Microbiome and Chronic Pelvic Pain

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11.1 Introduction

The causes of chronic pelvic pain (CPP) are multiple and different according to gender and age. It is self-evident that in the presence of pathologies such as endometriosis, dysmenorrhea, myofascial pain syndrome, ovarian remnant congestion, pelvic congestion, pelvic fibrosis, pelvis neurodystonica, cancer pain, adhesions after surgical procedures, radiation proctitis, ureteral obstruction, and others, all causes of CPP [1], a possible alteration of the microbiota in the hollow organs of the pelvic cavity (digestive tract, bladder, urinary tract, prostate, and vagina) may be not particularly relevant. On the contrary, when the origin of CPP is comparable to "dysfunctional" conditions, characterized by alterations of function, phenomena of micro-inflammation and activation of cellular and humoral immunity, such as vulvodynia, interstitial cystitis, prostadynia, or irritable bowel syndrome, furthers causes of CPP, the involvement of the microbiome may be critical for the genesis of disorders. In other words, when CPP is comparable to a visceral pain, the most recent scientific evidence shows that its cause is often to be found in an alteration of the microbiome and its relation to the host. Visceral pain recognizes various pathogenetic mechanisms and, in particular as regards hollow organs, distension, paroxysmal contraction, hypoxia, and inflammation phenomena, which, as we will see, are all mediated by the interaction with the microbiome and the molecules it

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produces or contributes to, which have the potential to exert strong effects on the cells of the bowel wall whose structure and function are in turn under the control of the central nervous system (CNS), peripheral nervous system (PNS), and autonomic nervous system (ANS).

11.2 Microbiota, Microbiome, Metabolome

The term *microbiota* defines the set of symbiotic microorganisms that cohabit in the human organism without damaging it. It has almost completely supplanted the obsolete and less appropriate "microflora," a term that recalls the plant kingdom in which bacteria were once classified. Based on recent revised estimates, the human microbiota comprises approximately 10^{13} prokaryotes (bacteria and archaea), as well as fungi and viruses with a contribution of 0.5 kg of the average adult's body weight, but with an extraordinary metabolic capacity, far exceeding that of human beings [2–5]. Of all these microbial components, bacteria have been the most thoroughly studied, but it has become increasingly clear that trans-kingdom interactions are just as important in influencing health and disease [6]. Many if not all human cell structures coexist with a more or less rich microbiota: organs as the lung, or the bladder, not to mention the fetus, which until a few years ago were considered examples of "sterile structures and systems," in fact turned out to have their own microbiota, and in many cases some of their pathologies have been associated with alterations of its composition. That applies for example to all recent works that describe the role of urinary microbiota in urinary tract and gynecological dysfunction [7–9]. Not discounting a number of shortcomings in the available studies, even blood does not seem to be excluded [10], whereas the only exception, to date, is represented by the CNS. There is no doubt, however, that the microbiota residing in our gastrointestinal tract is the hub upon which the modulation not only of all intestinal functions, but also of other organs, depends, including the CNS, even at the level of its "higher" functions such as mood and ideation. Harboring around 10–100 trillion prokaryotic cells at density of 10^{11}–10^{12} cells/mL, the human gastrointestinal tract is one of the most complex microbial ecosystem on the planet Earth, comprising only a few phyla (Firmicutes and Bacteroidetes) but hundreds of species, thousands of strains, and millions of bacterial genes with specific assemblies for each subject, like fingerprints. Added to this is the high degree of plasticity, i.e., the microbiota ability to change in response to several endogenous and exogenous factors, such as age, diet, geography, lifestyle, intake of drugs, and host inflammation [11].

