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A deeper understanding of the gut microbiota of different human races in search of disease specific microbial and metabolic biomarkers

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Author contributions

Sohini Mukhopadhyay, Tanya Pattnaik, and Palok Aich contributed to the study design; Palok Aich conceptualized the study; Sohini Mukhopadhyay and Tanya Pattnaik wrote the manuscript; Palok Aich revised and finalized the manuscript. All authors approved the submitted version.

Competing interests

The authors declare no conflicts of interest.

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Abbreviations

SCFAs, short-chain fatty acids; IBD, inflammatory bowel disease; T2D, type 2 diabetes; CD, Crohn's disease; UC, ulcerative colitis; HMO, human milk oligosaccharides; ASD, autism spectrum disorders; SLE, systemic lupus erythematosus;

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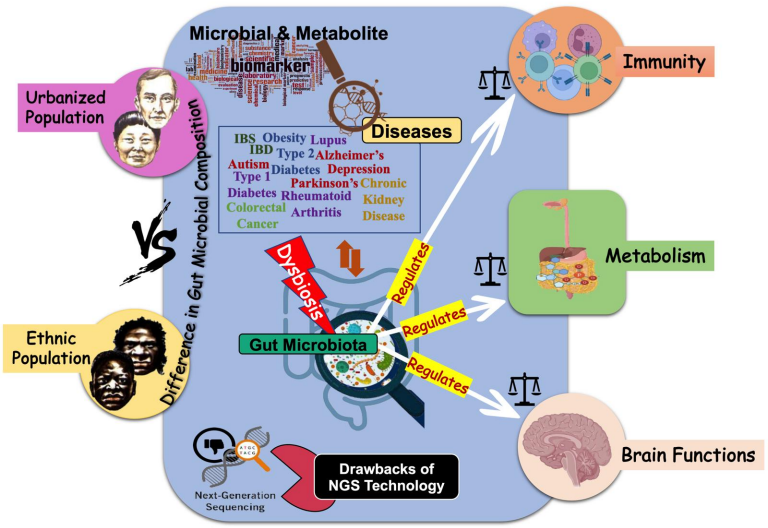
Abstract

Objective: Resident microorganisms live in dynamic harmony with their hosts to influence various physical and psychological health aspects. The majority of the resident microbes are found in the digestive tract, aka the gut of mammals. If and when perturbed, the composition of gut microbiota could jeopardize the physiological balance or homeostasis. In this article, we aim to establish how different diseases could be accompanied by notable changes in gut microbial composition and lend insight into microbial regulation of health. **Methods:** Literature search was done in PubMed using relevant keywords and summarized in tabular form as well as in narration. **Results:** We try to focus on the concept of microbial and metabolite biomarkers for diseases. We also try to capture the renewed perspective of good and harmful microorganisms in the context of host health. We have presented a comparative network analysis of microbial roles in select diseases. Recent findings also suggested that the growth of some traditionally disease-causing pathogenic microorganisms promotes health in other human communities. We have listed major taxa of gut microbes in communities worldwide, which signifies that gut microbiota can be healthy or harmful depending on the urbanization and ethnicity of the hosts. The traditional and current schools of thoughts are both limited by the technology of metagenomic studies; we have elucidated some of their shortcomings. **Conclusion:** Research in the field of gut microbiota must take into account the different populations and the changing narrative of healthy and harmful microbes.

Keywords: diseases and biomarkers, glitches of metagenomic approaches, gut microbiota, metabolic profiles, urbanised and traditional gut microbiota



Introduction

The Singapore summit of 2018 was an important event for many reasons. Still, one bewildering and recurring headline that interested the hoi polloi was that the North Korean leader carried with him his toilet. Amidst wild speculation, the actual reason for this measure was that nobody could have a chance to know the health status of the leader. One might wonder how much information can even be obtained from his fecal material, and the answer was enough for it to be a national security concern. The feces are more living than waste; the microbiome of the fecal samples can give away a lot about the host's immune, and systemic metabolic status [1].

Today, we know that gut microbiota encompasses 316 million genes, spanning over 25 phyla, with 2,000 genera and 5,000 species [2, 3]. Despite the growing ease of metagenome mapping, 20% of sequences do not match any known microbial genomes, indicating hidden taxa and unknown diversity. Of the total, 40% of sequences are not available in any functional databases, so there is a substantial gap in our understanding of the role of the microbiome, even if extensive research pours in from every corner of the world [4].

Not only inside the gut, but microbes are also found throughout the human body, mainly on the external and internal surfaces, including the skin, saliva, oral mucosa, and conjunctiva. Bacteria overwhelmingly outnumber eukaryotes and archaea in the human microbiome by 2–3 orders of magnitude [5]. The vast majority of the bacteria reside in the gut, more specifically in the colon, with an estimate of about 10^{14} bacteria, followed by the skin, which is estimated to be home to $\sim 10^{12}$ bacteria [6].

A mother's first gift to the newborn is a healthy smattering of microbes [7]. Most microbes are acquired during passage through the birth canal, while some are via breastfeeding and skin-to-skin contact [8]. This means that if the baby is delivered by cesarean section, they might miss out on a valuable maternal bacterial starter kit [9, 10]. Because a child's earliest years generally establish the composition of a gut community that may persist throughout adulthood, the resulting disruptions can have serious long-term health consequences [11, 12]. The gut microflora has more profound effects on the host's a) anatomical, b) physiological, and c) immunological development than microflora of other body parts [13-15].

The infant's gut microbiota undergoes a succession of changes correlated with a shift in feeding mode from breast or formula-feeding to weaning and the introduction of solid food [16]. Despite the relative similarities of the gut microbiota in mothers and their offspring, microbial succession in the gut is also influenced by numerous external, internal, and other host-related factors. External factors include the microbial load of the immediate environment, the type of food eaten, feeding habits, and the composition of the maternal microbiota. Also, dietary and temperature-related stresses can influence the succession of microbes. Internal factors include, but are not limited to, intestinal pH; microbial interactions; environmental temperature; physiological factors, such as peristalsis; bile acids; host secretions and immune responses; drug therapy; and bacterial mucosal receptors [17].

The large diversity, stability, resilience, and symbiotic interactions of the gut microbiota with the host can act as a "superorganism" that performs the host's most vital immune and metabolic functions [18-20]. Gut bacteria are crucial regulators of critical digestion along the gastrointestinal tract; commensal bacteria play an essential role in the extraction, synthesis, and absorption of many nutrients and metabolites, including bile acids, lipids, amino acids, vitamins, and short-chain fatty acids (SCFAs). Gut microbiota has a crucial immune function against pathogenic bacteria colonization inhibiting their growth, consuming available nutrients, and/or producing bacteriocins. Gut microbiota also prevents bacteria invasion by maintaining intestinal epithelium integrity. Microorganisms prevent pathogenic colonization by many competition processes: nutrient metabolism, pH modification, antimicrobial peptide secretions, and effects on signaling pathways [21].

Moreover, recent studies have identified a critical role for commensal bacteria and their products in regulating innate and adaptive immune cells' development, homeostasis, and function [22]. It is paradoxical to note that gut microbiota functions are highly preserved among individuals. In contrast, each individual's gut microbiota is characterized by a specific combination of bacterial species due to inter-individual and intra-individual variations throughout human life [23].

The conventionally healthy gut microbiota, specifically gut bacteria of a healthy human adult, comprises six significant phyla, Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, Fusobacteria, and Verrucomicrobia, with the two phyla Firmicutes and Bacteroidetes representing 90% of gut microbiota. The Firmicutes phylum comprises more than 200 genera, such as *Lactobacillus*, *Bacillus*, *Clostridium*, *Enterococcus,* and *Ruminicoccus,* while the *Clostridium* genus represents 95% of the Firmicutes phyla. Bacteroidetes consist of predominant genera such as *Bacteroides* and *Prevotella*. The Actinobacteria phylum is proportionally less abundant and mainly represented by the *Bifidobacterium* genus [23, 24].

Perturbation of the constitution and functionality of the healthy gut microbiome elevates disease risks. The adverse health outcomes, including inflammatory bowel disease (IBD), obesity, diabetes, cardiovascular disease, liver disease, colorectal cancer, and neurological disorders, can be at least partially attributed to undesirable functional alterations in the gut microbiome [25].

The gut microbiome is considered a new metabolic organ involved in regulating host metabolism. Their composition and abundance can be varied depending on internal factors (e.g., host genetics) and external factors (e.g., diet, lifestyle, and drugs) [26]. The change in gut microbiome composition has been reported to modulate the metabolic composition of the host. Accumulating evidence has proved that metabolites reside at the critical interface between the gut microbiome and the host's health status [27-29]. This study enlightens the role of microbiome and metabolites as a promising tool for disease diagnosis and prognosis.

Current reports suggest that the term healthy or diseased gut microbiota is undoubtedly vague in many contexts. One person's healthy gut microbiome might not be beneficial in another context. Several studies have identified a stark difference between the microbiota of urban populations and those of indigenous populations that lead traditional agrarian or hunter-gatherer lifestyles that resemble those of our early ancestors [30]. These differences seem to be attributable mainly to the loss of bacterial diversity, which might be linked to the lack of fiber in Western diets [31].

