

# Gut Microbiota Dysbiosis as Risk and Premorbid Factors of IBD and IBS Along the Childhood-Adulthood Transition

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**Abstract:** Gastrointestinal disorders, although clinically heterogeneous, share pathogenic mechanisms, including genetic susceptibility, impaired gut barrier function, altered microbiota, and environmental triggers (infections, social and behavioral factors, epigenetic control, and diet). Gut microbiota has been studied for inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS) in either children or adults, while modifiable gut microbiota features, acting as risk and premorbid factors along the childhood-adulthood transition, have not been thoroughly investigated so far. Indeed, the relationship between variations of the entire host/microbiota/environmental scenario and clinical phenotypes is still not fully understood. In this respect, tracking gut dysbiosis grading may help deciphering host phenotype–genotype associations and microbiota shifts in an integrated top–down omics-based approach within large-scale pediatric and adult case-control cohorts. Large-scale gut microbiota signatures and host inflammation patterns may be integrated with dietary habits, under genetic and epigenetic constraints, providing gut dysbiosis profiles acting as risk predictors of IBD or IBS in preclinical cases. Tracking dysbiosis supports new personalized/stratified IBD and IBS prevention programmes, generating Decision Support System tools. They include (1) high risk or flare-up recurrence –omics-based dysbiosis profiles; (2) microbial and molecular biomarkers of health and disease; (3) –omics-based pipelines for laboratory medicine diagnostics; (4) health apps for self-management of score-based dietary profiles, which can be shared with clinicians for nutritional habit and lifestyle amendment; (5) –omics profiling data warehousing and public repositories for IBD and IBS profile consultation. Dysbiosis-related indexes can represent novel laboratory and clinical medicine tools preventing or postponing the disease, finally interfering with its natural history.

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**Key Words:** microbial community ecology, gut microbiota dysbiosis scale, dysbiosis tracking, gastrointestinal disease predictors, –omics, meta-omics, and microbial biomarkers

## Meta-omics–based Dysbiosis Profiles of Gut Microbiota

Chronic diseases of the intestine, in particular, inflammatory bowel diseases (IBDs), are a major cause of morbidity and mortality in the developed world. Substantial evidence suggests that luminal commensal bacteria provide an antigenic stimulus, inducing immune response (dysregulation) and triggering the inflammation associated with IBD onset, in genetically susceptible individuals.<sup>1–5</sup> IBD has an established genetic component, and

genome-wide association studies have identified several immune system (IS) pathways mutated in susceptible hosts. Some of them are activated by infection-mediated syndromes<sup>1</sup> or by altered cellular responses.<sup>2</sup> The relative balance of beneficial versus aggressive commensal enteric microflora has been advocated to determine mucosal homeostasis versus inflammation.<sup>3</sup> This inflammation results in tissue damage and cell proliferation and infiltration, potentially changing the metabolism between normal and diseased tissues. Recent theory has highlighted aspects of the gut ecology, which exerts concerted actions and synergic commensal responses to pathogens.<sup>4</sup> Furthermore, because endogenous and external determinants (e.g., food, antibiotic treatment, and pathogens) modulate the gut microbiota in a complex way, only a synergic meta-omics or systems biology approach may provide a comprehensive understanding of the metabolic cascades, during early IBD and irritable bowel syndrome (IBS) in children. According to the developmental programming concept, the presence of “healthy” gut microbiota in early life and throughout childhood has a crucial role in establishing “safe” gut baseline profiling. The shift from controlled (symbiosis) to short-tempered inflammation (dysbiosis) preludes to overt gastrointestinal (GI) disorders in childhood and adulthood. The stratification of GI dysbiosis levels leads to the definition of intermediate stages, acting as prognostic and predictive cues in the disease dynamics.<sup>5</sup>

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Because of such complexity, it was recently suggested to consider the microbiota as a “tissue,”<sup>6</sup> overcoming the definition of superorganism<sup>7</sup> and leading to acceptance of its active metabolic role. The advent of high-throughput -omics-based methods has opened new avenues in the knowledge of the gut ecosystem by shedding light on its shape, modulation and interplay with microorganisms, food or other external stimuli.<sup>8</sup> Gut microbiota ecology is now recognized as a “fingerprinting” mark capable of describing the natural history of the disease, before its clinical manifestation, between remission and flare-up, or in different life stages, hence allowing recognition of early dysbiotic signatures. Epigenetic profiling in IBD suggests that perturbation of epigenetic factors can also be a major contributor to the development of the disease.<sup>9</sup> -Omics may provide cutting-edge cues for a comprehensive understanding of gut dysbiosis and homeostasis imbalance through ages. The generation of -omics-based dysbiosis profiles will reveal disease-associated perturbations at a much earlier disease stage. Furthermore, functional profiling, in terms of microbial pathways, may be used to generate a panel of biomarkers for disease prevention, diagnosis, and prognosis.<sup>5,10</sup>

## IBD Risk, Prevention, and Disease Management

From a clinical point of view, IBD prevalently includes ulcerative colitis (UC) and Crohn’s disease (CD), chronic relapsing inflammatory conditions that involve large and small bowels at the mucosa, submucosa, and muscle levels, respectively.<sup>11</sup> Although CD and UC are distinct disorders, they share abdominal pain, vomiting, diarrhea, rectal bleeding, and weight loss. CD and UC present with extraintestinal manifestations (i.e., arthritis, skin manifestations and eye diseases, anemia, pyoderma gangrenosum, primary sclerosing cholangitis, and nonthyroidal illness syndrome) to different extents.<sup>12–14</sup> Diagnosis should be based on a combination of history, physical examination, and in general is achieved by assessing laboratory (i.e., blood and fecal inflammatory markers), followed by endoscopic evaluation (esophago-gastro-duodenoscopy and ileocolonoscopy) with multiple biopsies of pathological lesions and Rx imaging. It is also clear that species belonging to the symbiotic gut microbiota are involved in the etiology and/or maintenance of inflammatory processes. Reduced microbial diversity, increased *Bacteroidetes* and *Enterobacteriaceae*, and decreased *Firmicutes* proportions were all observed in patients with IBD.<sup>15</sup> A clinical study reported that *Eubacterium rectale*, *Bacteroides fragilis*, *Bacteroides vulgatus*, *Ruminococcus albus*, *Ruminococcus callidus*, *Ruminococcus bromii*, and *Faecalibacterium prausnitzii* were 5- to 10-fold more abundant in healthy subjects than in patients with CD, while *Enterococcus* spp., *Clostridium difficile*, *Escherichia coli*, *Shigella flexneri*, and *Listeria* spp. were more abundant in the CD group.<sup>16</sup> Heritability of IBD seems to arise from the contribution of hundreds of common gene variations. One hundred sixty-three IBD susceptibility loci were characterized and related to 300 known genes, associated with cytokine production, lymphocyte activation, and response to bacterial infection, including nucleotide

oligomerization domain-2, interleukin (IL)-10 and CARD9 network, suggesting a close relationship between IBD and genes related to host–bacteria interaction and anti-inflammatory response.<sup>17</sup> In patients with intestinal inflammation, several events contribute to increase bacterial exposure, including disruption of the mucus layer, dysregulation of epithelial tight junctions (TJs), increased intestinal permeability, and increased bacterial adherence to epithelial cells. In IBD, innate cells produce increased levels of pro-inflammatory cytokines (i.e., tumor necrosis factor- $\alpha$ , IL-1 $\beta$ , IL-6, IL-12, IL-23) and chemokines. A marked expansion of lamina propria occurs, with an increased number of CD4<sup>+</sup> T cells, especially pro-inflammatory T-cell subgroups, which secrete increased levels of cytokines and chemokines with recruitment of leukocytes and chronic inflammation.<sup>18</sup> Medical treatment of IBD is personalized. The choice of drugs and route of administration (oral, rectal, injection, and infusion) relies on several factors, including type, localization, and severity of disease, as well as other historical and biochemical prognostic factors, and patient preferences.<sup>11</sup> Traditionally, depending on level of severity, IBD may require immunosuppression to manage symptoms, and anti-inflammatory steroids are used for controlling disease flares. Severe cases may require surgery, such as bowel resection, strictureplasty, or temporary or permanent colostomy or ileostomy. A relatively new treatment option is fecal bacteriotherapy<sup>19</sup> and fecal microbiota transplantation, which have been successfully used in a few cases.<sup>20,21</sup> Infections may contribute to IBD in some patients and, indeed, they may benefit from antibiotic therapy.<sup>22</sup> At present, therapeutic approaches to IBD focus on contrasting the effect of specific proinflammatory cytokines, such as tumor necrosis factor- $\alpha$ , inhibiting the entry of cells into intestinal tissues, and stimulating T-cell activation and proliferation. Additional investigational biological therapies include the blockade of costimulatory signals enhancing interactions between innate cells and adaptive cells, administration of epithelial growth factors, and enhancing tolerance through a variety of mechanisms.<sup>23</sup>

## IBS Risk, Prevention, and Disease Management

IBS affects about 5% to 20% of people worldwide.<sup>24</sup> Diagnosis is based on symptoms of chronic abdominal pain, discomfort, bloating, and alteration of bowel habits, in the absence of overt organic disease. Diarrhea or constipation may predominate, or they may alternate. Although there is no cure for IBS, there are treatments that attempt to relieve symptoms, including dietary adjustments, medication, and psychological interventions.<sup>24</sup> The most common theory on IBS etiology states that IBS is a disorder of the interaction between the brain and the GI tract, although it has been advocated that, at least in some cases, abnormalities in the gut microbiota are implicated in inflammation and altered bowel function.<sup>25,26</sup> Younger age, prolonged fever, anxiety, depression, and history of childhood physical and psychological abuse are often associated with the development of IBS after acute infectious gastroenteritis.<sup>27–29</sup>