The term *microbiome* refers precisely to the whole genetic patrimony possessed by the components of the microbiota, i.e., all the genes it harbors and can potentially express. The tremendous and incredible impulse of knowledge on the richness of the human microbiota, the complexity of its interactions with the host and its consequent role in the onset and progression of many intestinal and extra-intestinal pathologies, which we have witness in recent years, has been possible thanks to our progressively increasing ability to typify the microbial genome. The

classical basic microbiology approach, based on the growth of biological material in special, but still limited, culture media, in fact could not give a complete and accurate description of the intestinal microbiota. This is due, on the one hand, to the huge number of strains present at a variable abundance in a single fecal sample and, on the other, to the difficulty of cultivating anaerobic microbes and reconstructing the existing syntrophic relationships between them, which make up the vast majority of the intestinal microbiota. The current gold standard to get a complete picture of the microbiota, in terms of composition and functionality, is represented by next-generation sequencing technologies, which fall into two main categories, i.e., targeted sequencing (of hypervariable regions of the 16S rRNA bacterial gene) for taxonomic purposes or whole-genome shotgun sequencing to retrieve information on all genes encoded, up to the assembly of whole genome. Until a few years ago, sequencing one million DNA nucleotide bases costed around US$ 10,000, which made the mapping of such a complex microbial ecosystem unaffordable. Between 2001 and 2011, as a result of major technological advances, the cost for the same test decreased to US$ 0.10, making it possible to define the compositional and functional structure of the microbiota in large populations of healthy and/or diseased patients [12]. It is thanks to such progress that we know that our symbiont gut microbial communities contain more than five million non-redundant genes (i.e., 500 times the human genome): through which microbes provide us with a range of otherwise inaccessible metabolic capabilities and play a fundamental role in training and influencing our immune system [13, 14]. However, it is also worth noting that a large amount of functional diversity is still largely uncharacterized ("the microbiome dark matter"), with potentially other important contributions to human pathophysiology.

Finally, the term *metabolome* refers to the complete set of metabolic products found within a biological sample, i.e., all the compounds that are likely to be involved in the biological processes of an organism. This includes both the substrates necessary for biochemical reactions, and the products derived from them, and hormones and other signal molecules. Like the microbiota, the metabolome is an extremely dynamic entity, which is able to change in a very short space of time: like all biochemical reactions, the modification of a single element can lead to a completely different final result. For this reason, no method of analysis can actually reflect a complete picture yet: metabolomic (i.e., the study of metabolome) indeed provides a partial snapshot of metabolism, also depending on the type of analytical technique used. Despite this, interest in this field has registered one of the most important scientific "revolutions" of the last decades. In January 2007, researchers from the Universities of Alberta and Calgary completed the analysis of the human metabolome, identifying and characterizing about 2500 metabolites, 1200 active ingredients, and 3500 components of food origin [15]. This enormous variability contributed by the microbiome, especially the intestinal one which, being at the connection between diet and host physiology, can produce and/or contribute to a vast range of bioactive small molecules virtually influencing all aspects of human physiology and biology [16–18].

