vasodilator and it is present in CNS[52]. It is synthesized in neurons and it has an excitatory neuromodulator action in the enteric nervous system[53].

The source of HS could be enteric neurons, intestinal microflora and diet. Red meat, high protein intake and alcohol are associated with relapse in ulcerative colitis as a consequence of high luminal concentration of HS which could compromise use of the endogenously producted butyrate, a short-chain fatty acid that has been shown to contrast some ulcerative colitis pathologies.

The positive effect of probiotics on treatment of inflammatory conditions, including IBS, might be related to prevention of negative effects of HS.

Mast Cells

Mast cells act as a modulatory interface between neurons and their environment and this interaction is bidirectional. A local triggering cause induces peripheral release of substance P (SP) present within extrinsic sensory nerve terminals through an axon reflex. The SP acts on its receptors located on epithelial, inflammatory, neuronal and/or smooth muscle cells, principally during inflammation when NK1 receptors are much more present[54].

NK receptors are also located on mast cells and the exocytosis of their granule contents stimulate nerve endings, maintaining an inflammatory condition. The mast cells mediators include various pro-inflammatory substances (tryptase, histamine, platelet activating factor, prostaglandins, cytokines and leukotriens) which can stimulate residential macrophages on the one hand and intrinsic and extrinsic afferent neurons on the other hand[55].

There is an accumulating evidence that symptoms in a group of IBS patients are correlated to post-infectious sub-clinical inflammatory alterations in the gut wall and intestinal dysbiosis exists in at least a subset of patients, more probably like a subtle alteration than a gross bacterial overgrowth[56] determining an altered immune nerve signaling.

Various authors have observed a strong morphological association between mast cells and nervous fibers in biopsies taken from IBS patients[57]. This association has found a functional correlation in the observation that various mediators released from human intestinal mast cells stimulated with IgE, causes direct activation of guinea-pig and human enteric neurons[58]. This cocktail of various mediators led to an exaggerated neuroimmune signaling in IBS patients.

Recently, has been described that patients with IBS showed an abnormal IL-10/IL-12 ratio, suggestive for a pro-inflammatory condition[59].

The alleviation of symptoms and the normalization of the ratio of pro-inflammatory and anti-inflammatory cytokines was obtained with the administration of the probiotic microorganism *Bifidobacterium infantis 35624*. Likewise, symptoms in IBS patients were relieved with administration of the probiotic combination VSL3[60].

Beyond the immune modulatory action of probiotics it has been shown that the ENS might be another target, based on the finding that food supplementation with the probiotic yeast *Saccharomyces boulardii* alters the neurochemical phenotype of pig myenteric neurons evidenced by a decrease of calbindin expression, while other neuronal populations are not affected[61]. The probiotic-evoked change in phenotype seems to have some functional implications[62].

The authors found in mice that a perturbation of the gut microflora is associated with an increase of substance P-immunoreactivity and visceral hypersensitivity, while the administration of *Lactobacillus paracasei* normalized substance P-immunoreactivity and hypersensitivity.

In conclusion, the interactions between ENS and immune systems remains a very important topic and very interesting progresses has been achieved to shed light on the neuro-immune axis in the human gut, but there is still much that we do not know.

Serotonin as signaling molecule

Normal GI function relies on properly functioning brain-gut axis, which involves the coordinated interplay of the GI musculature, the CNS, the autonomic nervous system (ANS), and the enteric nervous system (ENS). Impaired function or coordination of any of these systems, or the communication between these system and the GI musculature, can lead to symptoms of dysmotility and altered sensory perception, which are characteristic of IBS and other GI motility disorders[63]. The neurotransmitter serotonin (5-hydroxytryptamine -5-HT) is a predominant signaling molecule in the ENS and the most part of serotonin (95%) is found in the gut[64].

Serotonin facilitates communication between muscles, secretory endothelium, endocrine cells, and the vasculature of the GI tract, playing a key role in normal GI tract functioning[65]. In addition, serotonin plays an important role in the communication between ENS and CNS.