Observations have identified a postinfectious variant of the syndrome, possibly associated with a reduction in microbiota diversity due to antibiotic use.<sup>30</sup> It has been shown that patients with IBS have fewer intestinal Bifidobacteria, *Collinsella aerofaciens*, *Coprococcus eutactus*, and *Clostridium cocleatum*, and an increase in Veillonella and Enterobacteriaceae.<sup>31–34</sup> Some studies have focused on protozoal infections as a cause of IBS.<sup>35–37</sup> In particular, 2 protozoa frequently observed in patients with IBS (*Blastocystis hominis* and *Dientamoeba fragilis*) have a high prevalence in industrialized countries, although their importance in IBS is still controversial.<sup>36–38</sup>

It has also been shown that a low FODMAP (Fermentable, Oligo-, Di-, Mono-saccharides, and Polyols) diet reduces IBS symptoms by a figure of 60% to 80%.<sup>39</sup> This diet restricts various carbohydrates that are poorly absorbed in the small intestine, as well as fructose and lactose, reducing IBS symptoms in a dose-dependent manner, while its effects on gut microbiota composition are still not fully understood.<sup>40–42</sup>

Probiotics may also have positive effects on the gut–brain axis in IBS, by countering the effects of stress on gut immunity and function.<sup>43</sup> Probiotics may exert their beneficial sequelae through preserving the gut microbiota, normalizing cytokine blood levels, improving the intestinal transit time, decreasing small intestine permeability, and treating small intestinal bacterial overgrowth of fermenting bacteria.<sup>44</sup> Drugs affecting the gut serotonin may reduce symptoms<sup>45</sup> and similarly, selective serotonin re-uptake inhibitors, frequently prescribed for panic and/or anxiety disorder and depression, affect the gut and brain serotonin and seem to improve symptoms and promote the global well-being of some patients with IBS.<sup>46</sup>

Gut activities are locally controlled by the enteric nervous system, a complex network of neurons along the lining of the esophagus, stomach, small intestine, and colon and connected with the brain by the vagus nerve, sacral parasympathetic and sympathetic afferents (Fig. 1).<sup>47</sup> Indeed, negative emotions (e.g., sadness, fear, and anger) are often associated with the development of acute GI infections.<sup>48</sup> Conversely, chronic GI inflammation exerts multiple effects on mood. Risk factors for the development of IBS indeed include adverse life events, depression, and fretfulness.<sup>47</sup> The gut microbiome synthesizes a vast array of neuroactive molecules including true neurotransmitters, such as serotonin, gamma-aminobutyric acid catecholamines, histamine and acetylcholine, and through fermentation, a panoply of short-chain fatty acids (SCFAs), and tryptophan, all of which have established and unclear effects on the nervous system.<sup>49</sup> As an example, gamma-aminobutyric acid is synthesized by many bacteria, especially *Lactobacilli*.<sup>50</sup> Direct and indirect effects of the gut microbiome on the intestinal epithelium, local mucosal gut-associated lymphoid tissue and their cytokines, enteroendocrine cells, and enteric nervous system collaborate to affect the afferent neuronal pathways to the brain. By means of complex interactions on the hypothalamic–pituitary–adrenal axis and, in particular, the central nervous system target structures, the gut microbiota influences both cognition and mood.<sup>51</sup> When the brain is alerted to

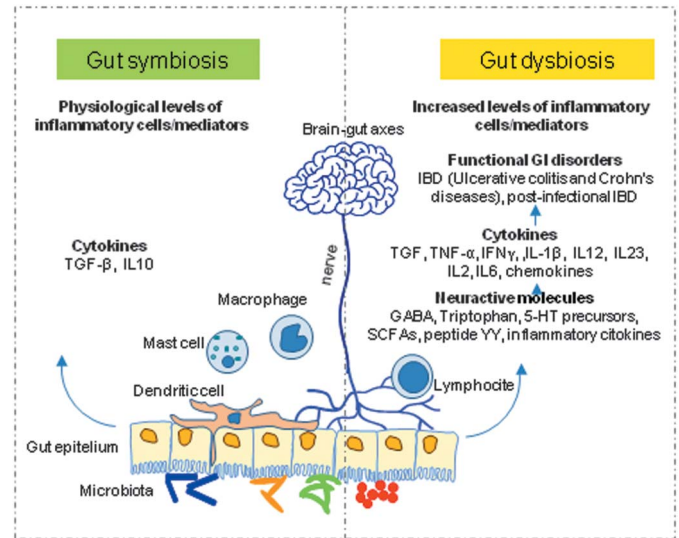


FIGURE 1. Key pathways involved in microbiota–gut–brain signaling. The gut microbiota can modulate the gut–brain axis through many pathways including endocrine, immune (cytokines), and neural (vagus nerve and enteric nervous system) pathways. Gut dysbiosis leads to increased levels of inflammatory cells/mediators. The modulation of systemic tryptophan levels is strongly implicated in relaying the influence of the gut microbiota to the brain. In addition, SCFAs are neuroactive bacterial metabolites from dietary fibers possibly modulating the brain and behavior.

inflammation by the IS through cytokines, the brain releases stress hormones and neurotransmitters, leading to a variety of neurological and psychiatric symptoms (e.g., anxiety, obsessions, compulsions, depression, fatigue, anhedonia, inability to sleep, etc.). Factors increasing the intestinal barrier permeability, with the breakdown of mucosal IgA and TJs, trigger IS abnormalities and autoimmunity, and influence the blood–brain barrier, resulting in neuroimmunity.<sup>51</sup>

## DISENTANGLING THE GORDIAN KNOT OF THE IBD–IBS DISEASES

### The Human Microbiota and the “Hygiene Hypothesis”

It is generally accepted that humans are born with a sterile gut.<sup>8</sup> However, new evidence suggests that colonization of the GI tract starts before birth, with the fetus ingesting amniotic fluid containing microbes.<sup>52</sup> Intestinal colonization is acquired during the first months of life, with aerobic and facultative anaerobic colonization, followed by obligate anaerobes and Bifidobacteria.<sup>53</sup> Establishment of the gut microbiota is recognized as a complex process influenced by factors at the level of the host and microbes themselves.<sup>54</sup> Humans, similar to all animals, are continuously exposed to a broad spectrum of intestinal microbes, including bacteria, eukaryotes, viruses, and archaea over millions of years of evolution.<sup>55,56</sup> High-throughput sequencing studies have

depicted bacterial diversity in the human microbiota and established that healthy individuals harbor distinct communities of bacteria dominated by Bacteroidetes and Firmicutes phyla.<sup>57–60</sup> Although sparsely studied, eukaryotes, including fungi, protists, and helminths, have appeared as part of our co-evolved intestinal community and all are components of the healthy microbiome.<sup>61</sup> The hygiene hypothesis argues that co-evolved microbial symbionts are important to human health.<sup>56</sup> The gut microbiota composition has been altered dramatically by adoption of highly hygienic habits, shifts in diet toward sterilized and processed foods, and use of antimicrobial drugs.<sup>62</sup> These aspects of modern lifestyles have reduced the diversity of components of microbiota, including bacteria,<sup>62,63</sup> worms,<sup>56</sup> and protists,<sup>61</sup> altogether resulting in “defaunation” of the human intestine. The distribution of autoimmunity and inflammatory diseases is tightly correlated with a transition to modern lifestyles,<sup>64</sup> and altered microbiota accompanying this transition is an important risk factor for diseases.<sup>64,65</sup> The hygiene hypothesis predicts that increased hygiene, use of antibiotics, and sterile food preparation result in isolation of the IS from positive microbial exposure, favoring susceptibility to immune-mediated disorders.<sup>66</sup> Absence of exposure to intestinal helminths and eukaryotic commensals (i.e., *Blastocystis* and *Dientamoeba*) has been recognized as a risk factor for allergic/autoimmune diseases including IBD.<sup>67–69</sup> Several mechanisms underlie the hygiene hypothesis: (1) lack of microbial burden in childhood, predisposing the host to allergic disorders due to a Th1/Th2 (T helper cells) imbalance; (2) defective maturation of regulatory T cells, as a consequence of modern lifestyles; (3) antigenic competition from infectious agents inhibiting responses to weak antigens; protection from allergic diseases through mechanisms independent of their constitutive antigens, leading to stimulation of nonantigen-specific receptors; and (4) development of an aggressive immune response caused by genetic hyperimmunity, which is also triggered by dysbiosis.<sup>70</sup>

## Dysbiosis in IBD and IBS: from “Extended” Microbiota Genotypes to Tissue Hypothesis Substantiation

### Homeostasis

Gut microbiota has several metabolic functions including production of vitamins and SCFAs, amino acid synthesis, bile acid biotransformation, hydrolysis and fermentation of nondigestible substrates and endogenous mucus.<sup>71</sup> Bacterial fermentation takes place in the cecum and colon, where SCFAs are absorbed, stimulating the absorption of salts and water and exerting a trophic effect on the intestinal epithelium (Fig. 2).<sup>72</sup> Commensal organisms prevent pathogenic colonization, competing for attachment sites and nutrients and secreting antimicrobials.<sup>4</sup> These mechanisms are relevant for reducing the level of bacterial products detrimental to the host (i.e., lipopolysaccharides, peptidoglycans, bacterial CpG-DNA motifs, and superantigens).<sup>73</sup> The indigenous microbiota is also essential for the development of IS, directly, by modulating regulatory T cells (i.e., T helper type 1 and 2 cells, and