11.3 Intestinal Dysbiosis and Its Functional Consequences

The human gut microbiome is typically defined by global ecological parameters such as richness, diversity, and evenness of its microbial communities: as nature teaches us, a high richness and diversity of species is an indicator of health of every ecosystem. In particular, a high biodiversity of the gut microbiome is universally recognized as a hallmark of intestinal health, being the guarantee of completeness, integration, and normality of the digestive, absorption, and nutritional processes that take place in the digestive tract, but that influence the health and functioning of all organs, brain in the first place. Conversely, reduced microbiota diversity has been observed in a multitude of infectious diseases, metabolic diseases, and inflammatory disorders. As anticipated above, the microbiome complexity and stability are influenced over time, from infancy to old age, in relation with many factors such as genetics, mode of birth, breast or formula feeding, geography and early childhood exposure, sex, age, hygiene, psychology/stress, diet/nutrition, physical activity, tobacco, alcohol, and drugs. In this respect, let's just think of the effects of prolonged and recurrent antibiotic therapies and the intake, often abused, of the Proton Pump Inhibitors (PPIs), which results in a zeroing of gastric acidity, a real entry barrier to food and drink microorganisms from the outside world, and therefore a very important physiological factor in maintaining the stability of the intestinal microbiota. In fact, all these factors may induce perturbations affecting the complexity and stability of the microbiome, potentially leading to *dysbiosis* [19, 20]. In practice, all aspects of behavior and interaction with the external environment have the potential to act on composition and gene expression of the microbial community: when the interaction between these factors severely compromises the biodiversity of the microbiome, with an impoverishment of normally present, health associated, species and/or an enrichment of pathobionts (i.e., opportunistic pathogens present in low abundance in healthy microbial ecosystems but able to thrive in inflammatory conditions), disorders and diseases may occur. Dysbiosis may be featured by specific compositional and functional attributes in different disease contexts [21]. In many different diseases, however, the dysbiotic gut microbiome shows a reduction in the proportion of anaerobes that dominate the healthy gut and increased amount of facultative anaerobes, including Proteobacteria and Bacilli. It is interesting to note, but nobody should be surprised, that such low-diverse, disease-associated microbiome resemble the gut microbiome of perfectly healthy infants [22, 23]. This may be explained by the concept of "secondary succession" where a dramatic change that wipes out a complex community (such as a forest fire) results in the observation of similar early succession, or "pioneer" species. These phenomena suggest important reflections on the physiopathology of the gut microbiome, on which we are not going to dwell in detail in this chapter, but which are of great interest. One is that microbiome diversity is physiologically scarce in the child and, with different microbial actors, even in the elderly and that "special" compositions have been found in centenarians, thus hinting that their longevity may be consequent to "that" special composition of the microbiome, and not the opposite [24, 25]. Another topic of great interest is the study of the intertwining of environmental factors that throughout life lead some individuals to

contract a pathology, others another and some none. Let's think, for example, of the relationship between gut microbiome, host metabolism and immunity and diet in the determinism of colorectal cancer. It has been accepted for some time that the important role the gut microbiome plays in nutrient processing and synthesis may affect colorectal cancer development through metabolite-mediated changes in immune and metabolic signals [26]. Moreover, increasing data indicate that gut microbes are pivotal in integrating environmental cues with host physiology and metabolism and may influence several biologic processes critical to carcinogenesis including the balance of intestinal cell proliferation and death. For example, consistent data indicate that *Fusobacterium nucleatum* and *Bacteroides fragilis* are enriched in patients with colorectal cancer, whereas butyrate-producing bacteria are depleted in cancer patients [27]. Adherence to the Mediterranean diet is confirmed to have beneficial effects precisely because it leads to increased levels of fecal short-chain fatty acids (SCFAs) in relation to the presence of *Prevotella* and some fiber-degrading Firmicutes, which are fundamental to a healthy condition [28]. On the other hand, the long-term consumption of a low-fiber diet has been shown to have deleterious consequences on microbiota diversity and abundance profiles, which may be transferred over several generations, and not reversed simply by following a high-fiber diet [29].

Evidences have so far demonstrated that dysbiosis is the most relevant etiopathogenetic element for intestinal pathologies such as functional gastro intestinal disorders (FGIDs), in particular Irritable Bowel Syndrome (IBS), Small Intestine Bacterial Overgrowth, infections as *Clostridium difficile,* and Inflammatory Bowel Diseases (IBD). In these pathologies, it had long been suspected that the microbiota and its influences on the integrity of the intestinal epithelial barrier were somehow involved, while it has only recently become clear that dysbiosis is also involved in the pathogenesis of other frequent pathologies such as Celiac Disease, Diverticular Disease, and diabetes/sugar intolerance [30, 31]. Finally, based on multiple evidence it became clear that the old approach that placed intestinal functions (secretion, motility, immunomodulation, production of endocrine substances and others) under the control of CNS and ANS, i.e., the so-called brain–gut axis, should be completely overturned. At the origin of many diseases, or, one could say, at the base of the health status as a whole, there is a microbiome–gut–brain axis, to be intended as bidirectional interactions between the brain and the gut, with the microbiome as a third key player.

