MICROBIOME, HUMAN HEALTH AND BIOMARKERS: A BRIEF REVIEW

Mitu De[1,*], Sayak Ganguli[2] and Santi Ranjan Dey[3]

[1]Associate Professor, Department of Botany, Gurudas College, Kolkata 54

[2]Group Leader, Amplicon Institute of Interdisciplinary Science and Technology, Palta

[3]Assistant Professor, Department of Zoology, Rammohan College, Kolkata 9.

[*]Corresponding author e-mail: mitude@rediffmail.com

Abstract

The human microbiome, consisting of the total microbial complement associated with human hosts, is an important emerging area for metagenomic biomarker discovery. Current evidences support that alterations in composition and/or metabolic activity of microbiome play pivotal role in the pathogenesis of many diseases and disorders. Changes in gut microbiota can modulate the peripheral and central nervous systems, resulting in altered brain functioning, and suggesting the existence of a microbiota gut-brain axis. Countless efforts are now underway to develop microbiome-based diagnostics, therapeutics, and services in both the academic and commercial arenas. This paper is a review on the association of microbiome and human health with development of biomarkers.

Keywords: microbiome, metagenomics, gut-brain-axis, biomarkers

Introduction

A biomarker is a measurable attribute associated with the clinical status of a patient. The National Institutes of Health (NIH) defines a biomarker as: "a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes or pharmacologic responses to a therapeutic intervention." Biomarkers can arise from genes, proteins, peptides and metabolites, the global biochemical approaches for biomarker discovery have been dubbed the 'omics' and there are a variety of technologies spanning genomics, proteomics and metabolomics, all of which are currently being applied to biomarker research.

In clinical research, the term 'biomarker' or 'biological marker' refers to a broad category of medical signs, or objective indications of medical state, that can be measured accurately and reproducibly and may influence and predict the incidence and outcome of disease [1]. Biomarkers provide a dynamic and powerful approach to understanding the spectrum of neurological disease/disorders with applications in observational and analytic epidemiology, randomized clinical trials, screening and diagnosis and prognosis [2]. However, in order for

a biomarker to be developed, it must be demonstrated that the marker is present before the onset of symptoms, and that it is specific to the disorder [3].

Biological markers (biomarkers) have been defined as "cellular, biochemical or molecular alterations that are measurable in biological media such as human tissues, cells, or fluids" by Hulka in 1990 [4]. More recently, the definition has been broadened to include biological characteristics that can be objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention [5].

The Human Microbiome

The human microbiota encompasses several thousands of fungi, eubacteria, archaea and viruses, with eubacterial cells alone totaling over 10 trillion and outnumbering our body cells 100 to 1 [6]. The microorganisms found in the human oral cavity have been referred to as the oral microflora, oral microbiota, or oral microbiome [7]. The major niches colonised by microbes in the human body are gut, mouth, genitals, skin and airways. The genome of these microbiota and their ecosystem constitute to form a microbiome. Not only the types and abundance of microbes are different in different organs, but also these may differ in different individuals. Factors such as diet, environment, host genetics etc. may be the reason behind the wide microbial diversity [8].

Dysbiosis

Current evidences support that alterations in composition and/or metabolic activity of microbiome play pivotal role in the pathogenesis of many diseases and disorders. Alteration in microbial communities (often referred to as *dysbiosis*) has been shown to be associated with diseases ranging from infectious (*Clostridium difficile* infection) to inflammatory (inflammatory bowel disease [IBD] and rheumatoid arthritis) and metabolic (diabetes and obesity) diseases, suggesting an important role for them in the pathogenesis of multifactorial conditions [9]. Remarkable new findings showed that our microbiome not only primarily affects the health and function of the gastrointestinal tract but also has a strong influence on general body health through its close interaction with the nervous system and the lung. Therefore, a perfect and sensitive balanced interaction of microbes with the host is required for a healthy body [10].

The human microbiome is classified into a core' microbiome and a variable' microbiome [11]. The core microbiome is shared among all individuals and is comprised of the predominant species that exist under healthy conditions at different sites of the body [11- 13]. The variable microbiome is exclusive to the individual and has evolved in response to unique lifestyle, and phenotypic and genotypic determinants. Some examples of dysbiosis implicated in some diseases are given in Table I.