T helper 17 cells),<sup>74–76</sup> or indirectly through the immunomodulatory effect of SCFAs.<sup>77,78</sup> Interestingly, germ-free mice display underdeveloped lymphatic systems, with fewer Peyer’s patches and isolated lymphoid follicles<sup>79,80</sup> and fewer intestinal dendritic cells.<sup>81,82</sup> The intestinal mucosa prevents injuries by signaling the innate IS through pattern recognition receptors, such as Toll-like receptors and nucleotide oligomerization domain-like receptors.<sup>83</sup> Pattern recognition receptors recognize and bind to microbes-associated molecular patterns,<sup>83</sup> including lipopolysaccharides, flagellin, peptidoglycan, and N-formylated peptides. Pattern recognition receptor activation initiates NF- $\kappa$ B pathways, mitogen-activated protein kinases, and caspase-dependent signaling cascades, leading to protective peptides, cytokines, chemokines, and phagocytes, triggering apoptosis, protecting response to commensal bacteria, and triggering inflammatory response to pathogens.<sup>84</sup>

Goblet cells secrete a layer of mucus limiting the exposure of intestinal epithelial cells to bacteria. Both the secretion of antimicrobial peptides (e.g.,  $\alpha$ -defensins) by Paneth cells and the production of immunoglobulin A (IgA) provide additional protection from luminal microbiota.<sup>82</sup> In healthy individuals, the lamina propria normally contains a diverse array of immune cells and secreted cytokines, including the anti-inflammatory mediators TGF- $\beta$  and IL-10 that down-regulate immune responses. In addition, pro-inflammatory mediators from both innate and adaptive immune cells limit excessive entry of intestinal microbiota and defend against pathogens.<sup>23,84</sup> Noninflammatory defenses, including phagocytosis by macrophages, probably assist against bacteria entering the lamina propria, minimizing tissue injury. A homeostatic balance is maintained between regulatory T cells and Th1, Th2, and Th17 effector cells.<sup>23</sup> The mucus layer, reflecting the balance between mucus secretion and bacterial degradation, represents an obstacle to the uptake of antigens and pro-inflammatory molecules.<sup>85</sup> Some evidence suggests that butyrate reinforces the colonic defense barrier by inducing secretion of mucins, trefoil factors, and antimicrobial peptides.<sup>86</sup> Some bacterial communities could strengthen the barrier at TJs and may be involved in cell and tissue development, regulating cell growth and differentiation.<sup>87</sup> Finally, indigenous microbes shape the development of the villi microvasculature, as demonstrated in germ-free animals colonized during or after the completion of postnatal gut development (Fig. 2).<sup>88</sup>

### Homeostasis Breakdown

When medications, psychological and physical stresses, radiation, abnormal peristalsis, diet, and other factors alter the microbiota composition, bacterial metabolic activity and/or local community distribution are also affected, triggering dysbiosis with a serious consequence to human health (Fig. 2).<sup>66</sup> Chronic inflammation may cause activation of IS, such as in obesity, type 2 diabetes, and nonalcoholic fatty liver disease. Indeed, plasma endotoxins are higher in patients with nonalcoholic fatty liver disease and are associated with small intestinal bacterial overgrowth and induction of hepatic Toll-like receptor-4.<sup>89,90</sup> Hence, gut permeability, which influences the systemic distribution of endotoxins, may further induce metabolic endotoxemia through



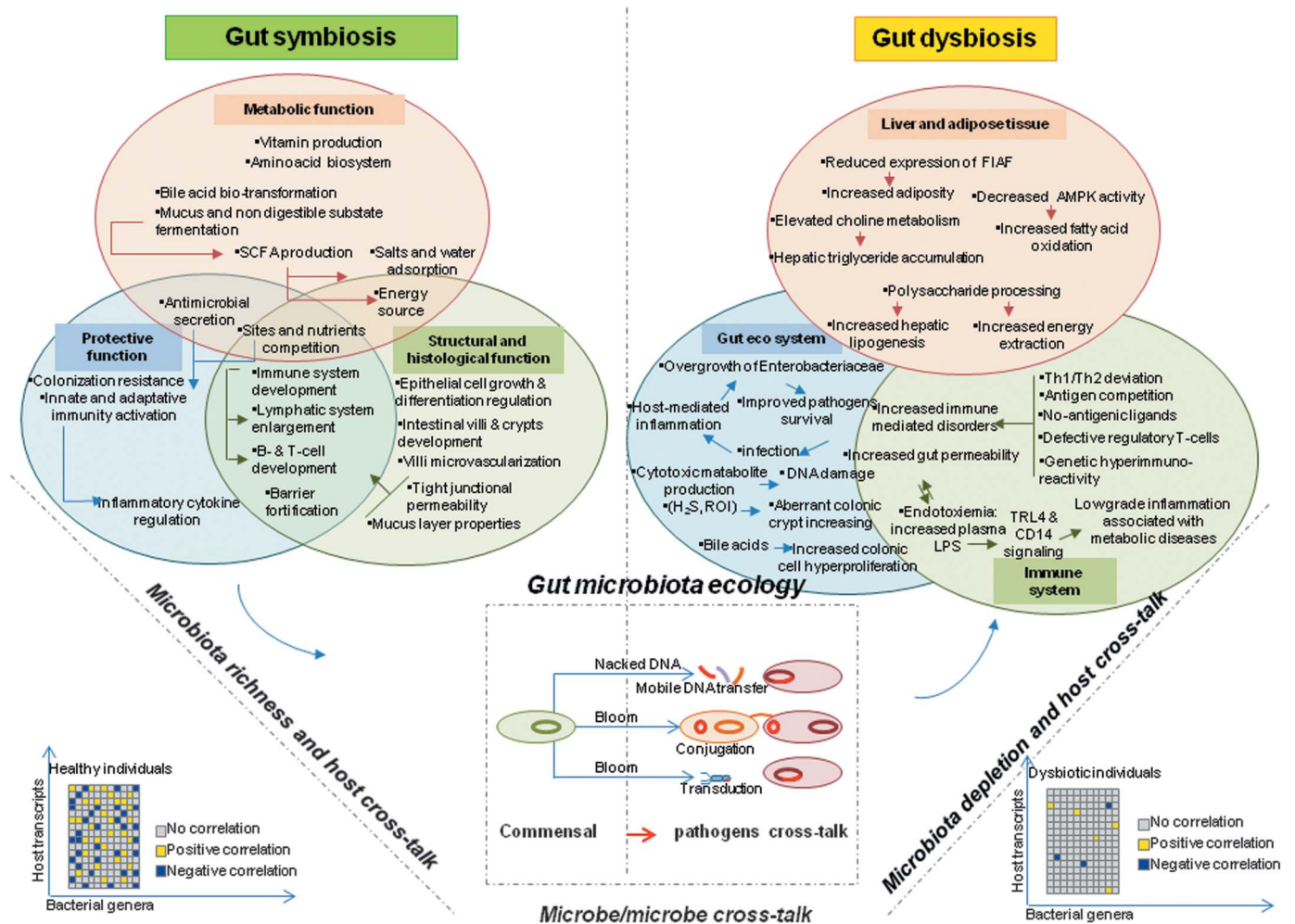


FIGURE 2. Microbial, metabolic, and cellular mechanisms' agents of symbiosis–dysbiosis shift under the host–microbe and microbe–microbe interplays. Gut microbiota has several metabolic, protective, structural, and mucosal functions. When symbiosis switches to gut dysbiosis, the imbalance involves the liver, adipose tissue, and the IS, and the gut ecosystem loses many bacterial species altering homeostasis.

disruption of TJ proteins.<sup>91</sup> Pathogenic infections might be facilitated by disruption of the intestinal ecosystem by environmental factors. Models of *Citrobacter rodentium*, *Campylobacter jejuni* infection, and chemically and genetically induced models of intestinal inflammation have shown overgrowth of Enterobacteriaceae in all models, indicating that inflammation-induced microbiota changes support colonization by aero-tolerant bacteria.<sup>92</sup> The inflammatory response, triggered by the invading pathogen, may function to enhance its colonization, further facilitating its virulence. Thus, an alteration of the gut microbiota, initiated by host and environmental factors, may participate in initiating diseases caused by infectious agents (Fig. 2). Because of the inherent plasticity of microbiota, derangement of gut barrier functions and metabolic activity could be exploited to develop biotherapeutics. Mechanisms of pre- and pro-biotics include remodeling of microbial communities and suppression of pathogens, suppression of pro-inflammatory factors, effects on epithelial cell differentiation, and proliferation and promotion of the intestinal barrier<sup>93</sup>

with controversial effects on IBD, metabolic syndromes, immunomodulation, and pathogen defense.<sup>94–96</sup> An increase in Bifidobacteria induced by nutritional supplements results in improved gut barrier, lower portal lipopolysaccharide levels, and lower inflammatory tone in ob/ob mice (Fig. 2).<sup>97</sup>