Preclinical and partly clinical evidence is increasingly convincing, indicating in the intestinal dysbiosis a relevant etiopathogenetic factor in neurodegenerative disorders such as Multiple Sclerosis [32] and Alzheimer [33, 34] and Parkinson's disease [35]. Incredibly, intestinal dysbiosis has also been reported in patients with neurodevelopmental disorders such as Autism [36, 37], Attention-Deficit Hyperactivity-Disorder [38], and Schizophrenia [39], and real psychiatric disorders such as anxiety and depression, and animal experiments strongly suggest that the correction of dysbiosis significantly affects symptoms and course of the disorder [40, 41]. Consistently, very recent studies have shown that the fecal microbiota transplantation improves the symptomatic picture in patients suffering from neuropsychiatric disorders [42].

11.4 Microbiome–Gut–Brain Axis

There are multiple ways, levels, and signaling mechanisms by which the microbiota can influence the interaction between the gut and the nervous system, including the brain. The components of this complex bond are a network of specialized targets/transducers cells in the gut wall functioning as an interface between microorganisms and the host lumen. This network consists of immune cells, enterochromaffin cells, smooth muscle cells, interstitial cells of Cajal, enteric neurons, epithelial cells, in particular dendritic cells [43]. In connection with external or internal disturbing factors, the brain acts by modulating the organization and functions of these cells via the branches of the ANS (i.e., through catecholamines and acetylcholine) and the hypothalamus–pituitary–adrenal axis (HPA). The microbiota is in constant bidirectional communication with this interface via multiple pathways, and these communication channels are modulated in response to perturbation of the microbiota, or the brain, by variations in the permeability of the intestinal epithelial barrier and the blood–brain barrier. In particular, to date, it is known that the intestinal microbiota can modulate the CNS through the following mechanisms: (a) synthesis of neuroactive microbial products (such as SCFAs); (b) stimulation of cytokine release by mucosal immune cells; (c) stimulation of the release of hormones (such as serotonin) by enteroendocrine cells, which enter the bloodstream and/or act on the surrounding nerves; and (d) direct stimulation of afferent fibers, such as the vagus nerve. Secondary bile acids and tryptophan metabolites are other microbiota-derived molecules with a role in influencing CNS neurotransmission but it is likely that many other mediators produced or contributed by the microbiota are able to cross the intestinal mucosa, enter the systemic circulation, and then cross the blood–brain barrier. Recently, a catalog of neuroactive potential of the human gut microbiome has been assembled, suggesting positive associations between butyrate producers (i.e., *Faecalibacterium* and *Coprococcus*) and higher quality-of-life indicators, probably mediated by SCFAs and the dopamine metabolite 3,4-dihydroxyphenylacetic acid [44]. However, it remains unclear whether the microbial-derived intermediates reach brain sites directly in sufficient local concentrations to modify distinct brain circuits, or whether microbial signals mainly communicate via neural pathways involving vagal and/or spinal afferents [45]. Brain–gut microbiome interactions are programmed during the first 3 years of life, including the prenatal period, but can be modulated throughout by diet and others factors as mentioned above.

11.5 Food and Microbiome–Gut–Brain Axis

With regard to diet, there is a fascinating relationship among food, immunity, and the microbiota. Diet is widely acknowledged as a pivotal determinant of the gut microbiota composition and function, capable of orchestrating the host–microbiome crosstalk, thus sustaining homeostasis or, on the contrary, contributing to disease susceptibility. Many dietary components are indeed known to interact with the microbiota, modulating the relative abundance of specific genera or the metabolite