Enterochromaffin cells (EC cells) are located within the mucosa of gastrointestinal wall and they synthesize and store serotonin. 5-HT is synthesized too by serotonergic neurons of myenteric plexus.

EC cells contain one form of rate-limiting enzyme in serotonin's biosynthesis, tryptophan hydroxilase-1(TpH-1), and enteric and central serotonergic neurons contain TpH-2, which is a different gene product. When the mucosa is stimulated by the material passing through the gastrointestinal lumen, serotonin is released by EC cells and it binds to its receptors (primarily 5-HT1P receptors) on intrinsic primary afferent neurons whose cell

bodies are located in submucosal and myenteric ganglia, as well as sensory neurons located in spinal (dorsal root) ganglia and vagal (nodose) ganglia, initiating secretion, peristalsis and vasodilatory reflexes, as well as stimulation of afferent signals to the brain and spinal cord. Serotonin also binds to 5-HT4 receptors on interneurons, which increase the transmission of the signals to motor neurons, resulting in augmented peristaltic activity.

Serotonin mediates the signaling between the ENS and the CNS and so modulates pain perception by binding to 5-HT3 receptors present on efferent sensory innervations coming from the vagus and the spinal nerves. We said that the serotonin signaling process is regulated by the SERT molecules which are produced by intestinal epithelial cells[66].

Human studies demonstrated that defects in serotonin signaling contribute to the physiology of IBS and other functional GI disorders. In patients with IBS intestinal biopsies have shown a significantly lower mucosal serotonin concentrations probably as a consequence of lower mRNA levels of tryptophan hydroxilase, which is the rate-limiting enzyme in serotonine synthesis[67].

In another study[68], was observed that in the biopsies of patients with IBS constipation variant (IBS-C) the levels of serotonin were higher than in patients with the IBS-D variant or in healthy controls. Increased understanding of the role of serotonin in gut physiology has determined the development of drugs designed to modulate its function in the GI tract. The use of serotonergic agents, in particular *Alosetron (5-HT3 receptor antagonist)* in women with IBS-D and *Tegaserod (5-HT-4 receptor agonist)* in the short-term treatment of patients younger than 65 years of age with chronic constipation, has provided contrasting results.

Actually the new prescribing label of Alosetron, approved by the US Food and Drug Administration (February 2005), includes a "black box" warning regarding: a) restricted indication for women with severe IBS-D who have not responded to conventional therapy and in whom anatomic and biochemical abnormalities have been excluded; b) the risk for serious GI adverse events, such as ischemic colitis; c) a new starting dosage; d) a requirement that physicians and patients subscribe to a risk-management program.

It is evident that serotonin signaling is altered in IBS-D, IBS-C and PI-IBS, but the cause and effect relationship of epigenetic changes in the elements of 5-HT signaling is unclear, because we do not know if changes in 5-HT signaling contribute to the modifications in GI function and sensation typical of IBS or vice versa elements of 5-HT are altered in response to an altered function and sensation. In other words we do not know whether 5-HT signaling changes in response to altered gut function or altered 5-HT signaling, can lead to changes in gut motilty. In fact, administration of low concentrations of SSRI fluoxetine (Prozac), increase the rate of propulsive motility at low concentrations, while slow motility at higher concentrations[69, 70].

In general, receptors which have been identified for 5-HT are: 5-HT1A-E,P, 5-HT2A,B,C , 5-HT3, 5-HT4, 5-HT5, 5-HT6, 5-HT7, in prevalence localized in the gut (5-HT1A, 5-HT1P, 5-HT2A,B,C, 5-HT3, 5-HT4, 5-HT7)[71].