### Microbe–microbe Interactions, Blooming, Contraction and Pathobiont Selection

The dense bacterial communities inhabiting the distal gut compete for a limited quantity of diet-derived or host mucus-derived carbohydrate available for fermentation.<sup>98</sup> Diet modifications alter the microbial community structure, while maintaining the dominance of obligate anaerobic *Clostridiaceae* and *Bacteroidaceae* over Enterobacteriaceae.<sup>99,100</sup> Enterobacteriaceae are unable to compete with obligate anaerobic bacteria for high-energy nutrients to support their growth by fermentation, with a disadvantage in acquiring fermentable nutrients during anaerobic growth. Indeed, *Clostridiaceae* and *Bacteroidaceae* use

glycoside hydrolases to hydrolyze complex carbohydrates, binding proteins to recognize carbohydrates at their surface, and active transport systems to import released oligosaccharides against a concentration gradient. By contrast, a paucity of secreted glycoside hydrolases makes Enterobacteriaceae ill-equipped to degrade complex carbohydrates, only relying on oligosaccharides passively transported across the barrier. This might partially explain the dominance of obligate anaerobic *Clostridiaceae* and *Bacteroidaceae* over the facultative anaerobic Enterobacteriaceae in healthy gut, which is reverted during inflammation, when antimicrobials (i.e., lipocalin-2, LIP-2) and other reactive species are released by the intestinal mucosa, favoring the propagation of Enterobacteriaceae, which can withhold nutrients with iron or zinc siderophore activity.<sup>101</sup>

When stimulated with pro-inflammatory molecules, the intestinal epithelium produces antimicrobials' reactive oxygen species, reactive nitrogen species, and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), superoxide radicals (O<sub>2</sub><sup>-</sup>), and nitric oxide synthase, thus changing dramatically the luminal environment.<sup>102–104</sup> Nitric oxide concentrations increased in colonic gas of patients with IBD, modifying the luminal environment of the large bowel.<sup>105</sup> Although the production of reactive nitrogen species and reactive oxygen species creates a hostile environment in close proximity to the mucosal surface, the generation of these radicals has important side effects. In fact, as reactive nitrogen species and reactive oxygen species are converted into non-toxic products, their by-products, S-oxides, N-oxides, and nitrates open new metabolic alternatives, favoring the growth of facultative anaerobic microbes including Enterobacteriaceae.<sup>98,106</sup> Therefore, after perturbations, the gut microbiota ecosystem can shift to a state of dysbiosis, in which commensal protective function, structural and histological role, and metabolic activities manifest impaired concerted mechanisms (Fig. 2). This can involve overgrowth (blooming) of otherwise under-represented or potentially harmful bacteria (i.e., pathobionts), induced by intrusion or disappearance of individual members (i.e., invading bacterial strains during maturation of infant gut microbiota)<sup>107</sup>; shifts in relative bacterial abundances by external stimuli; and mutation or horizontal gene transfer.<sup>8</sup> These alterations affect significantly the overall functionality of microbiota, by enhancing the fitness of certain keystone pathogens or keystone stabilizers.<sup>101</sup> Similarly, some pathobionts such as *Salmonella* spp. acquire virulence factors,<sup>108</sup> while symbiotic *E. coli* str. NC101, presenting with polyketide synthase-encoding genotoxic island, adheres to gut mucosa and blooms in patients with IBD.<sup>109</sup> Under antibiotic-induced growth, multidrug-resistant *E. coli* pathobionts (i.e., against ampicillin and neomycin) induce sepsis-like disease in antibiotic-treated mice through activation of the NAIP5–NLRC4 inflammasome route.<sup>110</sup> Interestingly, *Enterococcus* spp., *Streptococcus* spp., and Gammaproteobacteria members undergo gut blooming during allogeneic hematopoietic stem cell transplantation.<sup>111</sup> Disease-driven blooming has also been observed for adherent and invasive *E. coli*, with a higher prevalence within the ileal mucosa in patients with CD.<sup>112</sup>

## Host–microbe Signaling Interactions

Gut microbiota, with their metabolites and scaffold proteins, modulate key signaling pathways involved in intestinal mucosa inflammation, but the underlying molecular mechanisms of host–microbiota interactions are still unclear. When the balanced interaction between the GI tract and resident microbiota is disrupted, intestinal and extraintestinal diseases such as IBD develop.<sup>113</sup> Genetic and environmental changes of gut microbiota may contribute to defective host immune response. Indeed, both quantitative and qualitative levels of microbial dysbiosis have been reported in IBD.<sup>65</sup> The impact of gut microbiota on gut and systemic immune homeostasis has gained tremendous research interest over the last few years. Particular attention has been focused on the effects of a dysbiotic microbial community, which is characterized by increased intestinal mucosal-adhesive microbes and by intestinal mucosal barrier defective function.<sup>114</sup> Imbalance of barrier integrity, with increased antigen and bacterial uptake, is considered important to the pathophysiology of several intestinal disorders including IBD.<sup>115</sup> Epithelial barrier integrity is necessary for the maintenance of correct intestinal nutrient absorption while shielding the body from the gut lumen content, including dietary antigens and microbial products.<sup>116,117</sup> Epithelial integrity can be assessed by measuring electrical resistance of the mucosa, transmucosal flow of fluorescent molecules, or by analyzing the TJ integrity and the actin cytoskeleton shape.<sup>118</sup> The intestinal barrier is primarily regulated by the apical junctional complex consisting of TJs and adherens junctions. Intestinal junctions are selectively permeable, and intestinal permeability can be increased physiologically in response to luminal nutrients or pathologically by mucosal immune cells and pathogens.<sup>118</sup> Compromised intestinal barrier function is associated with an array of clinical conditions, both intestinal and systemic, including IBD and IBS.<sup>119–121</sup> The intestinal microbiota lives in intimate contact with the surrounding intestinal wall and both determine the gut health status. It is already known that enteric pathogens are able to modify intestinal permeability by affecting specific TJ proteins.<sup>114,122,123</sup> On the other hand, commensal and probiotic bacteria are known to improve the intestinal barrier functioning.<sup>124</sup> However, the presence of dysbiosis, as in patients with IBD where the proportions of intestinal microbiota are changed, alters host permeability by negatively influencing the junctional complex functioning.<sup>125–128</sup> The effects of the dysbiotic microbial community on cell permeability, in particular on junction complexes, are still unclear. Recent evidence suggests that microbiota modulates the actin cytoskeleton, which has a major role in assembling and maintaining cell junctions.<sup>122,129,130</sup> Stress fibers (SFs) are often the most prominent cytoskeletal structures in cells growing in tissue culture. Attention is growing on the effect of exogenous agents in inducing SF assembly and organization in cells. Very little is known about the relationship between bacteria and SF formation in the intestinal epithelial cells. The cytotoxic necrotizing factor 1, a protein from pathogenic *E. coli*, induces actin reorganization into stress and retraction fibers in human epithelial

cultured cells,<sup>131</sup> while in contact with some *E. coli* strains, the correct polarization of the epithelial cells is compromised by a cytoskeleton rearrangement due to the assembly of the SFs.<sup>132</sup> Further investigation is needed to analyze this issue in detail and to test the hypothesis that altered SF formation and organization can be used as markers of intestinal inflammation. Commensal microbiota plays an important role in regulating the expression of genes involved in some intestinal functions and in maintaining immune homeostasis. MicroRNAs (miRNAs) are highly conserved among species and seem to play a major role in both innate and adaptive immunity, as they can control the differentiation of various immune cells, as well as their functions. However, it is still largely unknown how microbiota regulates miRNA expression, thereby contributing to gut homeostasis and pathogenesis of intestinal inflammatory diseases. A recent concern has emerged about a possible role of epigenetic mechanisms on pathogenesis of chronic inflammatory disorders. Emerging data show that miRNAs are critical regulators of both innate and adaptive immune responses<sup>133</sup> and can suppress functional targets, including epithelial barrier function regulatory genes, hence playing a critical role in controlling the key pathogenic mechanism in IBD.<sup>133,134</sup> To further stress this relationship, recent results have shown that commensal microbiota affects miRNA expression.<sup>135,136</sup> However, to what extent miRNAs are implicated in microbiota-mediated host gene regulation and, in particular, in the pathogenesis of IBD is yet unclear.

Transcriptional profiles of the mucosa actively interact with colonic microbiota while this relationship is lacking in the colon of patients with UC.<sup>137</sup> Bacterial metabolism, such as butyrate production, directly affects mucosal gene expression. Lepage et al<sup>137</sup> reported of bacterial crosstalk between host and microbiota as a prominent feature of UC. The differentially overrepresented bacteria in the microbiota of patients with UC mostly consist of potentially pathogenic aerobic genera (*Rhodococcus*, *Shigella*/*Escherichia*, and *Stenotrophomonas*). Together with an increased level of transcripts related to the extracellular matrix, this suggests that the presence of these bacteria could result from a defect in barrier function in the UC gut epithelium, where they contribute to establish a vicious circle sustaining the inflammatory process. In particular, several butyrate-producing bacteria are more abundant in healthy controls than in patients with UC. Defective colonic epithelial oxidation of butyrate in UC has been implicated in the disease's pathogenesis.<sup>138</sup> Lepage et al<sup>137</sup> have shown a correlation between the abundance of *F. prausnitzii* and decreased mucosal expression of genes (e.g., MAP3K8, metallothionein) that is associated with immune and oxidative stress response in patients with UC, compared with their unaffected cotwins, although the genetic background was influencing the gut microbiota composition and diversity. Microbiota of monozygotic twins was more similar to that of dizygotic twins and unrelated individuals, suggesting a major role of genetic background in the presence of a comparable early environment. Surprisingly, microbiota similarity was still high in monozygotic twins discordant for UC. However, the microbiota of unaffected siblings from UC twin

pairs exhibited higher percentages of potentially protective bacteria, which could play a protective role in a deleterious environment. Unaffected siblings from UC-discordant pairs also showed less bacterial biodiversity in respect to healthy individuals, further supporting the heritability issue. Twins discordant for UC had different gene expression profiles, affected patients having lower proportions of potentially protective species.<sup>137</sup>