So, to define the healthy and diseased gut microbial composition more appropriately and robustly, researchers need a comparative larger and more global data set. Global and long-term studies would give a better-informed starting point for broadly understanding what a normal microbiome in a healthy individual can look like and thus make it easier to recognize disease-linked perturbations.

However, the methodology of microbiome analysis is still a hindrance in identifying the exact gut microbial composition of the host. Standard methods of microbiome analysis favor the identification of bacteria and are not as good at recognizing other common gut microorganisms. The other major drawback of the analysis technique is that this can detect only species up to the accuracy of genus level while ignoring subspecies and the specific strain of the microbiota. Hopefully, an alternative analysis technique will be a workaround shortly [32, 33].

In this context, the current review will anticipate a vast knowledge of a) the critical role of the gut microbiome in maintaining healthy homeostasis, b) the perturbing factors that break the homeostasis to cause innumerable acute diseases, c) the gut microbial composition of traditional human races, the hunter-gatherers to break the myth of healthy and diseased microbiota in this context, and d) last but not the least the current glitches of the microbiome analysis.

Microbiota, a key regulator of health

Long before, Lederberg even defined the term microbiome in 2001,

Antony van Leuwenhoek had observed the living creatures in his sputum and fecal samples [34, 35]. He studied their diversity and observed the difference between habitats and states of disease and health. The human microbiome occupies various niches of the human body. Communities of flora can be in nasal cavities, oral mucosa, on the skin, in the gut, and inside urogenital tracts [6, 36]. They modulate immunity and regulate hormones [37]. The skin microbiome is part of the first barrier, protects against pathogens, and its secretion modulates pH. Biofilms in the oral cavity help digestion and secrete essential vitamins [38]. In the urogenital tract, the microorganisms maintain pH and prevent diseases by various non-specific mechanisms [39]. The microbiome residing in our gut performs all these functions and more, making it one of our body systems' most vital and versatile components. The diversity of the flora in the gut modulates the immune response, neurobehavior, energy metabolism, digestion, and absorption. The microbiome biosynthesizes a) vitamins, b) steroid hormones, c) neurotransmitters, and metabolizes a) amino acids, b) bile salts, c) drugs, and d) xenobiotics [40].

Out of the 12 phyla characterized by the Human Microbiome Project, the four dominant phyla in the gut of a healthy individual, i.e., i) Bacteroidetes, ii) Firmicutes, iii) Actinobacteria, and iv) Proteobacteria and their metabolic products modulate the systemic homeostasis of the host in various ways [41]. They influence a) immune maturation, b) host cell proliferation, c) vascularization, and d) pathogen burden, along with bone density, energy biogenesis, and intestinal endocrine function [42, 43].

The microbiome communities differ by the location of localization and the organism's condition. Limitations to culturing a majority of commensal microbes still hinder our understanding of their functioning [44].

There are multiple ways in which microbiota influence different systems.

Gut microbiota and immune crosstalk

The gut is home to an alarming proportion of immune responding cells. Such proximity to the microorganisms in our body cannot be a coincidence. In the same way, it is not a coincidence that the critical window of gut microbiome colonization for infants is the same time duration in which babies are highly susceptible to infectious diseases. An elegant study elucidating the difference in the development of gut-associated lymphoid tissue in germ-free and specifically colonized mice solidified the hypothesis that microbiome diversity modulates the first line of defense of the host gut [45]. Immune cells can be primed under the condition of commensal activation, aided by SCFAs, which also modulate the production of cytokines [46]. The co-evolution of microbial diversity with the host system maintains the balance between the commensals of the gut and the host immune system [47]. The members of the gut microbiota then modulate the host's immunological state and response, among other things. Recent literature indicates that microbial colonization determines Th1/Th2 bias in mice, with specific phyla of bacteria instrumental in producing Th1 response [48]. The gut microbiota also forms a line of defense by competing with infectious pathogens for resources and exerting antimicrobial action through different immune cells [49]. Resident microbiota of the gut promotes the production of antimicrobial peptides: leptin, and defensins using pattern recognition receptors mediated mechanism [50, 51].

Whether a microorganism promotes, triggers, or protects against disease is highly contextual, depending on the host's immune activation, the region where the microbiota is localized, and the host's genetic landscape [43]. Evolution and modulation of environmental and pathological hosts need to cultivate fine-tuning among host-microbial interactions over time [52]. In the recent few decades, the increased use of antibiotics, drastic shift in diet and lifestyle, and almost total eradication of nematode infection in significant parts of developed nations have resulted in a sharp increase in autoimmune and inflammatory diseases, a result of a disrupted balance between the microbiome and host immunity [53, 54]. In a balanced, healthy microbial composition, the commensals combat the pathogens either in competition for nutrients or directly influencing the pathogenicity of the virulent by secretion of antimicrobial metabolites [51]. Commensals also trigger T and B lymphocytes against pathogens, promoting inflammation and autoimmune disorders when not under control [55, 56].

Brain and behavior

The gut microbiome weighs as much as a human brain and is undoubtedly as important, if not more, as the master organ itself [57]. By integrating the hormonal and neural pathways, the gut symbionts have established bidirectional communication with the brain, acting along what is known as the gut-brain axis [58]. Extensive research has uncovered numerous implications of this crosstalk between the brain and the gut microbial composition. A few decades ago, scientists solidified the connection between bacterial colonies and behavioral disorders when they observed that oral antibiotics directly alleviate the symptoms of encephalopathy [59, 60]. Since then, dysbiosis has been included as a symptom of neurobehavioral disorders. Systematic studies have cropped up characterizing the diverse states of microbial compositions at different disease stages. Probiotics and prebiotic treatments are increasingly considered a therapy to reduce stress and mood disorders [61]. Stress response in germ-free mice is more intense than in gut colonized by microbiome [62]. Depending on when the microbiota is restored, anxiety levels stagger to normal, indicating that microbiota controls neural response development and plasticity [63]. There is more than enough evidence to conclude that microbial composition in the gut and an individual's behavior are a product of one another. Years ago, a seminal study showing that *Bifidobacteria infantis* plays a vital role in metabolizing tryptophan proved that the gut microbiota directly challenges and controls the neurotransmitter quantity and action in the brain [64]. Tryptophan is the precursor for many important neurotransmitters implicated in mood disorders, and microbial endocrinology studies have since shown that gut commensals did indeed produce their neurotransmitters [65]. This discovery forced onto the field a new perspective. Not only did the microbial composition dictate changes in mood as a downstream effect, but it was actively producing the very molecules whose activity determined the person's behavior. The next obvious question was if the neurotransmitters were produced in the gut, how were they transported? The answer lay in a string of vagus nerve-centric research, in which the nerve was stimulated or dissected out in germ-free mice to conclusively lay the foundation for gut-brain axis communication [66].

While vagus nerve innervation connects the microbial effects to the central nervous system, the enteric nervous system is also receptive to microbial metabolites and their concentrations [67]. Another crucial effector is the hypothalamic-pituitary-adrenal axis for stress response [68]. The cortisol produced as a result of activation of this axis modulates the action of intestinal cells, which are directly in contact with and under the control of the microbiome [58, 69]. Specific colonization of the bacteria resulted in altered expressions of genes involved in stress response in different brain regions [70]. While studies like these are plentiful, still more requires understanding the exact mechanisms behind the effect of specific microorganisms, their metabolite products, and their downstream effects.

Digestion and metabolism

Bifidobacteria are supposedly the first phylum of bacteria to be colonized in the infant's gut, believed to be passed down from the mother's breastmilk [71]. It is essential because it aids in the digestion of the milk's sugars [72]. Polysaccharides, a complex sugar, have their complexity compounded by the array of multiple bonds they can form between themselves, resulting in the need for a similarly diverse set of enzymes to help in their breakdown [73]. The gut does provide for this, but the enzymes are not all humanely synthesized [74]. A large chunk of the proteins is contained in the symbionts' genomes that reside in our gut lumen [75]. Even after the complexity of the digestion process is overcome, the gut microbiome plays a still

important role in converting carbohydrates to energy, effectively bypassing specific consuming pathways [76]. Features like these make them invaluable to the process of primary degradation in the intestines. In the absence of any gut microbiota, we observe increased autophagy in the intestinal cells, as a response to nutrient stress. Supplementing with microbially digested metabolites rescues the germ-free mice from the energy deficit and autophagy [77].

Pyruvate formed during the catabolic processes is converted to intermediates. But surprisingly, only trace amounts of these intermediates are found in fecal samples. This decreased concentration is mainly because the commensals metabolize those molecules to produce short-chain fatty acids [78]. This SCFA is essential to fuel the body's total caloric use and has many other effects, as elucidated below [79]. Specific steps of these SCFA productions may result in undesirable byproducts, which are again consumed by microorganisms in the gut [80]. This cross-feeding is essential to maintain the process's kinetics and save the cells from toxic effects [81].