Table I. Dysbiosis implicated in a wide range of diseases

Disease/Disorders influenced by Dysbiosis	**Research Group & Year**	**Reference**
Inflammatory bowel disease	Li *et al.*, 2015	[14]
Diabetes	Tilg and Moschen, 2014 Qin et al., 2012	[15] [16]
Crohn's disease	Joossens *et al*, 2011 Hedin *et al*, 2014	[17] [18]

Obesity	Arslan, 2014,	[19]
Colorectal cancer (CRC)	Sobhani *et al.* 2011	[20]
Depression	Naseribafrouei *et al*, 2014 Smythies *et al*, 2014	[21] [22]
Autism Spectrum Disorder (ASD)	Macfabe, 2012 Li, Qinrui *et al*. (2017).	[23] [24]
Hypertension	Li J, Zhao F, Wang Y, Chen J, Tao J, Tian G, et al. . 2017	[25]
Liver Cancer	Qin *et al* 2014 Yu and Schwabe, 2017	[26] [27]
Atopic dermatitis.	Kong *et al*, 2012	[28]

Types of Biomarkers in Human Health

There are two major types of biomarkers: biomarkers of exposure, which are used in risk prediction, and biomarkers of disease/disorder, which are used in screening and diagnosis and monitoring of disease progression. Biomarkers used in risk prediction, in screening, and as diagnostic tests are well established, and they offer distinct and obvious advantages. The classification of many neurological diseases is based on either standardized clinical criteria or histological diagnoses [2].

Biomarkers depicting prodromal signs enable earlier diagnosis or allow for the outcome of interest to be determined at a more primitive stage of disease. Blood, urine, and cerebrospinal fluid provide the necessary biological information for the diagnosis. In these conditions, biomarkers are used as an indicator of a biological factor that represents either a subclinical manifestation, stage of the disorder, or a surrogate manifestation of the disease.

Biomarkers used for screening or diagnosis also often represent surrogate manifestations of the disease/disorder. The potential uses of this class of biomarkers include:

1) identification of individuals destined to become affected or who are in the "preclinical" stages of the illness,

2) reduction in disease heterogeneity in clinical trials or epidemiologic studies,

3) reflection of the natural history of disease encompassing the phases of induction, latency and detection, and

4) target for a clinical trial. The improvement in validity and precision far outweigh the difficulty in obtaining such tissues from patients.

Biomarkers are potentially useful along the whole spectrum of the disease/disorder process. Before diagnosis, markers could be used for screening and risk assessment. During diagnosis, markers can determine staging, grading, and selection of initial therapy. Later, they can be used to monitor therapy, select additional therapy, or monitor recurrent diseases [29].

Association of the Human Microbiome and diseases

Gut microbiota, consisted of trillions of microorganisms and modulated mainly by diet [30]. Microorganisms from the oral cavity have been shown to cause a number of oral infectious diseases, including caries (tooth decay), periodontitis (gum disease), endodontic (root canal)

infections, alveolar osteitis (dry socket), and tonsillitis [7]. Evidence is accumulating which links oral bacteria to a number of systemic diseases [31]. The gut microbiota is essential to human health and the immune system and plays a major role in the bidirectional communication between the gut and the brain. Based on evidence, the gut microbiota is associated with metabolic disorders such as obesity, diabetes mellitus and neuropsychiatric disorders such as schizophrenia, autistic disorders, anxiety disorders and major depressive disorders.

The human microbiome is one such factor, which not only has a pivotal role in maintaining health but also in regulating various inflammatory and metabolic pathways in a range of conditions including gastrointestinal (GI) diseases (inflammatory bowel disease, irritable bowel syndrome), arthritis (rheumatoid arthritis), cancer (colorectal cancer) and recently in chronic pulmonary diseases, such as asthma, Chronic obstructive pulmonary disease (COPD) and Cystic fibrosis (CF) [32, 33].

Characterization of the human microbiome

The Microbiome research has benefited vastly from the genomic revolution, allowing DNA-based identification of nonculturable bacteria inhabiting various body sites. The human microbiome, consisting of the total microbial complement associated with human hosts, is an important emerging area for metagenomic biomarker discovery [34]. Comparisons between healthy and diseased tissues have highlighted the importance of tasks such as class discovery (detecting novel subtypes of a disease) and class prediction (determining the subtype of a new sample) [35- 37].

The revolutionary change in our ability to understand the role of the microbiome came with the advent of next-generation sequencing that has allowed in-depth characterization of the gut microbiota using multi-omics approaches without the need to culture individual microbes, which in some instances can be quite challenging. The current methods use a spectrum of strategies to characterize the microbiome, the simplest being the marker gene approach using variable regions within the highly conserved 16S ribosomal RNA gene. This approach, although valuable in assessing alterations in microbial community structure, fails to provide resolution at species or strain level and does not provide sufficient functional insight into the community. Complimentary approaches including metagenomics (study of all genomes in an ecosystem), metatranscriptomics (characterization of gene expression from all microbes in an ecosystem), metabolomics (characterization of all small molecule metabolites in an ecosystem), and metaproteomics (characterization of all proteins in an ecosystem) provide greater insight into functional potential as well as the expression of microbiome-derived bioactive molecules necessary to understand the therapeutic implications for the microbiome [38].