5-HT3 and 5-HT4 receptors have proven to be possible targets for the treatment of diarrhea-predominant and constipation-predominant IBS, but they are not responsible for all the effects of 5-HT in the GI tract and other 5-HT receptor subtypes contribute to the pathophysiology of IBS. Alosetron and Tegaserod might be acting through antagonism of 5-HT2B receptors. Both compounds inhibit the excitatory effects of receptors in the human colon. Thus it is possible that the effects could be due to blockade of 5-HT2B rather than to the activity at either 5-HT3 and 5-HT4 receptors. 5-HT7 receptor subtypes may also be promising targets for the future which are expressed in both the small and large human intestines[72]. Tonini et al.[73]. observed that 5-HT7 receptors may modulate the nitric-oxide mediated intestinal accommodation and smooth muscle relaxation in guinea pig ileum.

Endogenous 5-HT was involved in smooth muscle accommodation in the preparatory phase of peristalsis by direct activation of 5-HT7 receptors.

Moreover, the authors suggest that abnormal stimulation of the 5-HT7 receptor may contribute to some clinical syndromes like IBS and that it could be a novel target to treat diarrhea-predominant IBS[73]. Functional 5-HT7 receptors are present in human microglial MC3 cells, suggesting that they are involved in neuroinflammatory processes[74].

It is possible imagine that 5-HT7 may be one of the supposed links between changes in serotonin signaling and metabolism on one hand and the symptoms experienced by post-infectious IBS patients on the other hand.

The 5-HT1P receptor seems to mediate a response that is different from that of any currently cloned 5-HT receptor.

The lack of molecular identity has inhibited the research on this target. Gershon and coll. published a series of abstracts to suggest that this receptor is in fact a heterodimer of the 5-HT1B/D and D2 dopamine receptor. The 5-HT2B are localized in both smooth muscle and nerves, and their activation can cause direct smooth muscle contraction in the human ileum and can enhance cholinergic-neuron mediated contractions in human colon[75]. The localization and the enhancement of 5-HT2B receptors, suggest that blockade of 5-HT2B receptors might reduce prokinesis.

In conclusion the role of 5-HT in neurologic physiology of gastrointestinal tract is indubitable and clear evidence exists for a role of disturbed mucosal 5-HT signaling in IBS, as well as in other FGIDs. It is clear that the sequence of events that induces the serotonin signaling can be modified and transformed in response to different physiological and pathophysiological conditions. It is very probable that altered 5-HT signaling collaborates to induce an abnormal gut function and to heighten the sensitivity in IBS patients.

Psychosocial factors and Central dysregulation

Psychosocial factors seem to be important in FGIDs although whether these factors directly alter gastrointestinal function is not clear[76-78].

In fact, it is possible that psychosocial factors may be involved in the ethiopathogenesis of FGIDs, not just influencing the frequence and severity of symptoms and health care seeking of the patients. In other words, are psychosocial factors just a consequence of symptom severity or are strictly related to FGIDs "per se"? Two population-based studies observed that IBS and psychosocial factors were associated and this association was present also in subjects who had not sought health care for their symptoms[79, 80].

Physical and sexual abuse are conditions frequently reported by women or children and psychosocial factors are mediating factors linking abuse with the development of fun-ctional gastrointestinal symptoms[81, 82]. Interestingly, the association between self-reported abuse and the impaired functioning in patients with severe IBS seems to be mediated by a general tendency to report various bodily symptoms[83]. Cognitive factors are considered important for IBS and other functional disorders. Patients with severe depression report more severe abdominal pain, whether for a particular tendency to catastrophic thinking, but also for interpersonal problems in a complex way.

For both Functional Dyspepsia (FD) and IBS, it clearly appears that severe chronic life stress threat together with the prolonged coping associated with the stressor, have significant effects on symptoms onset and relapsing over time[84]. It is interesting that psychological stress and other cognitive aspects can also be related to sensorimotor dysfunction in FGIDs patients[85].

For example, in IBS the severity of psychosocial disturbance parallels the degree of small bowel motor or sensory dysfunction[86], whereas exposure to psychological stress provokes rectal hypersensitivity[87].