Actinobacteria and bacteroidetes primarily break down the dietary carbohydrates and human milk oligosaccharides (HMO) into acetate and trace amounts of succinate and propionate. Firmicutes are dedicated principally to cross-feeding the byproducts and the production of SCFA. Different species metabolize the intermediates to other end products, which are metabolized by their sister species. Proteobacteria digest dietary proteins, which are especially rich in sulfur-containing amino acids, which are metabolized further as part of the sulfur detoxification process in the gut. Many species of Bacteroidetes metabolize aromatic amino acids to indoles which play a major role in intestinal gut barrier integrity maintenance [80].

Gut microbiota and metabolism

The importance of gut microbiota was initially shown in decades-old studies. Different metabolic disorders were characterized by distinctly different microbial populations [82]. By studying the action of short-chain fatty acids, the metabolized end products of gut microbiota, we are a little closer to understanding the effect of said microorganisms on the host's metabolism. Butyrate and propionate induce gut peptides that decrease food intake and increase satiety [83]. Acetate and propionate have antagonistic effects on adipose tissue function, e.g., acetate promotes lipogenesis, and propionate inhibits it [84].

The advancement of omics studies has generated rich omics data revealing the involvement of the microbial community in host disease pathogenesis through interactions with their host at a metabolic level. Metabolic dysregulation caused by the microbiome is believed to contribute to the development of gastrointestinal diseases like IBD, metabolic diseases such as type 2 diabetes, obesity, neuropsychiatric diseases like autism, and various chronic inflammatory diseases such as Lupus [27]. In addition, the diseases caused by gut microbial dysbiosis have a significant effect on the systemic level of the host. The gut microbial composition of the host at various diseased states is enlisted in Table 1 and Figure 1. We also enlisted (Table 1) the diseases whose pathogenicity triggers the development of other systemic disorders of the host.

Table 1 Microbial signatures of gut dysbiosis related diseases in human

Category	Disease	Increased taxa	Decreased taxa	Related other diseases	References
Gastrointestinal	IBS	*Veillonella, Streptococci, Ruminococcus, Enterobacteriaceae, Lachnospiraceae, Escherichia coli,*	*Lactobacilli, Faecalibacterium, Bifidobacteria, Collinsella, Clostridium leptum*	Anxiety, depression	[115–117]
	IBD	*Ruminococcus gnavus, E coli, Fusobacterium, Bacteroidetes fragilis*	*Faecalibacterium prausnitzii, Lachnospiraceae, Bifidobacterium, Roseburia hominis*	Anxiety, obesity, celiac disease	[118–123]
	Celiac's disease	*Bacteroides - Prevotella, E. coli*	*Bifidobacterium, Clostridium histolyticum, C. lituseburense, and Faecalibacterium prausnitzii, Lactobacillus*	IBD, diabetes (type 1), rheumatoid arthritis, Crohn's	[124–128]
Metabolic	Obesity	*Ruminococcaceae, Rikenellaceae, Desulfovibrionaceae*	*Akkermansia muciniphila*	Diabetes, depression, IBS	[129–133]
	Diabetes (Type 2)	*Desulfovibrionaceae, Clostridium, Bacteroides caccae, Akkermansia muciniphila*	*Roseburia, Faecalibacterium prausnitzii*	Obesity	[134, 135]
	Hypertension	*Eggerthella, B. plebeius, Akkermansia*	*Roseburia, Faecalibacterium*	Diabetes, obesity, IBD	[136–138]
Neuropsychiatric	Autism	*Lactobacillus, Clostridium, Bacteroidetes, Desulfovibrio, Caloramator, Sutterella, Sarcina*	*Bifidobacterium, Akkermansia muciniphila*	IBS	[139–141]
	Depression	*Oscillibacter, Parabacteroide, Klebsiella, Paraprevotella, Veillonella, Desulfovibrio, Parasutterella, Paraprevotella*	*Coprococcus, Lactobacillus, Escherichia/Shigella, Clostridium XlVa, Dialister, Howardella, Pyramidobacter, Sutterella*	IBS, obesity	[142, 143]

Table 1 Microbial signatures of gut dysbiosis related diseases in human (*Continued*)

Category	Disease	Increased taxa	Decreased taxa	Related other diseases	References
Neuropsychiatric	Alzheimer's Parkinson's	*Escherichia/Shigella* *Akkermansia, Oscillo-spira, Bacteroides*	*E. rectale, Bifidobacterium* *Blautia, Coprococcus, and Roseburia*	IBD, depression	[144, 145] 146
cancer	Colorectal cancer	*Bacteroides fragilis, Enterococcus, Escherichia/Shigella, Klebsiella, Streptococcus, Peptostreptococcus, Dorea, Faecalibacterium, Fusobacterium*	*Roseburia, Lachnospiraceae, Bacteroides, Coprococcus*		[147, 148]
Inflammatory	Rheumatoid arthritis	*Lactobacillus salivarius, Fretibacterium, Selemonas, Bacilli, Collinsella* (could be causative)	*Faecalibacterium prausnitzii, Prevotella copri, Faecalibacterium, Blautiaccoids, and Flavobacterium*	IBD, Crohn's, ulcerative colitis	[149–151]
	Lupus	*Lachnospiraceae, Clostridiaceae, Blautia, Ruminococcus gnavus*	*Bifidobacterium, Erysipelotrichaceae, Odoribacter, Alistipes*	IBD, depression	[152–154]
	Diabetes (Type 1)	*Clostridium, Bacteroides, and Veillonella*	*Lactobacillus, Bryantella, Bifidobacterium, Turicibacter, Blautia coccoides/Eubacterium rectale, Prevotella*	Celiac disease, cardiovascular disease	[125]
Uremic	Chronic kidney disease	*Lachnospiraceae, Ruminococcaceae, RikenellaceaeII*	*Lactobacillaceae, Prevotellacea*	Colitis	[155]

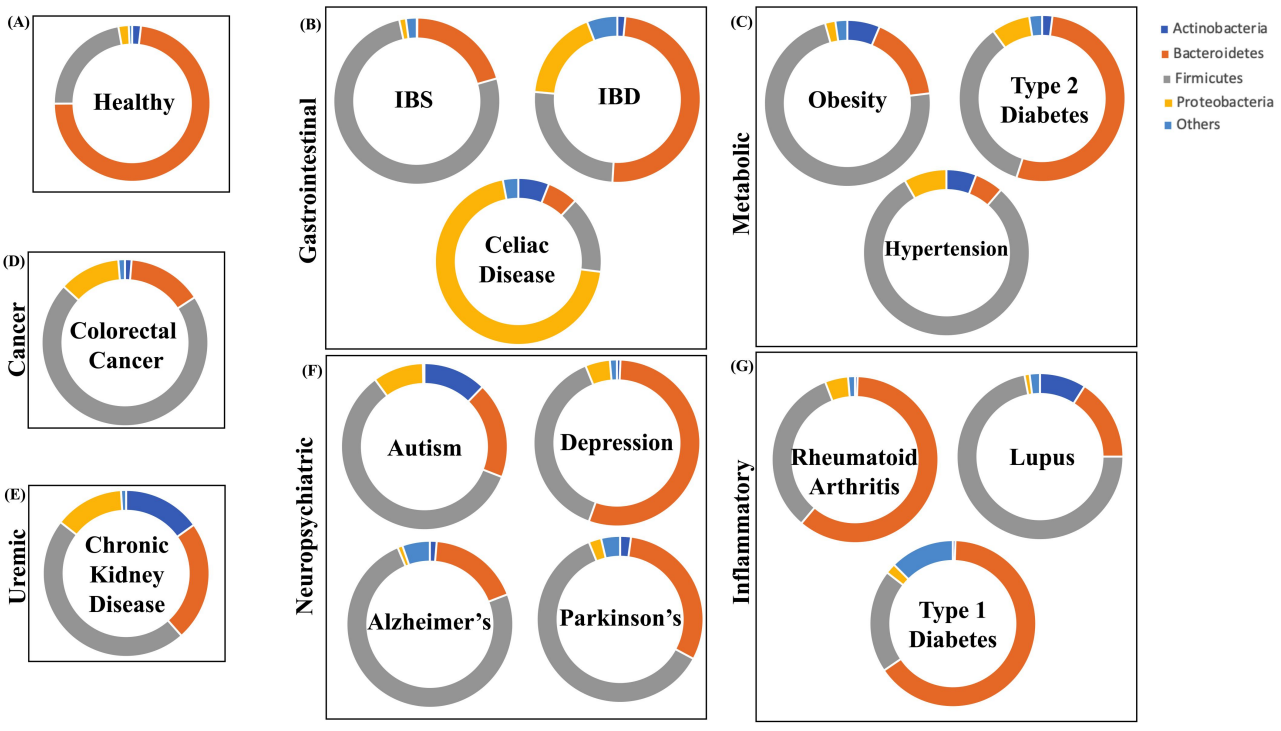


Figure 1 Donut plots of the gut microbial composition of healthy and diseased individuals at the phylum level. The ratio of a different phylum of healthy [183] and diseased individuals are quite different from each other. Gut microbial dysbiosis is the leading cause of the following diseases- A. IBS [184], B. IBD [185], C. Obesity [186], D. Type 2 diabetes [187], E. Hypertension [188], F. Colorectal cancer [189], G. Chronic kidney disease [190], H. Autism [191], I. Depression [192], J. Alzheimer's [193], K. Parkinson's [194], L. Rheumatoid arthritis [195], M. Lupus [196], N. Type 1 diabetes [197]. We categorized all the diseases into six different types based on the type of disease outcome.