Together with a reduced complexity of mucosal microbiota, the observed loss of crosstalk between host gene expression and bacterial profiles suggests that these key elements, influencing disease manifestation and progression, are at least in part under genetic control. Human gut microbiota participates in epithelium maturation, host nutrition, protection against pathogens and regulation of gut epithelial cell proliferation,<sup>139</sup> host energy metabolism,<sup>140</sup> and immune responses.<sup>76</sup> The mucosa senses pathogens and is unresponsive to food antigens and commensals, thus maintaining the integrity and normal function of the intestine.<sup>141</sup> Gut immune and inflammatory responses involve the transcription factor NF- $\kappa$ B, which through a highly conserved regulatory pathway drives expression of genes involved in pro-inflammatory processes at the site of infection or tissue damage, also controlling cell survival, proliferation, and differentiation induced by a wide range of noxious stimuli.<sup>142</sup> NF- $\kappa$ B signaling is a critical element of gut homeostatic immuno-inflammatory function and both its deficiency and hyperactivation are linked with chronic IBD.<sup>143–145</sup> Lakhdari et al,<sup>146</sup> by means of the high-throughput functional screening of metagenomic libraries, explored the novel NF- $\kappa$ B modulatory potential within human intestinal microbiota, opening a new strategic path toward the identification of bacterial strains and molecular patterns presenting potential therapeutic interest.

## Modulating the Microbiota Through Nutritional Interventions

Microbiota is modulated through diet and nutritional habits<sup>147,148</sup> even if, as the composition of gut microbiota seems to be rather stable over long periods of adulthood,<sup>149</sup> its richness may be individually different. Reduced richness of gut microbiota has been found in patients with IBD.<sup>137,150,151</sup> Reference genome mapping has allowed the assessment of the different phylogenetic composition of microbial communities, hence providing the description of low gene content and high gene content individuals, displaying significant differences in several metabolic pathways.<sup>147</sup> In particular, the 2 groups differed in terms of SCFA production and mucus degradation potential, hydrogen/methane/hydrogen sulphide production potential, oxidative stress management potential, and *Campylobacter/Shigella* abundance, suggesting that low gene content individuals harbor inflammation-associated microbiota. In another study,<sup>148</sup> the impact of an energy-restricted high-protein diet on the gut microbiome was investigated. After consuming an energy-restricted diet, gene richness significantly increased in the low gene content group, while in the high gene content group, no significant change in gene richness or diversity was found over the course of the study. Increases in gene richness were significantly associated



with decreases in total fat mass, hip circumference, total cholesterol, and LDL cholesterol, supporting the hypothesis that correcting microbial richness may result in improvements in metabolic derangements.<sup>148</sup>

Given that the human microbiome influences the risk of developing diseases,<sup>152</sup> can we modulate the microbiome to a health baseline pattern and will this decrease the individual risk of developing diseases?

Research regarding the link between microbiome and disease has the potential to revolutionize the way of screening, diagnosing, and treating patients in the near future. It seems that gut microbial communities and ISs co-evolve over the lifespan. Scientists are collecting information on the way in which the metabolic phenotypes do reflect functions encoded in host genomes and gut microbiomes. Taken together, these observations raise the question of how the metabolism of consumed foods affects our ISs. The dietary intake of macronutrients and micronutrients shapes the microbial community structure, which, in turn, changes the nutritional value of the consumed food. The link between nutrient metabolism and IS occurs at several levels, ranging from endocrine signaling to direct sensing of nutrients by immune cells. For example, leptin, a pleiotropic cytokine, regulates appetite, modulating both innate and acquired IS,<sup>153,154</sup> while low butyrate levels modify the cytokine profile of Th cells<sup>155</sup> and promote intestinal epithelial barrier integrity.<sup>156</sup> A few metabolic sensors help to coordinate immune responses, when they are absorbed in the intestine as unmodified dietary components. They interact with immune cells or act as microbial signals in the form of microbes-associated molecular patterns that modify local mucosal immune responses through innate signaling pathways, such as the inflammasome or Toll-like receptors, as dietary components (i.e., SCFAs), providing signals using which the IS can monitor the metabolic activities of microbiota.<sup>157</sup>

## UNDERLYING PARADIGMATIC STRATEGIES TO PREVENT IBD AND IBS BY GUT MICROBIOTA DYSBIOSIS TRACKING

### Using -omics Suites for Gut Dysbiosis Data Generation and Integration: Technology Meets Diagnostics and Clinics

Direct or indirect biomarkers of nutritional status coupled to host and microbiota genetics and epigenetics should be investigated in collaborative research, aiming at correlating IBD/IBS onset and development from childhood to adulthood.

High-throughput technological platforms may assist in the generation of nontargeted and targeted metabolomics (MB), metaproteomics (MP), and metagenomics (MG) profiling from human biospecimens to define the relationship between disease phenotypes, host genetics and inflammation, nutritional status, and microbiome configuration. To identify shotgun metabolite scaffold (*untargeted level, discovery*) and at lower levels

(*targeted, validation*) IBD/IBS-related biomarkers, -omics scientists should use and validate either targeted or untargeted MB, overcoming the limitations of current applications.<sup>158,159</sup> For MP, shotgun label-free workflows with the minimal number of handling steps (*fast analysis*) and maximal sensitivity (*broad and deep analysis*) might be used for high-throughput and in-depth characterization of fecal and mucosa metaproteomes. Handling of samples can be performed according to procedures previously optimized,<sup>160,161</sup> while pipelines, such as multiple-reaction monitoring–based or selected reaction monitoring–based targeted MP, can be set up in homebrew workflows and, furthermore, optimized by validation steps.<sup>162</sup> Selected reaction monitoring is a targeted high-throughput mass spectrometry technique for accurate and robust multiplexed quantification of several tens of proteins in complex samples, ideally adapted to validate sets of biomarker candidates over large sample cohorts. This technology has been used successfully for assessing diagnostic and prognostic cancer biomarkers.<sup>162,163</sup> Using selected reaction monitoring, the selected signals can be confirmed and quantified in specimens from patients with diverse forms of IBD/IBS and from controls. The number of samples should be settled as the best compromise between cost limitation and technical and statistical robustness<sup>164–171</sup> and represents an unprecedented large-scale deciphering of MP. The MP strategy allows the analysis of whole proteomes of half of a patient subset with CD to discover global signatures without an a priori approach (shotgun label-free proteomics) and to confirm these signatures in the remaining half through a high-throughput targeted and independent approach.<sup>161</sup> Untargeted label-free liquid chromatography–mass spectrometry (LC-MS/MS)–based shotgun proteomics aims at discovering *remarkable IBD/IBS-associated functional patterns* in fecal microbiota and provides a global qualitative and semiquantitative view of gene expression levels under health and disease, without any a priori assumption of the metabolic and cellular functions of patients with IBD/IBS. The pipelines may rely on high-resolution tandem MS instrumentation and benefit from recent advances in this field. Large aliquot parts of trypsin digests of proteomes extracted from bacterial communities can be injected into ultra performance liquid chromatography and acquired MS/MS spectra interpreted by interrogating annotated metagenomic databases, specifically reorganized to increase the efficiency of peptide identification and quantification (e.g., X!TandemPipeline and MassChroq open-source softwares developed by INRA, PAPPISO Platform, <http://pappiso.inra.fr/bioinfo/>). Metaproteomic variables or clusters of variables, grouping and subgrouping participants into homogeneous clinical phenotypes, can be extracted using different statistical methods already implemented in large-scale programs (e.g., MetaHit) or generated *ex novo*.<sup>164–171</sup>

Among the host-state-associated protein signals extracted above in *fecal microbiota*, the *targeted proteomics approach* may select proteins and representative peptides best adapted for confirmation through multiple-reaction monitoring, giving priority to peptides identified in label-free shotgun MS data and associated with target functions with biological plausibility, in the context of



IBD/IBS risk and onset. Similarly, in biopsies, *remarkable IBD/IBS-associated functional traits of mucosal microbiota* can be discovered by using untargeted proteomics. Label-free, gel-free LC-MS/MS-based shotgun proteomics allow untargeted approaches on biopsies to provide a global qualitative and semi-quantitative view of gene expression in health and disease, without any a priori assumption of metabolic and/or cellular functions accompanying IBD and IBS,<sup>172</sup> adapting original pipelines previously developed in animal models.<sup>163</sup> Remarkably IBD- and IBS-associated functional traits of mucosal microbiota can be confirmed then by using targeted proteomic approaches. Among the associated extracted protein signals, -omics scientists can select proteins and their representative peptides through multiple-reaction monitoring experiments, giving priority to mucosal peptides targeting functions possibly associated with IBD and IBS phenotypes, by using advanced SWATH-related equipments, assisted by bioinformatic tools for high performing data interpretation, before uploading mucosal metaproteomes into advanced informatics infrastructure for secured data storage and archiving.