landscape, with considerable ultimate effects on human health [46]. Though conflicting data are sometimes reported and more mechanistic work is called for, diet–microbiome–host research has great translational potential in the clinic, and it is likely that the interpretation of several diet-related signs and symptoms should be sought in the nexus with the gut microbiome. Foods, mainly plant-, fruit-, and animal-derived carbohydrates and proteins and fats, rapidly affect the composition and metabolic capacities of commensal microbiota. From an ecological point of view, altered environmental conditions exert selective pressure on various species, leading to competition for the most fit to survive and replicate. Through their enzymatic machinery, microbes convert dietary components into a series of molecules (e.g., SCFAs) that, once adsorbed, can reach the brain, so as to manipulate the host's eating behavior, generating cravings or dysphoria for certain nutrients. In addition, the microbiota can signal through structural components (i.e., microbe-associated molecular patterns—MAMPs) acting as ligands of Toll-like receptors, and inflammasomes, or NOD activators. From the host's perspective, the food supply is scarce and linked with geographic, seasonal, and ethnic parameters. Evolution has produced a highly optimized mutualistic system in which the maximum capacity of energy is extracted from a given amount of food while intestinal homeostasis is maintained. Consequently, animals have evolved mechanisms to modify the microbiota for their own benefit, such as via the mucus barrier and antimicrobial peptides (AMPs) [47]. It is worth remembering that the same quantities of ingested food can be processed differently, with a different number of extracted calories, depending on the individual-specific configuration of the microbiota and its metabolic capacities; in the case of dysbiosis related to persisting eating disorders, the resulting weight gain is then maintained in a self-sustaining vicious cycle. In other words, the substances produced by the gut microbiota for the same food ingested and therefore the signals that can cross the intestinal epithelial barrier and reach the brain can be profoundly different in different individuals, and result in distinct health outcomes [48]. Just think, for example, of the possibility that ingested carbohydrates are transformed into ethyl alcohol by an individual's microbiota and another not: absorbed alcohol may produce hepatic steatosis similar to that of drinkers, i.e., non-alcoholic hepatosteatosis, even in the absence of alcohol consumption [49]. Based on this increasing body of evidence, in the coming years we expect a growing number of studies on diet–microbiota interactions and health leading to the development of microbiome-targeted functional foods, with beneficial and even therapeutic effects for several disease conditions. Foods might one day be used in clinical medicine to prevent and treat diseases. The theory of "you are what you eat" finally is supported by scientific evidence.

11.6 Microbiome and Visceral Pain

Abdominal pain characterized by bloating and distention has been attributed to visceral hypersensitivity to mechanical and chemical stimuli. Many studies draw attention to a role of the gut microbiome in regulating intestinal sensation: they are mostly gnotobiotic studies showing transfer of the visceral hypersensitivity