We categorized the microbiome dysbiosis related diseases into 6 sub-categories such as i) gastrointestinal diseases, ii) metabolic diseases, iii) neuropsychiatric diseases, iv) inflammatory diseases, v) cancer, and vi) uremic diseases based on the disease outcome. We chose 5 different diseases from the six sub-categories to investigate how the altered gut microbial composition controls the host's metabolism, which further plays a significant role in the severity of the disease outcomes [85, 86].

Regression and correlation analysis between the different genera of the gut microbiome and metabolite concentration from various disease conditions helped us find some signature microbes and metabolites that are very much disease specific. Enrichment of the disease-specific signature microbiota or metabolites in the host system can be used as a unique marker for the diagnosis and prognosis of the particular disease in the near future. In the coming section, we will discuss some signature microbial genera and metabolites that can be adopted as useful biomarkers for diseases.

To investigate the signature biomarkers of the diseases, we collected the data from already published articles and reanalyzed them as per our requirements. We predicted the biomarkers for the following diseases- i) IBD, ii) Obesity, iii) Type 2 diabetes, iv) Autism, and v) Lupus.

Predicted microbes-metabolites biomarkers for IBD

Inflammatory bowel disease, most commonly known as IBD, is a disease of the human gastrointestinal tract. Dysbiosis of gut microbiota ultimately activates flares of inflammatory reactions in the human gut. Based on the localization of the inflammation in the gut, IBD can be categorized into Crohn's disease (CD), where the inflammation use to occur in the upper GI tract, and Ulcerative colitis (UC) is the inflammation of mainly the colon and some other portion of the lower GI tract [87-89]. In this context, it is very important to understand the interactions between the microbiome and metabolites of the host that ultimately causes differential inflammatory outcomes at different parts of the GI tract. We used the publicly available datasets to understand the relationship between microbiome and metabolome in IBD disease progression.

The Spearman correlation analysis of microbiome and metabolome data revealed a strong correlation between 15 different bacterial species with 14 discriminant metabolites in CD patients. 7 different species, *Faecalibacterium prausnitzii, Oscillospira eae, Oscillospira guillermondii, Anaerobranca zavarzinii*, *Veillonella montpellierensis*, *Ruminococcus albus* and *Alkaliphilus crotonatoxidans* belonging to the Firmicutes phylum, 4 species, *Desulphonauticus Autotrophicus, Serratia entomophila*, *Escherichia albertii, and Candidatus Endobugula sertula* to the Proteobacteria phylum, another 3 species, *Dysgonomonas wimpennyi, Rikenella microfusus*, and *Parabacteroides johnsonii* to the Bacteroidetes phylum and finally only one, *Bifidobacterium adolescentis* to the Actinobacteria phylum showed a strong association with different metabolites level of CD patients. More specifically, a strong correlation was seen between species *Oscillospira eae* with the metabolites 5β-coprostanol, 3-methyladipic acid, citric acid, methylamine, 2-hydroxy-3-methylvaleric acid, PC (16:0/3:1) and urobilin; species *Oscillospira guillermondii* showed correlation with 5β-coprostanol, methylamine, and PC (16:0/3:1); species *Desulphonauticus autotrophicus* was associated with 5β-coprostanol, 3-methyladipic acid, citric acid, methylamine, and PC (16:0/3:1) putrescine and cadaverine production and finally, the abundance of *Faecalibacterium prausnitzii* was correlated with the metabolite phenylethylamine (Figure 2A, B; Supplementary 1) [90-92].

Evaluating the bacterial species and metabolites relationship in UC patients revealed strong correlations between *Pedobacter kwangyangensisvs* and *Dysgonomonas wimpennyi* with 3-methyladipic acid, 5ß-Coprostanol, 2-hydroxy-3-methylvaleric acid, citric acid, and methylamine and TMAO. 3-methyladipic acid and production also correlated with the species *Akkermansia muciniphila* and species *Alkaliphilus crotonatoxidans,* respectively (Figure 2A, C; Supplementary 1) [90-92].

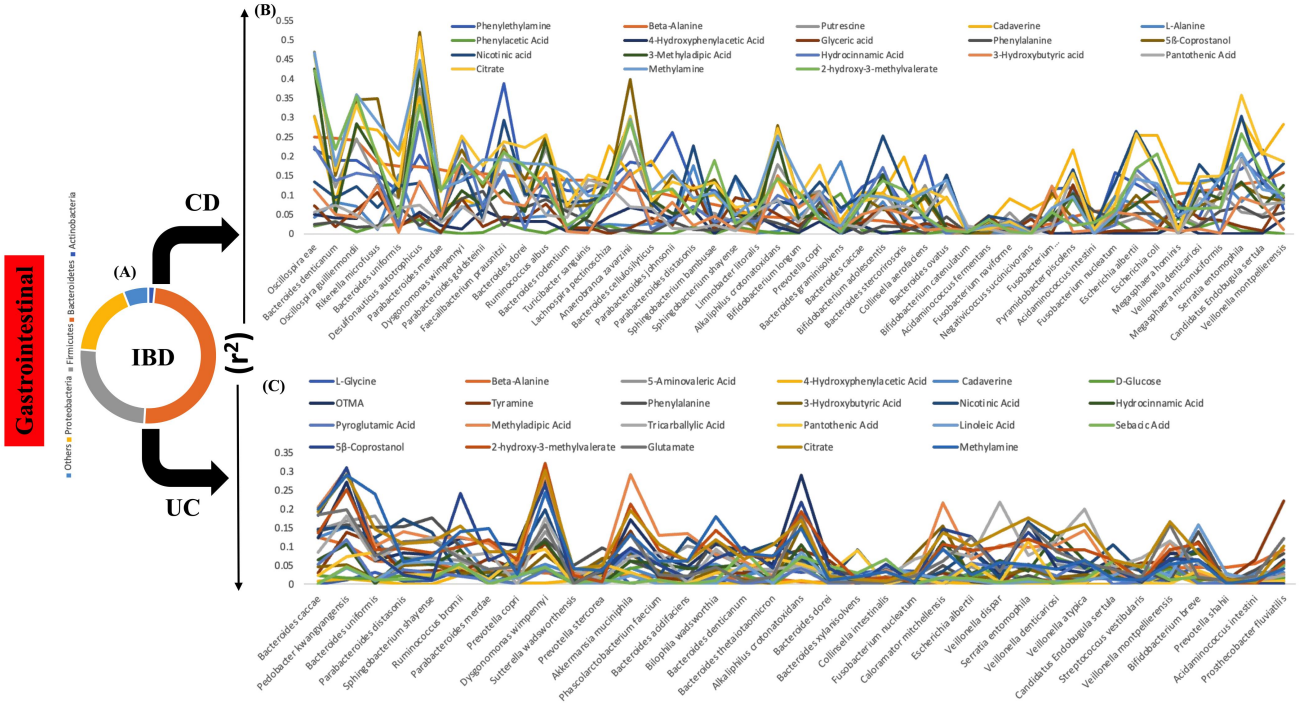


Figure 2 Global correlation of the altered microbiome and metabolomic profile of IBD. Here we depicted the altered gut microbial and metabolic changes due to IBD. We tried to establish a correlation between disease related microbial and metabolic composition to get an idea which particular genus is responsible for what kind of metabolic changes. Panel (A) demonstrates the IBD-related gut microbial changes of the host at the phylum level, and the also the correlation (Spearman) between altered gut microbiota and altered metabolism of the host due to Crohn's disease (CD) (B) and Ulcerative colitis (UC) (C).

Predicted microbes-metabolite biomarkers for the metabolic disorders

Obesity

Obesity or overweight is a global problem of the current century. The situation is like that; 1 in every 5 persons is considered obese in the present scenario. Numerous comorbidities are associated with obesity, reduced life expectancy, and increased mortality. In this situation, it is necessary to investigate some disease-associated biomarkers for the early diagnosis of the disease. As obesity is a metabolic disorder, it is best to find some metabolic biomarkers and the producers of the metabolites, i.e., mainly gut microbes.

Reanalysis of various public data set showed that genera *Coprococcus, Desulfovibrio, and Ruminoclostridium* were strongly correlated with butyrate and trimethylamine productions, *Erysipelotrichaceae* and *Butyricimonas* were associated with arabinose production, and *Ruminococcaceae* and *Erysipelotrichaceae* were related with galactose production (Figure 3A, C; Supplementary 1) [93, 94]. The strong association between the mentioned genera and metabolites with obesity provides us enough confidence to use them as promising biomarkers for the disease.