Also, fecal and urine samples could be analyzed for MB patterns, by gas chromatography–mass spectrometry, ultra performance liquid chromatography–high-resolution MS, ultra performance liquid chromatography–MS/MS, and proton nuclear magnetic resonance (<sup>1</sup>H-NMR)–based processes' protocols in both untargeted and targeted modes (multi-analyte detection), to define volatile and nonvolatile organic compounds. Moreover, lipids and pro-inflammatory proteases can be detected directly in biopsies by time-of-flight secondary ion MS imaging, again to establish without any a priori assumption key metabolic and/or cellular functions accompanying IBD and IBS mucosal microenvironments.

Finally, next-generation sequencing by full-MG profiling can confer entire microbial gene scaffolds, abundances, clusters of orthologue groups definition, and gene representation of entire fecal microbiota contents, providing reference sequence databases, even for metaproteomes' annotations. Indeed, full microorganism sequencing may represent the reference MG method (*up level, discovery*), while specific gene-targeted MG (*bottom level*) can be performed as a diagnostic tool for bacteria, fungus, and parasite description. Diagnostic gene-targeted MG can cover the gut bacterial microbiome (16S rRNA), parasitome and the so-called eukaryotome (18S rRNA),<sup>173</sup> and mycetome (ITS 1-4 fungal markers and 25S-28S rRNA) components. Remarkably, bottom level MG can be used to describe phylotypes from either fecal or mucosal microbiota, cataloguing relative operational taxonomic units abundances at different taxonomic levels, as microbiota fingerprinting in both diseased and healthy individuals.<sup>174</sup>

However, culturomics-based approaches also remain strategic to investigate the whole spectra of bacterial communities.<sup>175</sup> Indeed, based on a culturomic-based strategy and in vitro and in vivo pathogenicity assays, selected bacterial and yeast microorganisms, after harmfulness scaling characterization, can be tested at enterocytes' level, with respect to host inflammation

and immune response. Furthermore, invasiveness in vitro assays, antibiotic susceptibility, biochemical and substrate utilization tests, and virulence factor characterization can be used to define the phenotypical characterization of bacterial and yeast strains in either IBD or IBS gut mucosal microbiota. Therefore, the relationship between gut commensal microbiota and enterocytes<sup>176,177</sup> can be highlighted by evaluating the effects induced by the exposure of selected "harmful" versus "harmless" commensal bacteria on the morphological and functional features of enterocytes by in vitro investigation. Hence, amongst functional mucosal biomolecules, pro- and anti-inflammatory oxylipins (i.e., resolvins, protectins) and poly-unsaturated fatty acid metabolites can be characterized by hydrophilic interaction LC-MS/MS and nuclear magnetic resonance, generating metabolic profiling reflecting intra- or extra-cellular mucosal interplays between host and microbiota. Once isolated fractions of harmless microbes have been characterized, their potential application in food/pharma industries can be exploited,<sup>178</sup> with emerging systems biology approaches for probiotic value assessment.<sup>179</sup> Therefore, selected microbes, considered as harmless, can be characterized toward their potential industrial application. For selected bacterial strains, safety can be assessed *in silico* and in vitro, according to the European Food Safety Authority Guidelines (<http://www.efsa.europa.eu/fr/scdocs/doc/223.pdf>; <http://www.efsa.europa.eu/en/scdocs/doc/732.pdf>; <http://www.efsa.europa.eu/it/search/doc/2393.pdf>) and WHO Guidelines (<http://www.fda.gov/ohrms/dockets/dockets/95s0316/95s-0316-rpt0282-tab-03-ref-19-joint-faowho-vol219.pdf>) for their further potential evaluation in food.

Furthermore, genomics and metatranscriptomics approaches may assist to evaluate host genetic signatures associated with pediatric and adult IBD<sup>180</sup> and IBS.<sup>181</sup> Therefore, IBD- and IBS-related genetic loci, methylation profiles, and miRNA expression levels in inflamed versus non-inflamed gut mucosa and versus healthy controls can be highlighted by using SNP panels of selected loci for IBD and IBS from tissue samples. For patients with IBD, postzygotic variations can be detected and compared with zygotic variation in blood samples.<sup>182</sup> Indeed, mucosal postzygotic modifications, induced by microbiota–tissue biological diversity and metabolic change, could be regarded as overt IBD profiles.<sup>182</sup> Also, next-generation sequencing can be used to detect *de novo* mutations or mosaicism in sporadic patients without a priori hypothesis on specific mutated genes. Genome-wide methylation and transcriptomic profiles can be used to quantify differences in epigenetic profiles for patients with both IBS and IBD, by comparing unaffected versus affected gut mucosa at intraindividual and interindividual levels.

Finally, IBD and IBS candidate biomarkers in feces, biopsies, and urines can be identified based on MB, MP, MG, genomics profiles and metatranscriptomics discovery and confirmation experiments. Data from meta-omic analyses, diet and lifestyle records can be merged in descriptive and predictive statistical profiling to allow data mining. After defining IBD and IBS disease-related biomarkers, the multi-level integration of -omics data sets and markers can provide early dysbiosis profiles

and will lay out data, metadata structures, and algorithms needed for designing a multi-omics systems biology-based prevention program for either IBD or IBS.

The MG, MB, MP, genomics, transcriptomics and phenomics data sets, integrated into unified resources, such as web portals or a centralized bioinformatic service core, available to researchers and clinicians, because compliant with existing standards (<http://www.isa-tools.org/>), enable the deployment of the predictive profiling into effective prevention programs. A standard for generation, storage, and annotation of -omics data can be adopted, with a proactive action for alliance with the bio-sharing and translational resource communities (ISA Commons, RDA Alliance, and transSMART), in order that data and metadata formatting complies with most used schemes and sample metadata guidelines (e.g., Sequence Read Archive and Genomic Standards Consortium) and are submitted to on-line repositories including EBI MG services. In particular, we propose to adopt the open source ISA metadata tracking tools for the already available schemes for proteomics and possibly introducing ISA compliant descriptors (<http://www.isa-tools.org/>). In the derivation of statistically significant associations between MB, MP, MG data and clinical, physiological, immunological, genetic, and epigenetic data, standards also need to be adopted to describe the results for basic and advanced data analysis (e.g., richness, composition, and biodiversity, as well as meta-omics profiles).

The main expected result is a dysbiosis score-based tool (model and biomarkers, possibly described as multi-level network) aimed at predicting different gut dysbiotic types/profiles relying on multilevel -omics data, integrated with IBD and IBS clinical (phenomics) information. Starting from the unified resource, early dysbiotic patterns (e.g., trajectories leading to dysbiosis) will be differentially analyzed and stratified for main determinants of variability. The tool will use the identified predictive biomarkers and the disease phenotype to derive a score describing dysbiosis status on a continuous scale. Biological-clinical evaluation of the meta-omics profiles and additional data testing will be used in the validation phase. The resultant data sets will be deposited in annotated (“curated”) searchable databases, overcoming the issues generated by the difficult utilization of “big data” in translational preventive medicine (Fig. 4).<sup>183</sup> A major challenge will be to obtain cellular and molecular biomarkers for profiling of the innate and adaptive ISs, including biomarkers of mucosa-associated barrier function.

Given the small quantities of biomaterials available from biopsy specimens, especially in children, international clinical and -omics consortia will be necessary to foster the advancement of dysbiosis tracking as a preventive strategy or therapeutic recommendation in IBD and IBS control.<sup>113</sup> Clearly, microbiota composition and activity need to be complemented by host metabolic phenotype maps to unveil dysbiosis spectra in high-risk subjects and produce reliable disease risk assessment.<sup>184</sup> The idea is growing that metabolite diversity, possibly exceeding gut microbial diversity, should be investigated with respect to endogenous inflammation, mediated by environmental *stimuli*, developing

a new frontier of metabolics-immunology integrated at the level of microbial or microbial-mammalian co-metabolites and host response in the onset and development of disease (Fig. 3).<sup>185</sup> Also direct and indirect biomarkers of nutritional status<sup>186</sup> coupled with host and microbiota genetics and epigenetics should be investigated to fully consider IBD/IBS onset and development from childhood through adulthood.

Tracking of gut dysbiosis, assisted by assembled evidence from host genetics, host transcriptomics, gut microbiota composition and function, and their interactions will contribute to develop novel preventive concepts, discover dysbiosis biomarkers, and provide innovative tools for IBD/IBS risk detection, targeted prevention, and therapy in the framework of a system medicine approach. To fill existing gaps into the new integrated -omics framework on the gut microbiome and IBD/IBS host inflammation mechanisms, researchers and clinicians should extensively investigate disease indicators.

To fill existing gaps into this new -omics framework on the gut microbiome and IBD/IBS inflammation mechanisms, an integrated approach is needed, including discrete characterization of microbiome gene clusters,<sup>185</sup> tissue-targeted metabolite and lipidomic mapping, selectively fungi–host and fungi–prokaryotes interaction pathways by MS imaging, transcriptional host and microbial networks but also small-molecule phenocopies, gene clusters, important actors in the host–microbiota interplay, testing microbes, and metabolites as the new candidates of dysbiotic tracking.<sup>187–190</sup>

### Laboratory, Clinical Data, and Knowledge Management: From Biobank to Databank Through Open Access Integrated Data

To manage big data, anonymized clinical contents (phenomics) should be made available by setting specific Open Data endpoints able to monitor data sources (data producing facility, data type, and data format) and data processing (data collection, filtering, transformation, sharing and routing functions required to classify and assign disease phenotypes) before integration.