Hypervigilance is another factor that influences symptom reporting by IBS patients during rectal distension test[88]. In FD cognitive factors influence both symptom induction and sensorimotor dysfunction[89, 90]. Chronic stress induce in rats an alteration of mucosal defences against luminal aggressive factors like bacteria. Intestinal permeability is increased during stress via a cholinergic mechanism that requires the active presence of mucosal mast cells. Other mediators of stress-induced gastrointestinal sensorimotor responses are norepinephrine and corticotrophin releasing factor (CRF). Human data suggest that IBS patients are sensitive to CRF effects on colonic motility and infusion of CRF increases rectal sensitivity[91].

CRF-1 receptor mediates stress-induced increases in colonic contractility and CRF-2 mediates stress induced gastric hypomotility and surgical ileus[92, 93]. Brain-gut interactions play a fundamental role in the regulation of digestive processes, in the modulation of immune system and in the co-ordination in the gastrointestinal apparatus of the global emotional and physical state.

Disturbed brain gut-interactions likely provoke the generation of symptoms of FGIDs., alterations of immune activity in IBS and various eating disorders.

Positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) permitted the observations of various brain sites activation in consequence of pain stimulation in health and IBS. The results reported by different studies on processing of pain in IBS patients are often divergent , but, taken together, existing literature suggests that IBS patients respond to visceral distension with an increased activity in some sensory areas[94], in subcortical regions associated with affect, arousal and autonomic response and in cortical areas involved in attention and affect[95].

It has been demonstrated that male and female have some differences in relation to activation of brain regions[96] and the activation patterns differ between IBS subgroups based on the predominant bowel habit[97]. A very recent rectal distension study using fMRI, demonstrated that IBS patients with a history of abuse complained about more pain, greater MCC/PCC activation, and reduced activity of a region implicated in pain inhibition and arousal (sACC)[98]. Such studies emphasize the importance of external stressors, cognitive and emotional biasing as well as peripheral injury in GI pain[99].

Studies on CRH receptor antagonists in FGIDs demonstrate the strong modulatory role of the HPA axis in gut sensorimotor function and the possibility that derangements of its normal function occur in patients with FGIDs. In patients with IBS have been observed autonomic alterations such low basal cardiac vagal tone and that has particular relevance to pain sensitivity[100].

Autonomic Nervous System (ANS) Dysfunction and Hypothalamic-Pituitary-Adrenal axis

The ANS is a fundamental part of the emotional motor system and represent a bidiretional, body-brain interface that integrates afferent bodily inputs and central motor outputs for homeostatic-emotional processes[101]. In particular, CNS communicates with the gut through the parasympathetic and sympathetic pathways of the ANS by modulation of the ENS, as well as via the hypothalamic-pituitary-adrenal (HPA) axis. Sympathetically mediated mechanisms are implicated in different chronic pain syndromes[102] and animal and human data indicate a vagally mediated inhibition of visceral nociceptive sensory inputs[103] and in this way the ANS might modulate visceral sensory perception. The exact mechanisms by which sympathetic and parasympathetic nervous systems modulate pain is unknown.

The pro-nociceptive action of the sympathetic nervous system may relate to the release of prostaglandins and/or catecholamines from sympathetic nerve terminals which are in close proximity to the terminals of damaged primary afferent nerves and this could result in the direct activation of afferent fibers that have developed α-adrenergic receptors[104].

Early life events can definitively influence the development of corticotrophin-releasing hormone (CRH) systems, which mediate the expression of behavioural, emotional, autonomic, and endocrine response to stress.

In the animal studies, maternal deprivation in infancy is associated with enhanced neural CRH gene expression and increased stress reactivity.

These animals, in adulthood, show a greater activation of the HPA axis, sympatho-adreno medullary systems, central monoaminergic systems and greater vulnerability for stress induced illnesses.

In patients with Functional Dyspepsia (FD) has been proposed that abnormal proximal accommodation could be caused by a vagal defect[105], but studies inducing nausea and gastric dysrhythmias, have proposed the possibility of central neurohumoral dysfunction in the pathogenesis of FD[106].

Various studies have documented autonomic dysfunction in IBS, but the results seem to be contradictory depending on different experimental designs and different populations in study.