Type 2 diabetes (T2D)

Type 2 diabetes is another global burden for the healthcare system. This is also a metabolic disorder and is highly associated with the occurrence of obesity.

Available evidence showed that the gut microbiota and metabolic content were significantly altered in T2D patients. The short-chain fatty acids (SCFAs) and some SCFA-producing bacteria were also remarkably changed, such as diacylglycerol.

Genera *Prevotella* and *Prevotellaceae* UCG-003 in Bacteroidetes and genera *Streptococcus*, *Weissella*, *Veillonella*, *Pseudobutyrivibrio* in Firmicutes were correlated with metabolites linolenic acid and LPC (18:2). Families Lachnospiraceae and Ruminococcaceae were associated with the production of acetate and LPC (18:2). On the other hand, the concentrations of bile acids (cholic acid, glycoursodeoxycholic acid, chenodeoxyglycocholate, and glycocholic acid) and SCFAs (acetate, propionate, and butyrate) were correlated with families of Lachnospiraceae, Ruminococcaceae, Planococcaceae, and Prevotellaceae, etc. Genera of Lachnospiraceae and Ruminococcaceae families were also correlated with the production of lipids and bile acids (Figure 3B, D; Supplementary 1) [95-97]. So the mentioned microbes and metabolites are suitable resources and promising biomarkers in the future for the detection of T2D.

Predicted microbes-metabolites biomarkers for neuropsychiatric disorder- Autism

Autism or autism spectrum disorders (ASD) are a clinically and genetically heterogeneous group of neurodevelopmental disorders characterized by socio-communicative difficulties as well as repetitive and restrictive behaviors. Researchers noticed that a large proportion of autism subjects have gastrointestinal dysfunctions, with diarrhoea, constipation, and abdominal pain as the most common symptoms. It has also been noticed that autism patients have altered gut microbial and metabolic profile.

Available reports suggested that 6 microbial genera of the gut and 16 different metabolites are strongly associated with the disease progression. The abundance of the microbial genera and metabolites were also very nicely correlated with each other. Data suggested that the genus *Lactobacilli* was correlated with the production of the metabolites fumarate, acetate, leucine, ethanol, isoleucine, phenylalanine, alanine, *Akkermansia* was associated with leucine, methionine, alanine, ethanol production, *Bifidobacteria* showed correlation with metabolites acetate, leucine, isoleucine, phenylalanine, orotate, alanine, tyrosine, uridine, methionine, 1,3-dihydroxyacetone, *Bacteroides* was responsible for the production of leucine, isoleucine, alanine, fucose, uridine, the abundance of *Prevotella* was correlated with propionate, fumarate, N-methylhydantoin and finally genera *Suttrella* had a strong association with metabolites acetate, leucine, alanine, fucose, isoleucine, phenylalanine, tyrosine, aspartate, fucose, ethanol (Figure 4A, C; Supplementary 1) [98]. So these 6 genera and their associated 16 metabolites could act as promising biomarkers for the diagnosis of autism at an early stage.

Predicted microbes-metabolites biomarkers for inflammatory disorder- Lupus

Systemic lupus erythematosus (SLE) or lupus is a multifactorial autoimmune disease that can cause damage to many organs and has a global prevalence. The manifestations of lupus are very diverse and are related to immune system defects and subsequent systemic inflammation. Gut microbes play a remarkable role in maintaining the immune homeostasis of the host. As lupus is an immunological disorder, there is a high chance of altering the gut microbial composition of the lupus patients. The altered gut microbial profile is also related to the host's altered metabolic profile.

Previous correlation data of gut microbiome at genus level and altered lipids concentrations showed a strong association with the lupus pathogenicity. The majority of the bacteria that were correlated with altered lipid levels belong to the Firmicutes phylum. In this phylum, *Lactobacillales,* and *Erysipelotrichales, Clostridiales* are the taxa accounting for the effective correlations with disease progressions. Other phyla, including Bacteroidetes, Actinobacteria, Fusobacteria, Proteobacteria, and Tenericutes, were also correlated to the lipids. *Proteobacteria,* which contains various pathogens, including *Escherichia- Shigella,* and *Sutterella,* were also related to altered lipid levels. The lipids, significantly correlated with the disease were mainly bile acids (deoxycholic acid, glycocholic acid, isohyodeoxycholic acid) and arachidonic acid (Figure 4B, D; Supplementary 1) [99]. Altered lipid metabolism and a high abundance of the mentioned genera could be used as suitable diagnostics tools in the coming days.

Gut microbial alteration can potentially change the host's healthy homeostasis at the systemic level, which ultimately causes multiple health issues simultaneously. So the diseases caused by microbial dysbiosis are interconnected regarding gut microbial composition and host metabolic changes. In this current study, we tried to discover the diseases caused by microbial dysbiosis and share similar changes in microbial and metabolic composition and diversity.

Reanalysis of the public datasets of different diseases showed that the microbial and metabolic composition of type 2 diabetes patients has the highest similarities with other diseases like obesity, autism, and lupus. We tried to shortlist some common metabolic biomarkers for microbially and metabolically connected diseases. For example, drastic changes in butyrate level can be a signal of either obesity or type 2 diabetes or both. Similarly, changes in propionate and acetate can be the marker for autism and type 2 diabetes, glycocholic acid, and cholic acid as the marker for lupus and type 2 diabetes, and changes in phenylalanine levels can be promising markers for IBD, autism, or both (Figure 5; Supplementary 1). We further performed a predicted network analysis of microbes and metabolites with the diseases that share common metabolic biomarkers (Figure 6). The predicted microbial-metabolic network can be used as a propitious tool for disease diagnosis.

The concept of a healthy and diseased microbiome is not universal. The advancement of microbiome research shatters the concept of healthy gut microbiota. We will be highlighting this in the next section of the article.

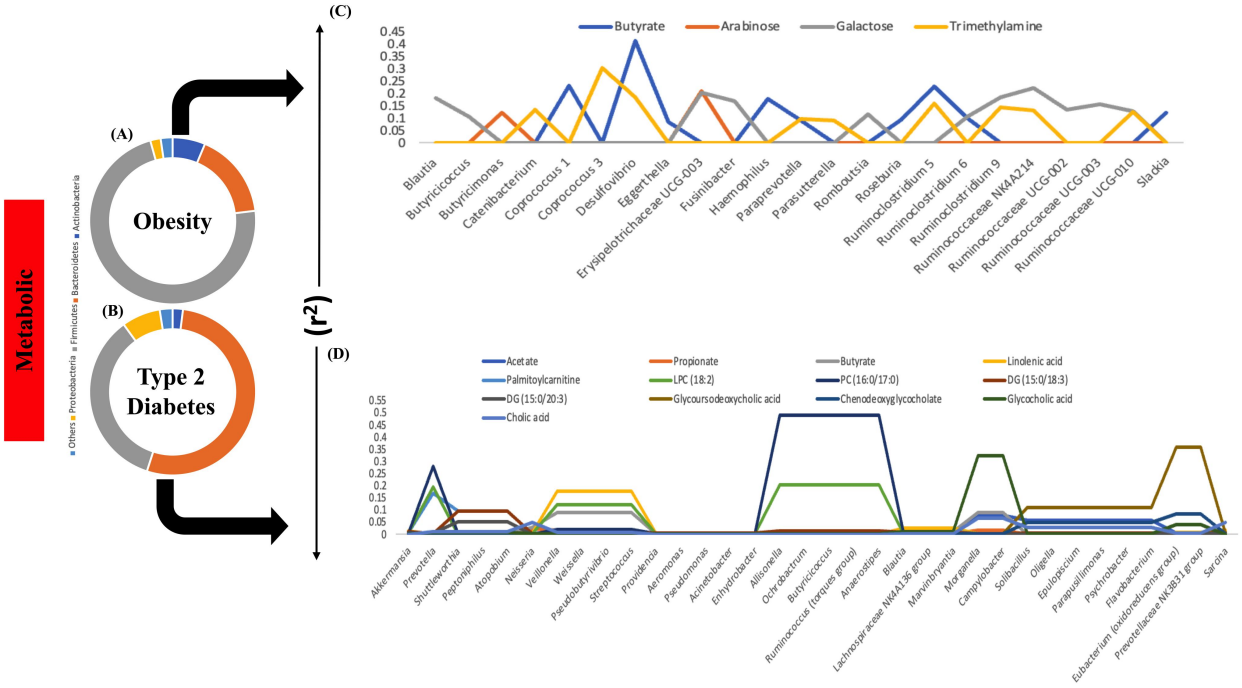


Figure 3 Global correlation of the altered microbiome and metabolomic profile of obesity and type 2 diabetes. Obesity and type 2 diabetes related microbial and metabolic changes are demonstrated here. Correlation study explained the specific genus responsible for specific disease related metabolic changes. Changes in the gut microbial composition of obese (A) and diabetic (B) hosts in phylum level and the correlation (Spearman) between altered gut microbiota and altered metabolism of the host due to Obesity (C) and Type 2 Diabetes (D).