The data management must guarantee also data sharing, open (or not) mining, platform curation and preservation, as previously experimented by the Metagenopolis project (<http://www.mgps.eu/index.php?id=homepage>). A preliminary design of the proposed data flow, available to both clinicians and scientists, is provided in Figure 4. A centralized “datastore” can collect and filter data from data generators (-omics, meta-omics, diagnostic laboratories, and clinical centers, through electronic case report forms) and can communicate them to the data analysis platforms (Fig. 4). In this respect, Bambino Gesù Children’s Hospital (OPBG) has started reference work for the description of extended microbiota genotypes associated with a programming phase during growth and chronic diseases.<sup>191</sup> The initiative represents one of the first attempts to translate into the clinical practice MG and more generally meta-omics–based diagnostic pipelines to provide compositional and functional signatures of gut-associated diseases.<sup>192</sup> Currently, meta-omics integrated analyses on neonate/children gut

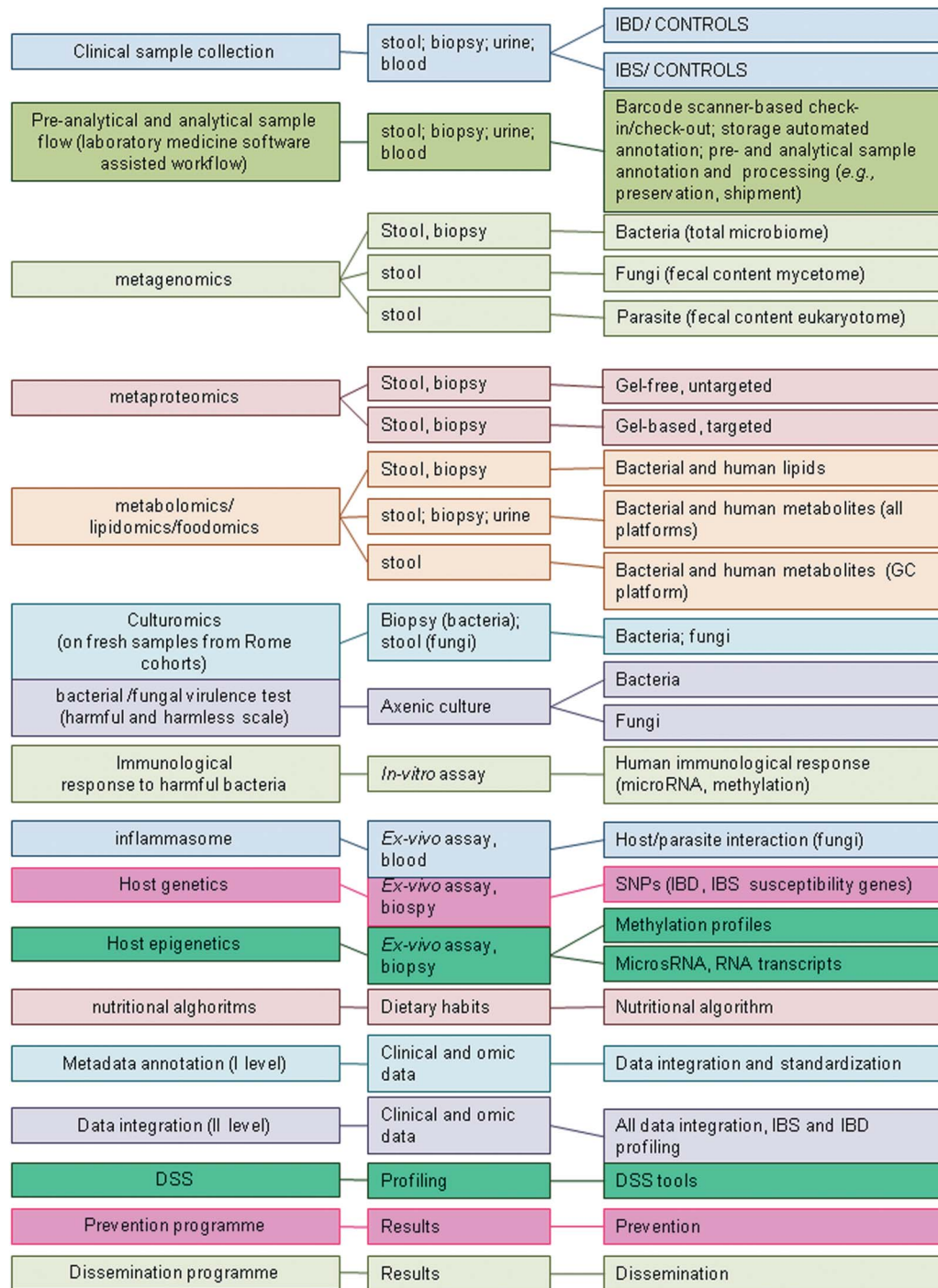


FIGURE 3. Scheme of the -omics pipelines to unveil dysbiosis patterns. This scheme reports the different -omics pipelines, the type of samples, and the goals of each activity aiming at describing gut dysbiosis.

microbiota under physiological and pathological conditions (“programming,” dysbiosis, and disease) are routinely performed at OPBG. The comprehension of a “healthy” gut microbiome in early life stages, immediately after birth and through the entire childhood, has a crucial role in establishing a good nutritional practice in childcare and pediatrics, especially in endorsing

healthy development and aging. Although on adults several studies on gut microbiota have been already produced, much work still remains to be carried out to improve the understanding of its role in neonates and children. The OPBG gut microbiota activities aim at describing endophenotypes associated with physiological conditions (symbiosis) and different gut-related diseases,



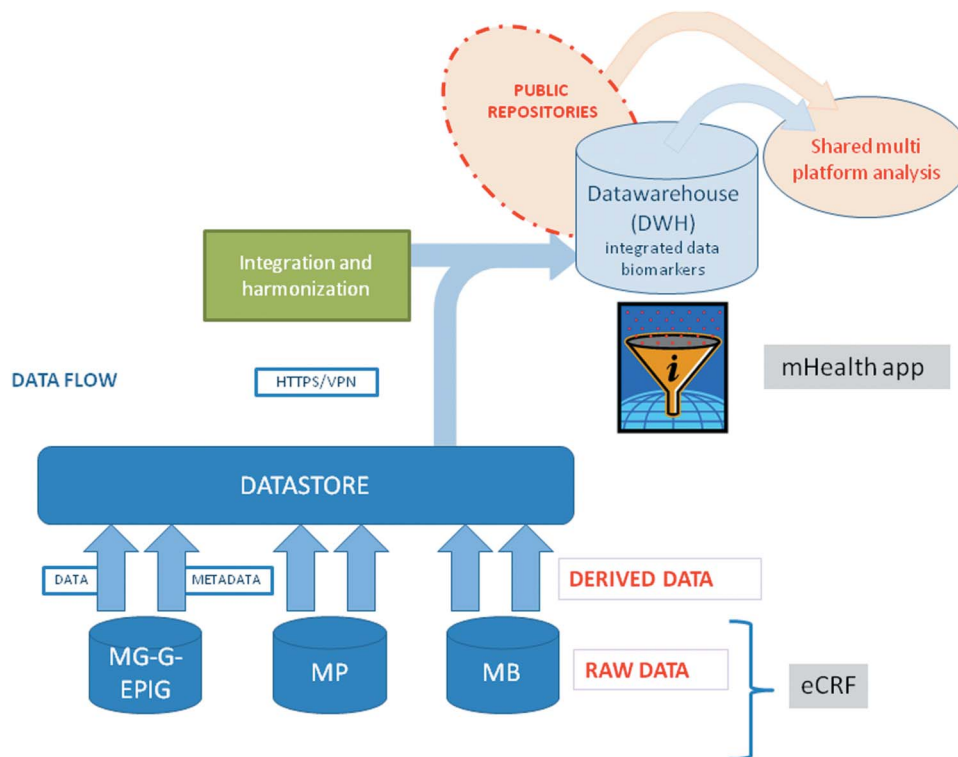


FIGURE 4. Workflow of -omics data for IBD and IBS dysbiosis tracking: datastore, data trafficking, and derived data warehousing.

including intermediate states of dysbiosis leading to disease, by providing individualized microbiota signatures (e.g., operational taxonomic units description and enterotypes) and function-related meta-omics charts (e.g., microbial protein clusters of orthologue groups' patterns and metatypes) of "healthy" and diseased children. Once the enterotypes have been identified and associated with endophenotypes, the meta-omics charts are used to identify functional factors leading to gut perturbation under different conditions, highlighting differences among healthy, dysbiotic, and diseased statuses. Differences in the gut microbiome under physiological or clinical conditions are selected as potential clues of perturbation/disease.