phenotype after transplantation of gut microbiota from patients with FGIDs, generally IBS, into germ-free mice [50, 51]. Recent studies confirm that the gastrointestinal microbiota profile is altered in patients suffering from chronic or recurrent visceral pain [52, 53]. A correlation between visceral hypersensitivity and an increase of *Escherichia coli* abundance followed by induction of hypersensitivity in response to *E. coli* gavage in mice was found [54]. As this study and many others show, disruption of the gut microbiota in early life is associated with long-term changes in visceral sensitivity, emphasizing the importance of the gut microbiome in the neurodevelopment of pain pathways [55]. However, the exact mechanisms by which bacteria affect visceral perception and sensation still need to be determined, and it remains difficult to discern whether these changes are causative or deleterious to the host, or whether the altered microbiota signature is an appropriate response to tissue injury, inflammation, or damage in the host. Certainly, preclinical studies including prebiotic, probiotic, and antibiotic interventions, fecal transplantation, and the use of germ-free and specific-pathogen-free animals have illuminated our understanding of the role of the microbiota suggesting a few putative mechanisms. These include microbial induction of epithelial μ-opioid and cannabinoid receptors as shown after oral administration of *Lactobacillus* strains in rodents [56], regulation of central and peripheral neuronal pathways [57, 58], antinociceptive effects from inhibition of transient receptor potential vanilloid as shown with the administration of *Lactobacillus reuteri* in rats [59], microbial metabolites, particularly organic acids, or by-products such as nitric oxide altering sensation and affecting colonocyte cytoskeleton contraction and the subsequent tight junction opening [60], and microbial-derived bioactive molecules such as γ-aminobutyric acid (GABA) as shown with the administration of GABA-producing *Bifidobacterium dentium* [61]. There are others studies showing that probiotics blunt nociceptive response to colorectal distention [62, 63], but the translation of findings from animal models to human beings remains a challenge. For instance, rectal administration of butyrate has been found to increase colonic hypersensitivity in rats, but decrease visceral sensitivity in healthy subjects [64, 65]. In summary, the data suggest that intestinal dysbiosis, whether it is already present in the early stages of life and therefore affecting the development of pain pathways or whether it occurs later in life in relation to environmental factors, stress, diet, or others as mentioned above, may be the element that determines a low threshold of visceral pain, are very convincing. However, it will probably be very difficult to identify the substances produced or contributed by the intestinal microbiome, or microbiomes from other hollow organs in the pelvis, which are responsible for the onset and modulation of pain, as these can vary from individual to individual in relation to the resilience of their microbial ecosystem to short- and long-term perturbations in the host environment. Not least, the dynamics of the gut microbiome across the life span, with hallmark characteristics in the different phases of life, has to be accounted [66].

11.7 Microbiome and Colonic Motility

We have to consider a further mechanism trough which dysbiosis, particularly in the large bowel, can influence the onset of chronic recurrent visceral pain. Only recently, thanks to the works by the Australian group directed by the physiologist Marcello Costa, it has been possible to understand that propulsion in the large bowel is consequence of two neural mechanisms. The first is the content-independent spontaneous colonic migrating motor complexes that occurs cyclically. The second is a content-dependent, adaptable mechanism controlled by the mechanical activation of enteric neural activity. Mechanosensory enteric neurons (located in the myenteric plexus) have essential mechanosensitive nerve endings in the circular muscle. Distension or stretch of the colon activates these sensory neurons to initiate polarized neural pathways that result in oral contraction and anal relaxation. These pathways do not require the mucosa but can be modulated by sensory nerve endings that project into the mucosa [67]. Enteric neural circuity can efficiently propel content with a wide range of physical properties. This content-dependent activity can be modified in terms of force of contraction and speed of propulsion depending upon consistency and volume of the colonic contents [68]. In others words, bolus size and its consistency affects propulsion speed suggesting that propulsion is not a simple reflex, according to the classic theory about intestinal peristalsis [69], but rather a more complex process involving an adaptable neuromechanical loop [70]. Consistency depends on the degree of fluidity of the intraluminal colonic contents, which in turn depends on the degree of absorption of fluids along the colon. But consistency is due also to the dry-component of the formed stools, and this is for the 60-80% composed by alive microbial cells originating from the colon microbiota [71]. So, when a relevant impoverishment of the microbial biomass occurs in the large bowel, that significantly influences colonic propulsion capacity. The resulting decrease in transport of the intraluminal contents can generate dysmotility phenomena with the onset of spasticity in some colonic tracts and distension/dilatation in others. It is well known as both these conditions cause visceral pain, particularly in individuals having a decreased threshold as a consequence of dysbiosis and changes in the microbiome–gut–brain axis as discussed above. It can be said that an empty colon has no motor activity and this is very important for explaining many cases of constipation where disturbances of defecation were consequence of profound imbalance in diet habit, antibiotic consumption. It should be noted that for fibers and prebiotics the mechanism of action is not due to a "mass" effect resulting from a recall of water produced by the polysaccharide molecules of which they are made up, but their action, which favors evacuation, derives from the fact that they constitute the main metabolic substrate for the colon microbiota, and that, as we have seen, the biomass constitutes the dry weight of the feces [72]. It follows that the primary objective of every therapy for constipation is certainly to achieve defecation but not "di per sè," but as a way of rebalancing the ecosystem in the intestinal lumen [73]. In our study, we demonstrated that in a population of patients with severe functional constipation it was enough to restore a regular colonic content