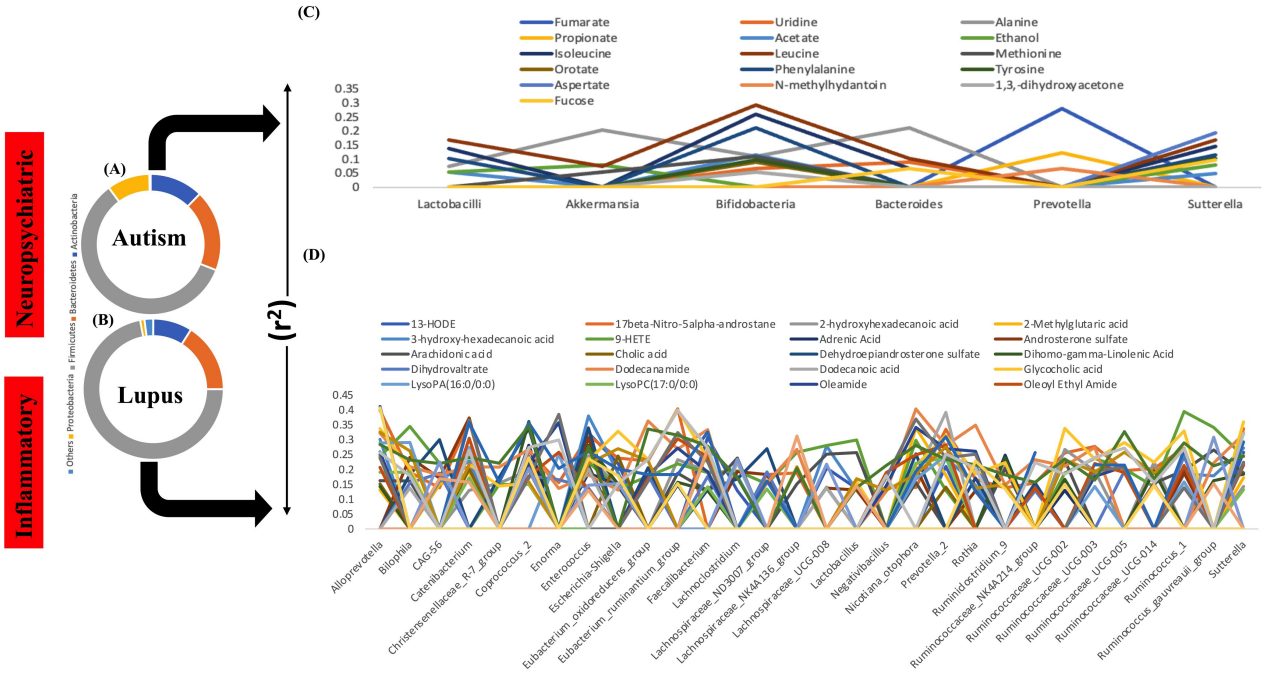


Figure 4 Global correlation of the altered microbiome and metabolomic profile of Autism and Lupus. Autism and Lupus related microbial and metabolic changes are demonstrated here. Correlation study explained the specific genus responsible for specific disease related metabolic changes. Phylum level composition of altered gut microbiota of Autism (A) and Lupus (B) affected host and the correlation (Spearman) between altered gut microbiota and altered metabolism of the host due to Autism (C) and Lupus (D).

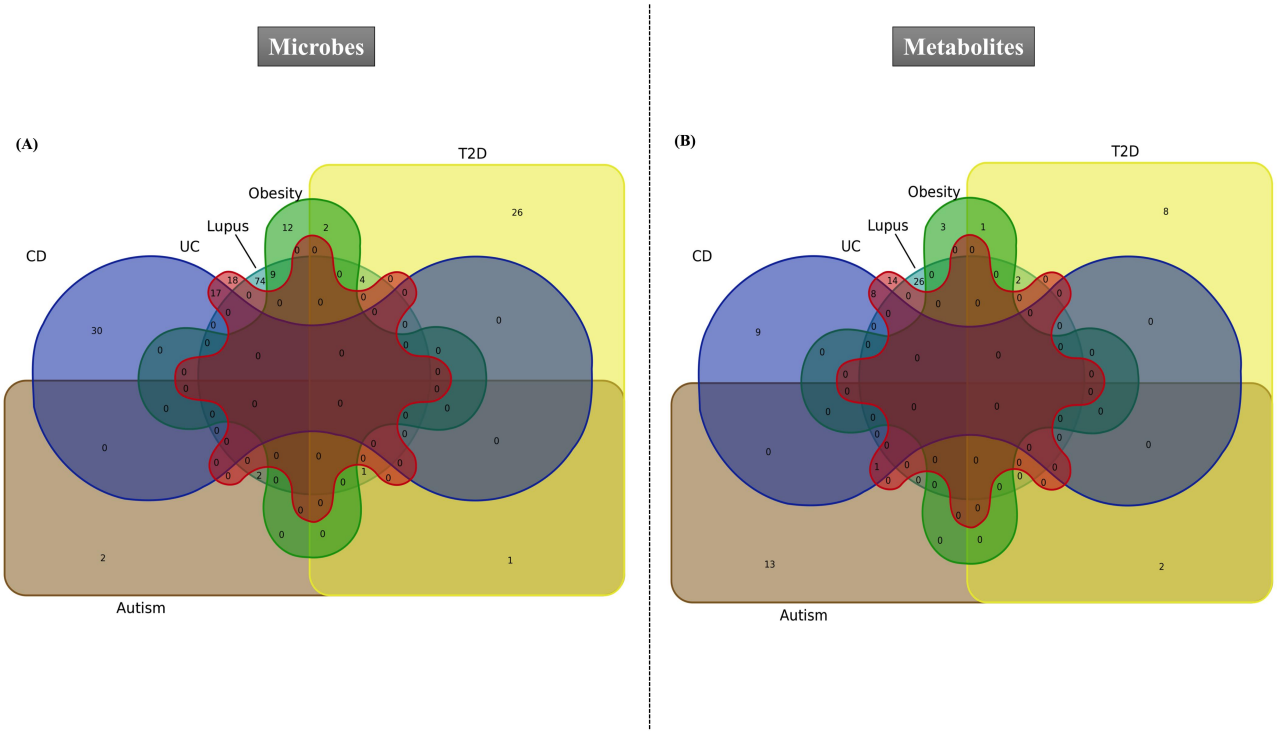


Figure 5 Venn diagram of unique and common microbes and metabolites among various diseased conditions. Different gut microbiota related diseases share can share certainly similar gut microbial and metabolic composition. In this figure we tried to figure out the number of common and unique microbial and metabolic composition across various diseased conditions. (A) represents the common and unique microbes present in the gut under different diseased conditions; (B) describes the common and unique metabolites compositions under various diseased conditions.

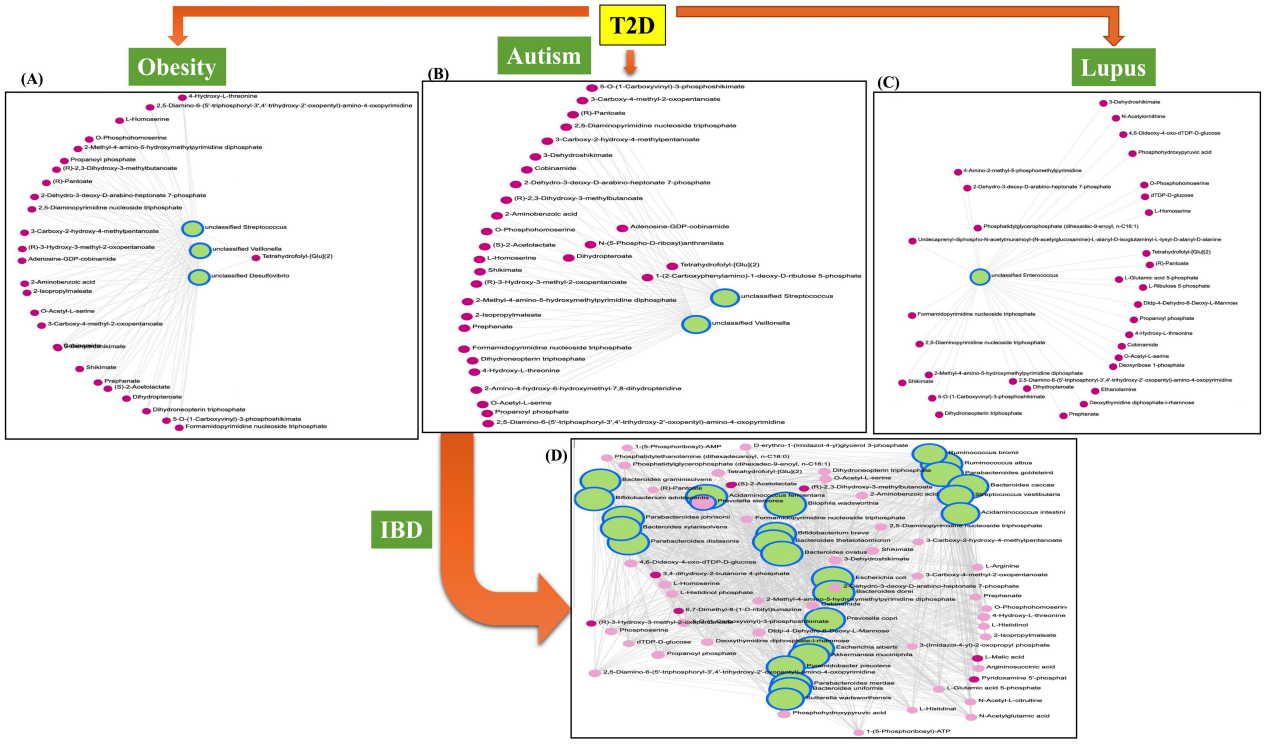


Figure 6 A predictive model of affected metabolites in various diseased conditions based on their gut microbial composition and inter-relation between different diseases in terms of microbiota and metabolism. From microbial and metabolic correlation data, we sorted the diseases which are interrelated gut microbially and metabolically and predicted some similar microbial and metabolic changes between Type 2 diabetes (T2D) and (A) Obesity (B) Autism (C) Lupus and in between Autism and IBD (D).