At a local level, OPBG has endorsed a team of leading clinicians, -omic scientists, and laboratory technicians to establish an *Italian consortium for systems medicine* aiming to intervene into medical research, diagnostic solutions, and clinical management of chronic diseases with onset at the age of early development. Scientists at OPBG have developed the concept of "extended genotype" aiming to produce operational pipelines for diagnostic routine toward non-invasive microbial ecology diagnostics and have developed original pipelines for meta-omics integrated studies in pediatric diseases<sup>163</sup> ([http://www.cell.com/abstract/S0092-8674\(12\)00629-0#Comments](http://www.cell.com/abstract/S0092-8674(12)00629-0#Comments)). The clinical OPBG framework nowadays allows the clinical interpretation of gut dysfunctions, critically evaluating relationships between gut and other organs or districts (e.g., mouth, upper and lower respiratory tract, liver, adipose tissue, and brain). The symbiotic and

dysbiotic cohorts (reference individuals) provide a large number of samples that constitute a reference pediatric biological bank, available to national and international consortia. All meta-omics data associated with patient fingerprinting are integrated by correlations. The OPBG Biobank presently includes more than 3000 fecal and 500 biopsy samples collected from inpatients affected by different pediatric diseases that are processed to assess gut and other site microbiota alterations and provide disease-related signatures (nonalcoholic fatty liver disease, cystic fibrosis, obesity, metabolic diseases, juvenile idiopathic arthritis, etc.).<sup>193</sup>

### From Dysbiosis Integrated Data to Predictive IBD and IBS Signatures: An mHealth App Support

The integration of meta-omics and phenomics with nutrigenomics (diet) and foodomics (food matrix) profiling is used to create a more comprehensive and exhaustive score-based tool (i.e., dysbiosis scale) for the prediction of gut inflammation types, corrected for all variability determinants (single patient path and -omics data multiplatforms, multiplatform Health app [mHealth app]). The signatures are used to target the disease network analysis (integrative network analysis by multilevel methods). This analysis allows the definition of most informative biomarker sets required for predicting disease onset and increasing the model fitness. The predictive signatures of IBS and IBD are using defined risk groups (e.g., I level familiar pedigree or relatives),<sup>194,195</sup> considering age-specific factors as appropriate (Fig. 5, Panel A). Accordingly, an

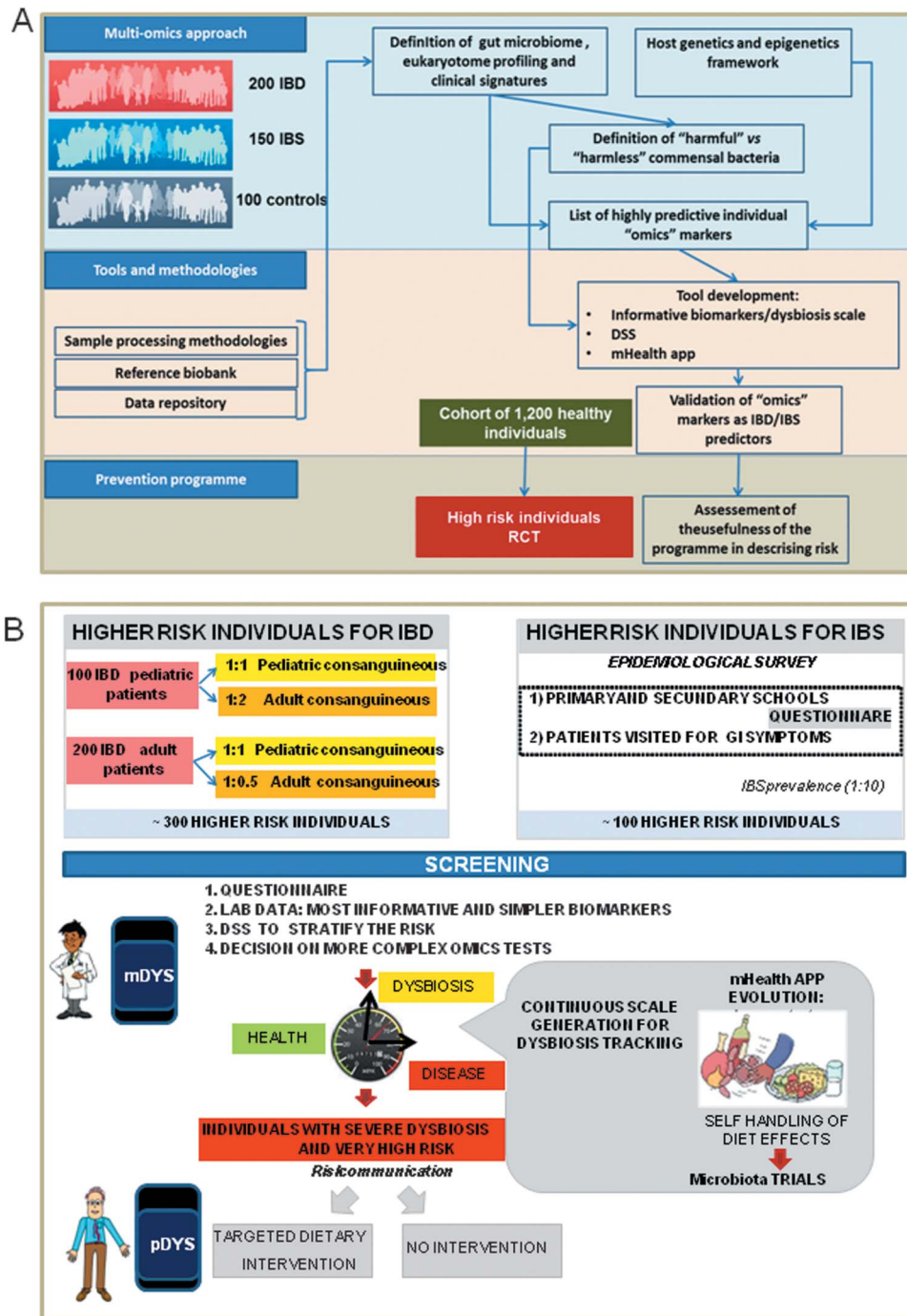


FIGURE 5. Dysbiosis patterns for preventive programs. Panel A. Methodologies applied to describe the dysbiosis scale. Panel B. Schematic diagram describing the clinical strategy for recruitment of IBD and IBS individuals at higher risk, -omics-based screening of at-risk individuals, and design of dedicated dietary prevention trials.

mHealth app for dysbiosis detection (mDys) will be upgraded into an mHealth demonstrator as a novel tool for advanced prevention strategies. The demonstrators will be used for self-management of score-based dietary profiles, as support to personalized dietary interventions, and shared with clinicians as a possible prevention tool prototype (pDys). The app will provide feedback to patients at risk

and in remissions to plan microbiota modification-based intervention. Hence, the prevention program may foresee a multistep screening approach including laboratory assays (targeted-omics assay) easily associable to discrete/defined biomarkers. In addition, this program will allow the identification of individuals with overt dysbiosis profiles, followed by more specific and complex assays,

also assisted by the mHealth app. Finally, the program will make possible to track dysbiosis before overt clinical disease, when clinically unaffected individuals still benefit from preventive strategies, and to avoid further flare-ups in patients in remission (Fig. 5, Panel B).

To validate the predictive ability of the identified biomarkers to correctly assess the risk of IBD, patients' first-degree relatives should also be enrolled.

For recruiting a cohort of subjects at increased IBS risk, children and adults with general GI symptoms could be selected, screened at first step by using questionnaires designed by pediatricians and gastroenterologists. Prevention programs, based on diet modulation in individuals at high risk, could be designed and assessed, also using dietary self-management through the pDys app, to generate score-based dietary advice. Then, data could be collected at each time point during dietary trials to assess potential changes in dietary habits, in relation to evolution of overt disease IBD phenotypes and clinical symptoms. For the IBS-risk groups, characterized by strong psychological risk cofactors, possibly because of microbiota alterations along the gut-brain axis, the impact of home-based cognitive therapies in the symptom amelioration and in the disease prevention should also be considered (Fig. 5, Panel B).

## Conclusions and Clinical Perspectives

IBD and IBS share a number of common causative features consisting of genetic predisposition, impaired gut barrier function, altered microbiota, and environmental triggers. Clinical heterogeneity within IBD and IBS has long been recognized, and evidence is growing that IS dysregulation, gut microbiota dysbiosis, and genetic constraints could explain differential age-dependent phenotypes. With reference to host-gut microbiota interplay, we propose ecological dynamics and functional alterations of gut microbiota as triggering factors of disease onset and progression.

The herein proposed dysbiosis tracking will enable the identification of (1) profiles describing gut perturbation and inflammation, (2) possible risk predictors of IBD or IBS, (3) reservoir of disease-specific, and (4) health-specific biomarker candidates. Through the application of tools to analyze biomarkers reflecting the complex molecular events taking place in the early phases of IBD and IBS in the routine clinical setting, we suggest to identify high-risk individuals through a multi-omics approach, overcoming the current lack of prevention programs tackling IBD and IBS.

We aim to assess the efficacy of targeted dietary interventions in restoring healthy microbiota function, the dysbiotic variations of which trigger the physio-pathological onset of the disease. The omics-based predictive assays and targeted dietary interventions may have the potential of developing prevention programs. A recent survey indicates that patients with IBS are interested in learning about diets limiting IBS symptoms.<sup>196</sup> We expect a similar reaction in individuals/parents recently informed about their own/their children's risk of developing a severe chronic condition such as IBS/IBD. However, poor compliance to dietary indications, especially in the long term, is well known.

To promote adherence, virtuous behaviors can be improved by means of mHealth Apps, providing personalized indications.

A scoring system for dietary patterns, linked with -omic signatures, can be regarded as a "new generation" predictor of IBD/IBS development and evolution. Dietary profiles can be made available through a web-interface by an App tool, targeting personalized management of dietary profiles and supporting data collection for integrative multi-step diagnosis and targeted dietary modulation.

This approach may contribute to develop novel preventive concepts, to discover new dysbiosis biomarkers, and to provide innovative tools for IBD and IBS risk detection, targeted-prevention, and therapy.

The *big -omic data* deposited in annotated searchable databases, under sharing conditions, allow data consultation by clinical and scientific communities. In this way, international -omics scientist and clinician consortia will forward the advancement of dysbiosis tracking as preventive strategy and therapeutic recommendation for controlling IBD and IBS.

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