Also regarding the HPA axis the results are divergent, because researchers have demonstrated attenuated[107], normal[108] and augmented reactivity[109] of the HPA-axis in IBS patients in response to various stressors, as well as an association with a proinflammatory cytokine release[109]. It remains unclear whether the autonomic alterations in the FGIDs are a primary phenomenon or merely reflect the bidirectional interactions of CNS-ENS dysregulation.

Visceral hypersensivity

In normal conditions gut is not a source of conscious sensory experiences. Unpleasant sensations are only felt acutely or perceived as painful when stimuli exceed those in the physiologic range.

The typical colic appears when supraphysiologic distension with spinal mesenteric afferents occurs, while ischemic pain occurs when blood flow in the mesenteric district falls below the physiological level. These stimuli are generally acute, but many patients without organic diseases complain of chronic abdominal symptoms, especially pain. It is evident that in certain circumstances it is possible to feel stimuli that are normally non-noxius (allodinynia) or increase afferent discharge to noxius stimuli (hyperalgesia). These nociceptive hyperalgesic phenomena are all grouped under the title of *visceral hypersensitivity (VH)* and are considered the hallmark of IBS and other FGIDs.

In 1973, Ritchie et al demonstrated that IBS patient were more sensitive than normal subjects to balloon distension of the colon[110]. Subsequently, intolerance to gastric distension was also observed in patients with functional dyspepsia[111].

From the data obtained in control subjects indicating that 40 mmHg is the normal tolerance threshold, about 90% of patients with IBS were classified as suffering from VH, but severe hypersensitivity (less than 28 mmHg) was found only in one third of subjects and was highly specific for IBS. When distension is done with ascending magnitude, subjects complain of a lo-wer pain threshold than controls, but if the same volumes are presented in a random order, all mean thresholds (IBS patients and controls) are very similar[112].

This condition is partly attributable to so-called *hypervigilance*, or excessive attention to or fear of painful stimuli from the gut, and partly due to a true hypersensitivity demonstrating an altered elaboration of visceral stimuli. Visceral sensations are transmitted from the gut via afferent nerves traveling through the spinal cord to the brain., where the pain characteristics are perceived and elaborated.

The sensitization could happen either within the dorsal horn of the spinal cord or in the brain.

The excitability of this way is so intense that pain attributable to visceral distension is seen over more dermatomes than normal. Functional imaging in irritable bowel syndrome, utilizing fMRI and PET, show an increased flow of blood in particular in the anterior cingulated cortex during colonic stimulation, but differing patterns of brain activation are characteristic. These differing patterns could reflect increased afferent input from rectum and/or greater emotional response based on previous experiences, for example child abuse. Most part of these studies show that the brain signal responding to intestinal distension is greater in patients with IBS than in controls.

This enhanced brain response could depend on different conditions: 1) could be determined by an increased signal arriving from the peripheral gut, 2) could depend on an amplification of a normal signal during its travel from gut through the spinal cord, 3) could be an amplification in the brain, 4) could be induced by some defects in the descending inhibitory mechanisms[113].

Many data from literature seem to confirm that peripheral mechanisms are involved in the transmission of an enhanced signal to the brain (for example inflammation in PI-IBS), but all these data do not give an absolute proof of this concept, because we have not valid instruments to evaluate the signal at level of gut or spinal cord and brainstem in humans.

In FGIDs the severity of visceral hypersensitivity does not correlate with the severity of clinical symptoms and it is possible to conclude that VH is a heterogeneous condition in patients with functional gastrointestinal diseases, but the current knowledge does not allow further differentiation of the subgroups of patients on the basis of symptom phenotype[113].

Besides, VH has been sometimes documented along the entire GI tract in FGIDs patients (33%), but in general patients with FD have gastric but no rectal hypersensitivity and IBS patients have rectal but not gastric hypersensitivity. In about one third of patients is associated a *somatic hypersensitivity* and in this condition probably depends on the fact that the modulation of a peripheral signal take place at level of spinal cord or in the brain[113].

In humans pain and discomfort are conscious experiences which result from a cerebral elaboration of the visceral afferent signals which are influenced by cognitive, emotional, motivational factors as well as memories and by the particular contest.