The myth of a healthy gut microbiome

Nowadays, microbiome ecologists grapple with a significant problem in defining the healthy microbiome, especially the gut microbiome. The vast diversities in human genetic composition, dietary habits, and geographic distribution worldwide add more complexity to understanding the healthy gut microbiome. Archiving the healthy and diseased gut microbiome is mainly done based on research in western and developed countries. The traditional definition of a healthy gut microbiome becomes vague when research crosses the boundary of the westernized developed countries.

Racial influences on gut microbial compositions

The Human Microbiome Project first investigated whether differences in microbiome ecology and health-related outcomes are associated with ethnic, racial, and national categories, such as Black, White, Asian, Mexican, and Puerto Rican or not. One of their project reports concludes that "ethnic/racial background proved to be one of the strongest associations" of metabolic pathways and microbes with clinical metadata [100, 101].

The concept of 'race' emerges in human microbiome ecology as 'biosocial race' in which the socio-cultural diversity in human groups is taken to induce differences in health-related biological traits in these groups. Whereas the biological component of race, basically the difference in microbial characteristics of groups, has some degree of autonomy from social factors, as it also depends on the host's genome and can be transmitted through biological inheritance channels [100].

Presently researchers have focused on different explanatory interests regarding the differential microbial composition of different races. One set of investigations focuses on developing interventions for traditional, indigenous, or non-western populations with seemingly high disease susceptibilities. The targeted study populations include post-colonial areas outside Europe and North America [102, 103]. The second group of studies explained, the reason behind the 'impoverished western microbiome'. The subtending assumption of these studies focuses on the evolution of the human microbiome; the diversity of microbial species decreased when human 'civilizations' passed from foraging and rural farming to urban and industrialized western lifestyle that includes the overuse of antibiotics and high-fat diets [104-106].

In recent days, race or ethnicity studies on human microbiomes related to the Latin American tribal population have been a rapidly growing area. Researchers identified the indigenous non-westernized microbiomes of healthy ethnic individuals [100, 101, 107]. Surprisingly the results indicate that most of the healthy gut microflora of the ethnic populations are the major disease causing factor for the civilized population. For a better understanding of the above mentioned fact, we have mentioned the gut microbial composition of ethnic human races and their food habits in detail in Table 2.

Table 2 Global distribution of various ethnic human races, their food habits, gut microbial composition, and the traditional disease-causing taxa, which are very much indigenous for ethnic human community

Community	Dietary composition	Major taxa of the gut microbiome	Traditionally unhealthy taxa	References
Hadza Hunter-gatherers of Tanzania	Meat (Dik-dik, Giraffe, Galago, Bee larvae), Honey, Baobab, Berries, and Tubers	*Prevotella, Eubacterium, Oscillibacter, Butyricicoccus, Sporobacter, Succinivibrio and Treponema*	*Prevotella*- Autoimmune disease, gut inflammation, diabetes, insulin resistance *Succinovibrio*- Malnutrition	[156–159]
Yanomami Hunter-gatherers of Amazon	Snakes, Wild Pigs, Monkeys, Deer, Jaguars, Insects, Larvae, Fish, Crabs, Wild Honey, Roots, Palm fruits	*Escherichia, Klebsiella, Ralstonia, Neisseria, Desulfovibrio, Cutibacterium Akkermansia, Treponema, Brachyspira*	*Brachyspira*- IBS, Diarrhoea *Ralstonia*- Cause chronic kidney disease in colitis patients	[160–162]
Papua New Guineans Rural Population	Sweet potato, Plantain, Cassava, Rice, Sago, Taro, Banana, Yam, Pumpkin, Kumu, Beans, Tulip, nuts, Coconut, Pitpit, Mango, Meat (Fresh Fish, Chicken, Lamb, Cassowary, Bandicoot)	*Prevotella, Bifidobacterium, Slackia, Propionibacterium, Streptococcus, Staphylococcus, Eubacterium, Erysipelotrichaceae, Clostridium senso stricto, Sarcina, Enterococcus, Lactobacillus*	*Prevotella*- Autoimmune disease, gut inflammation, diabetes, insulin resistance *Erysipelotrichaceae*- Obesity	[157, 158, 163–165]
Bassa Population of Nigeria	Tubers (Yams, Cassava), Grains (Guinea Corn, Millet, Maize), Fruit (Banana, Mango), Fermented Maize-Millet-Sorghum, Soups (e.g., Ayoyo from *Corchorus* Leaves and Kuka from Leaves of *Adansonia Digitata*), Soup Condiments (Okra, Melon), Fish, Very rarely meat (Goats, Chicken)	*Prevotella,* other Bacteroidales members (including *Prevotella* and an unknown S24-7 genus), *Bulleidia*, *Eubacterium*, *Cetobacterium*, *Succinivibrio*, and unclassified *Peptostreptococcacea, Phascolarctobacterium, Treponema, Ruminobacter, Butyrivibrio*	*Prevotella*- Autoimmune disease, gut inflammation, diabetes, insulin resistance *Succinovibrio*- Malnutrition *Peptostreptococcacea*- Non-alcoholic fatty liver disease	[157–159, 166, 167]
Fulani Nomadic of Nigeria	Raw and Cooked Milk, Fermented Milk, Local herbs, Maize, Millet, Yam, Rice, Cabbage, Bitter leaf	*Prevotella* 9, *Clostridium sensu stricto* 1, *Faecalibacterium, Eubacterium rectale group, Campylobacter, Prevotella 2*	*Prevotella*- Autoimmune disease, gut inflammation, diabetes, insulin resistance *Eubacterium rectale*- Induce colitis and colorectal cancer *Campylobacter*- Foodborne enterocolitis	[157, 158, 168–170]
Malawi Tribes of East Africa	Corn, Cassava, Fish, Egg, Meat, Chicken, Fruit (Sugar cane, other fruit juices), Crackers, Yogurt, Cheese milk, Mayonnaise, Coffee	*Prevotella, Dialister, Succinivibrio*	*Prevotella*- Autoimmune disease, gut inflammation, diabetes, insulin resistance *Dialister*- Osteoporosis *Succinovibrio*- Malnutrition	[157–159, 171, 172]

Table 2 Global distribution of various ethnic human races, their food habits, gut microbial composition, and the traditional disease-causing taxa, which are very much indigenous for ethnic human community (*Continued*)