using a symbiotic product for improving defecation disturbances [74]. Finally, colonic dysmotility and so visceral pain due to spastic and dilatation aspects in the large bowel may be the direct consequence of dysbiosis, independently by change in the intraluminal content volume, because function and neuroplasticity of the enteric nervous system are influenced directly by intestine metabolome: butyrate may affect neurochemical coding of myenteric neurons and the contractile activity in the rat colon upon long-term exposure. We can speculate that reduced concentration of butyrate in the gut lumen, inducing alterations in cholinergic neurons of myenteric plexus, is only an example among many how colonic motility can be influenced by microbiome imbalance [75].

11.8 Perspective

There is no doubt that the administration of specific probiotic strains, for which, as for antibiotics, a precise target and therefore a clear therapeutic indication have been identified, is the right means to correct dysbiosis and restore balance in the intestinal ecosystem, thus affecting the threshold of appearance of visceral pain. In this regard, it is worth mentioning the success of probiotics, such as *Lactobacillus reuteri* and *Lactobacillus rhamnosus*, and the synbiotic combination of Fructo-Oligo-Saccharide and seven probiotics in treating the infant colic: two meta-analyses including more than 400 infants found that *L. reuteri* significantly reduced crying time in formula-fed infants by nearly 1 h a day, making it the most extensively studied microbiome-targeting therapy for colic [76–79]. Future treatment strategies for the alleviation of chronic or intermittent visceral pain should take into consideration the personalized microbiota alterations by identifying subsets of patient with a distinct microbiota profile, and developing targeted approaches to restore specific populations of beneficial bacteria. In this regard, the fecal microbiota transplantation (FMT) deserves a separate discussion. We have seen that the human gut microbiota is not a mere assembly of microorganisms, but a highly organized integrated network of cells interacting intensely with each other as well as with the host, which could be thought of as an additional organ within the human body. Based on the available literature, the possibility of transferring this "organ" from a healthy individual, i.e., endowed with a high-diverse intestinal microbiota, to an individual whose microbiota is impoverished, unbalanced, and unable to oppose the action of pathogens has proved to be highly effective and statements on FMT indications, donor selection, preparation of fecal material, clinical management and fecal delivery, and basic requirements for implementing an FMT center are already well established [80]. The first and most documented clinical application of FMT is recurrent *Clostridium difficile* infections (rCDI) in which it is currently used as a last-resort treatment after failure of multiple courses of antibiotics [81], but beyond rCDI, FMT has been evaluated as a treatment option in a variety of gastrointestinal diseases, such as Inflammatory Bowel Diseases [82, 83], non-alcoholic steatohepatitis, alcoholic hepatitis, and hepatic encephalopathy [84, 85]. The evidence that FMT

can be useful in the treatment of disorders as IBS [86] and constipation [87] is very interesting, confirming what we have discussed, i.e., in this disorder intestinal dysbiosis and altered interaction with gut mucosa are pivotal. Moreover, extremely interesting it is that manipulation of gut microbiota through FMT seems to be effective also in conditions outside the GI tract, such as Autism and mood disorders [88] and the Metabolic Syndrome [89]. It is intriguing to speculate that FMT could prove to be an interesting approach for the treatment of patients with severe CPP, when all other therapeutic options have failed, precisely for these its effect, both on the intestinal mechanisms of visceral pain and of influencing the brain functions and mood stability. In fact, it is well known that problems of anxiety, depression, sexual abuse, psychiatric disorders, and often personality disorders are overrepresented in the population of patients with CPP. Studies about this possibility are very desirable, and they can take advantage of a longitudinal systems-based disease model with complementary brain imaging in order to integrate central, peripheral, and behavioral alterations before, during, and after treatment [90].

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