Conscious perceptions of some changes at gut level, when arrived in the brain, are filtered, modulated and elaborated in light of previous experiences. Symptom-related anxiety alter the perceptual responses to pain stimuli via multiple brain mechanisms including descending and opioidergic modulation networks[114, 115]. Significant clinical experiences have been made to identify brain areas, circuits and mechanisms interested in the facilitation and inhibition of the subjective pain experience[114].

Significative is the role of the emotional context during the central modulation of the pain stimuli arriving from the body and the data of literature stress the importance of attentional processes in the modulation of sensory informations[116-118].

The third possible explanation for visceral hypersensitivity is a defective functioning of descending anti-nociceptive pathways which originate in the brainstem and inhibit afferent discharge in spinal pain pathways. ***In conclusion***, in the cascade mechanisms provoked by visceral stimuli at the gut level, resulting in peripheral and spinal sensitization, central sensory modulation is likely a crucial factor in the pathophysiology of visceral hypersensitivity in FGIDs patients.

Stress and Psychological Factors

Frequently patients suffering from FGIDs refer that stress exacerbates their symptoms. The percentage of patients with symptoms certainly related to the stress is very limited (11%)[119].

In some cases the correlation between the consequences of severe stress, like post-traumatic stress disorder, and the developing of functional disorders is evident[120]. In FGIDs severe chronic life stress threat derived from divorce, housing difficulties, business failure, mobbing, death and so on, as well as the prolonged coping associated with the stressor, have caused the onset[121] and then the persistence of symptoms[122].

Psychological factors and quality of life are strictly correlated with the severity of gastrointestinal symptoms in IBS patients and severity of psychological problems is clearly related to visceral hyperalgesia[123].

The effects of chronic stress on the gut produce some alterations in mucosal defense against bacterial aggression and reduce intestinal permeability via a cholinergic mechanism that requires the presence of mucosal mast cells[124].

In conclusion bowel symptoms as a consequence of acute stress have been reported in many patients with FGIDs, but the variance in gastrointestinal symptoms is poorly significant. On the contrary, chronic psychological stress and its effect on visceral sensitivity seem to have an important role.

Conclusions

Functional gastrointestinal diseases have been characterized by the presence of chronic or recurrent symptoms referable to the gastrointestinal tract, in the absence of currently recognized biochemical or structural alterations.

Indeed, patients are characterized by a broad spectrum of gastrointestinal symptoms, frequently overlapping between them.

FGIDs are identified only by symptoms, therefore various symptom-based classifications have been proposed and are known as Manning criteria, Rome I, Rome II, Rome III. FGIDs include, IBS, functional bloating, functional constipation, functional diarrhea, and unspecified functional bowel disorders[125].

Although FGIDs have been dismissed by many clinicians as non-organic problem, recent studies suggest, for instance in IBS, some organic components, in particular interesting the complex interactions between ENS, ANS and CNS.

There is large wave of interest in the brain-gut axis and in the relations existing between brain and gut, between nerves and hormones, between gut inflammation, ENS and CNS.

Many peptides in the GI tract are present both in endocrine cell and in nerve fibers, and many peptides are found in both the brain and the GI tract.

Patients with FGIDs do not fit the typical biomedical model of disease because FGIDs seem to address their etiology to some of the component of nervous system.

The importance of nervous system for gastrointestinal functions in health and disease is more and more emerging, but today we have not a complete idea of the pathophysiological role of enteric nervous system, and accurate and affordable technologies for in vivo studies of physiology are still required.

Despite of the actual limitations of our knowledge, the actually used classifications of FGIDs, which are symptom based, are no longer defensible.

Considered that symptoms reported are heterogeneous, involving different portions of the gut, frequently overlapping and evaluated that extra-GI symptoms (fibromyalgia, fatigue, urogenital dysfunction) are very frequent, investigators and clinicians must remember that *"gut may only be an involved organ in the context of a systemic problem and not necessarily the site of the primary pathology"*[126].

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