Community	Dietary composition	Major taxa of the gut microbiome	Traditionally unhealthy taxa	References
Amerindians Tribe of Guyana	Corn, Cassava, Fish, Egg, Meat, Chicken, Fruit (Sugar cane, other fruit juices), Crackers, Yogurt, Cheese milk, Mayonnaise, Coffee	*Prevotella, Bacteroidales, Dialister, Succinivibrio*	*Prevotella*- Autoimmune disease, gut inflammation, diabetes, insulin resistance *Dialister*- Osteoporosis *Succinovibrio*- Malnutrition	[157–159, 171, 172]
BaAka Rainforest Hunter-gatherers of Central Africa	Gozo, Bitter manioc leaves, Koko leaves, Peanut sauce, Blue Duiker meat	*Prevotellaceae, Treponema, Sutterella, Anaerovibrio,* and unclassified members of the Clostridiaceae and Cyanobacteria	*Prevotella*- Autoimmune disease, gut inflammation, diabetes, insulin resistance *Sutterella*- Autism spectrum disorder	[157, 158, 173, 174]
Bantu Tribes of Central Africa	Sorghum, Maize, Millet, Legumes, Cucurbits (Squash, Melons), Eggs, Seasonally available fruits, Goat, Chicken, Fish, Cattle meat	Ruminococcaceae, Mogibacteriaceae, *Faecalibacterium*, *Leuconostoc*, *Lactococcus*, Christenellaceae, *Dialister*	*Ruminococcus*- Autism spectrum disorder *Dialister*- Osteoporosis	[172–174]
Tribes of Botswana	Sorghum, Maize, Millet, Corn, Legumes, Cucurbits (Squash, Melons), Foraged plants, Eggs, Seasonally available fruits, Goat, Chicken, Fish meat	*Prevotellaceae, Spirochaetaceae (Treponema), Succinivibrionaceae, Anaerovibrio*	*Prevotella*- Autoimmune disease, gut inflammation, diabetes, insulin resistance *Succinovibrio*- Malnutrition	[157–159, 175]
Himalayan Tribes of India	Native tubers, Greens, Fruits from the jungle, Wild honey, Fish, Occasional game, Yogurt, Snails, Fermented millet and corns, foraged plants	*Treponema, Prevotella Clostridium sensu stricto, Catenibacterium, Lactobacillus, Bulleidia, Sarcina, Enterococcus, Eubacterium, Oribacterium, Mogibacterium, Mitsuokella, Allisonella, Weissella, Papilbacter*	*Prevotella*- Autoimmune disease, gut inflammation, diabetes, insulin resistance *Catenibacterium*- Colorectal cancer *Mitsuokella*- Type III obesity	[157, 158, 176–178]
Tribes of Assam, India	Rice, Vegetables, Fish, Meat, Legumes, Whole grains, Fruits, Tubers	*Prevotella, Faecalibacterium, Eubacterium, Clostridium, Blautia, Collinsella, Ruminococcus, Roseburia*	*Collinsella*- Cause inflammation, non-alcoholic steatohepatitis *Ruminococcus*- Autism spectrum disorder *Prevotella*- Autoimmune disease, gut inflammation, diabetes, insulin resistance	[157, 158, 174, 179, 180]
Tribes of Manipur, India	Rice, Vegetables, Fish, Meat, Legumes, Whole grains, Fruits, Tubers, Fermented bamboo shoot, Fermented soybean, Fermented mustard seeds and leaves, Dried and smoked fish and meat	*Prevotella, Faecalibacterium, Eubacterium, Clostridium, Blautia, Collinsella, Ruminococcus, Roseburia, Bacteroides, Dialister, Veillonella*	*Collinsella*- Cause inflammation, non-alcoholic steatohepatitis *Ruminococcus*- Autism spectrum disorder *Prevotella*- Autoimmune disease, gut inflammation, diabetes, insulin resistance *Dialister*- Osteoporosis	[157, 158, 172, 174, 179, 180]
Tribes of Sikkim, India	Rice, Boiled vegetables, Fish, Meat, Legumes, Whole grains, Fruits, Tubers, Fermented bamboo shoot, Fermented soybean, Fermented mustard seeds and leaves, Dried and smoked fish and meat, Milk and milk products	*Prevotella, Faecalibacterium, Eubacterium, Clostridium, Blautia, Collinsella, Ruminococcus, Roseburia, Bacteroides, Dialister, Bifidobacterium, Lactobacillus*	*Collinsella*- Cause inflammation, non-alcoholic steatohepatitis *Ruminococcus*- Autism spectrum disorder *Prevotella*- Autoimmune disease, gut inflammation, diabetes, insulin resistance *Dialister*- Osteoporosis	[157, 158, 172, 174, 179, 180]
Tribes of Telangana, India	Rice, Vegetables, Fish, Meat, Legumes, Whole grains, Fruits, Tubers	*Prevotella, Faecalibacterium, Eubacterium, Clostridium, Blautia, Collinsella, Ruminococcus, Roseburia*	*Collinsella*- Cause inflammation, non-alcoholic steatohepatitis *Ruminococcus*- Autism spectrum disorder *Prevotella*- Autoimmune disease, gut inflammation, diabetes, insulin resistance	[157, 158, 174, 179, 180]
Tribes of West Bengal, India	Rice, Leafy vegetables, Roots, Starchy tubers, Seeds, Fruits, Nuts gathered from forest areas, Wild animals meat	*Ruminococcaceae, Succinovibrio, Bacteroides*	*Ruminococcaceae*- Lean body mass *Succinovibrio*- Malnutrition	[159, 174, 181, 182]

Difficulties/drawbacks of the current metagenomic approach

The advancement of the metagenomic approach is a blessing for researchers in studying the overall details of the human gut microbiome, but not without limitations.

Lack of subspecies and strain level information and individual microbial expression

The major drawback of the metagenomic technique is that this can detect only species up to the accuracy of genus level while ignoring subspecies and the specific strain of the microbiota. It is also difficult to detect the individual microbial expression from the consortium of the gut microbiota [32, 33].

Higher sequence coverage

The nutrient-rich environment of the gut is the main reason for the larger genome size of the bacteria present in the gut. The metagenomic approach requires much higher sequence coverage. This approach's costs and time are much higher than the other techniques [108].

Quality and quantity of the DNA sample

The technique demands very high quality and a sufficient amount of DNA samples. The quality of the DNA becomes compromised due to the presence of the human contaminant in the DNA sample. Almost 50%-90% of the available DNA sequence contains a human contaminant. The unavailability of the raw samples is the major limitation to obtaining the high quantity of DNA samples [109].

Use of different DNA extraction kits

The sample's DNA yield and bacterial DNA composition varied significantly between commercially available kits. Other DNA extraction kits cause the enrichment of different bacterial taxa, which are highly kit-specific. It is challenging to compare the data obtained from different DNA extraction kits and laboratories [110].

Use of different DNA extraction methods

The DNA extraction method influences the community structure of the gut microbial samples. Sometimes the inter-individual variation exceeded the variation resulting from the choice of extraction method. The main challenge is to compare the data across studies applying different DNA extraction methodologies [111].

Quality of functional annotations

The most crucial parameter of metagenomic sequence fragments is the underlying functional annotations. A significant proportion of the metagenomic data cannot be assigned a function due to a lack of close matches in reference databases [112].

The problem of OTU-based data analysis

OTU-based metagenomic analysis provides lower taxonomic resolution of the data, broadly impacting the alpha diversity estimations of the gut microbial community [113].

Drawbacks of using different NGS platforms for metagenomic study

454 Gs Flx + (Roche). Expensive, the high error rate in homopolymers, short sequencing reads, requires extensive bioinformatics analysis [114].

MiSeq/HighSeq (Illumina). PCR bias, incapable of characterizing unknown species from the sample [114].

5500 SOLiD (Life technologies). Short coverage and a very long process [114].

PacBio RS (Pacific Bioscience). Expensive, high error rate, complex installation [114].

Ion torrent (Life Technologies). Technology is not developed correctly; the instrument is under development [114].

Conclusion

Human gut microbiota evolves throughout life, but the core native microbiota is shaped in early childhood, mainly within 4-36 months. Scientists call microbiota healthy when it supports the homeostasis in the host's immunological, metabolic, and neurological functions. A dysbiotic state of the microbiota by any factors, e.g., unbalanced diet, stress, antibiotic use, or other environmental factors, ultimately causes life-threatening systemic diseases. Altered microbiota and associated metabolic changes made this microbiome and metabolites a powerful tool for diagnosing diseases.

Reanalysis of various disease-associated altered microbiome and metabolites data strongly supported the hypothesis that disease specific altered microbiota has a strong influence in determining the overall metabolic status of the host. Microbiota-derived metabolites are one of the key factors in determining the disease severity. Altered metabolite compositions are very much disease specific, and a particular group or genus of the microbes is responsible for producing specific metabolites. Using this concept, in the current study, we tried to find some disease-specific metabolites biomarkers for diagnosis purposes, e.g., altered short-chain fatty acids, mainly butyrate, acetate, and propionate, can be used as biomarkers for metabolic or neuropsychiatric diseases like obesity, type 2 diabetes, autism or the person can be affected by all the three diseases together. On the other hand, glycolcholic acid, cholic acid, and phenylalanine can be used to diagnose inflammatory diseases like lupus and IBD, as well as diseases like type 2 diabetes or autism. These metabolites have an excellent prospect of being used as the biomarker of early disease diagnosis, and the method is cost-effective too.

The conflict arises when the researchers establish the composition of healthy human microbiota irrespective of the environmental factors, food habits, and different races of humans. The signature healthy gut microbiota is very much specific to human races, their food habits, and other environmental factors such as industrialization, the pattern of lifestyle, etc. The myth of healthy gut microbiota became shattered when researchers started identifying the gut microbial composition of ethnic human races worldwide. They are not exposed to industrialization and have different lifestyles and food habits than westernized countries. More interestingly, the gut microbial composition, categorized as diseased gut microbiota for westernized people, is considered healthy for traditional human races. So, the concept of determining healthy gut microbiota becomes vague in this context.

Technological advancement in gut microbial research is a blessing for researchers to dig the area deeper and find the exact mechanism of how the gut microbial world maintains the host system's homeostasis. But every technology has its glitches. In the last part of the review, we discussed the technological glitches of gut microbiome research. More technological advancement in the current techniques may help scientists overcome all the problems shortly.

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