The human microbiome project (HMP)

This international effort emanates from a confluence of ongoing technical and computational advances in the genome sciences, an evolving focus of microbiology on the properties and operations of microbial communities, and the notion that rapid, and marked, transformations in human lifestyles are not only affecting the health of the biosphere, but possibly our own health as a result of changes in our microbial ecology. The human microbiome project (HMP) reflects the fact that we, humans are supraorganisms composed of human and microbial components. HMP is designed to understand the microbial components of our genetic and metabolic landscape, and how they contribute to our normal physiology and disease predisposition. It is a global and interdisciplinary project that promises to break down the

artificial barriers between medical and environmental microbiology [11]. So influence of the microbiome on human development as well as disease/disorder pathogenesis and progression is a significant area of interest [39]. Countless efforts are now underway to develop microbiome-based diagnostics, therapeutics, and services in both the academic and commercial arenas.

Oral microbiome and human health

The oral cavity, or mouth, includes several distinct microbial habitats, such as teeth, gingival sulcus, attached gingiva, tongue, cheek, lip, hard palate, and soft palate. Contiguous with the oral cavity are the tonsils, pharynx, esophagus, Eustachian tube, middle ear, trachea, lungs, nasal passages, and sinuses [7]. The oral cavity is a large reservoir of bacteria of more than 700 species or phylotypes and is profoundly relevant to host health and disease [40, 41]. Due to its topological position, bacteria in the oral cavity are influenced by various factors such as personal hygiene [42], diet [43] and smoke [44]. Studies have shown that different oral structures and tissues are colonized by distinct microbial communities. Oral microbiome has recently been recognized as a primary mediator for human health [45, 46].

While oral microorganisms exist in a symbiotic capacity, maintaining relationships with the host based on mutual benefits, some can transition to pathogens when they breach the barrier of commensalism, causing disruption of oral homeostasis, or "dysbiosis" [31, 40]. Despite advances in our knowledge of the healthy oral microbiome, the functional aspects that lead to dysbiosis remain largely unknown [47]. What is now clear, however, is that oral diseases arise as a result of a change in the proportion of certain species with greater pathogenic potential within the indigenous flora [31].

The Microbiota-Gut-Brain Axis

The gut microbiota is essential to human health and the immune system and plays a major role in the bidirectional communication between the gut and the brain [48]. Changes in gut microbiota can modulate the peripheral and central nervous systems, resulting in altered brain functioning, and suggesting the existence of a microbiota gut-brain axis [49]. The collective microbiota of the gut whose DNA contributes to the metagenome have links with inflammatory bowel disease (IBD), liver disorders, ankylosing spondylitis, neurodegenerative diseases, obesity and associated noncommunicable diseases (NCDs) including diabetes mellitus, hypertension, atherosclerosis, coronary heart disease, and neurodegenerative diseases beside other condition [50-52]. The association between oral diseases and systemic conditions has encouraged the exploration on the influence of oral dysbiosis and the pathogenesis of oral cancers. The role of gut microbiota in promoting gastric cancer is well established [53].

The Human Oral Microbiome Database (HOMD)

The human oral microbiome has been extensively studied, is being examined as part of the Human Microbiome Project, and will continue to be examined in the future using ever more powerful sequencing technologies. The Human Oral Microbiome Database (HOMD) was created for taxonomic framework, with oral taxon numbers to facilitate communication between investigators exploring the diversity of the oral microbiome, as seen by 16S rRNA gene-based methods. It is critical that investigators can point to curated stably designated taxa rather than taxonomically undefined clone sequences in disseminating research findings [7]. The HOMD can be useful to the entire Human Microbiome Project and infectious disease communities.

Current evidences support that alterations in composition and/or metabolic activity of gut microbiota play pivotal role in the pathogenesis of obesity and related disorders [54]. The intimate association between man and microbe over the course of a lifetime has profound implications on human health, including metabolism, immunity and the gut-brain axis. Microbes in the tongue often travel around the oral cavity to colonise other regions, facilitated by saliva. Microbes in the tongue include *Veillonella atypica, Porphyromonas gingivalis, Selenomonas* spp., *Actinobacillus actinomycetemcomitans, Prevotella intermedia, Capnocytophaga* spp. and many more [55]. Unique microbes residing in the oropharynx include *Streptococcus pyogenes, Streptococcus pneumoniae, Haemophilus influenza* and *Haemophilus parainfluenzae* .

The backbone of the HOMD is its set of reference 16S rRNA gene sequences, which are used to define individual human oral taxa and to create the phylogenetic and taxonomic structures of the database. The initial reference set of 16S rRNA gene sequences for the HOMD consisted of over 800 full-length sequences (each of which is greater than 1,500 bases) of named oral bacteria from the oral microbiological literature and strain and clone phylotypes generated from our sequencing and cloning studies. After entering and aligning these sequences in our RNA database, neighbor-joining trees were generated.

The HOMD includes 619 taxa in 13 phyla [7], viz.
Actinobacteria, *Bacteroidetes*, *Chlamydiae*, *Chloroflexi*, *Euryarchaeota*, *Firmicutes*,

Fusobacteria, *Proteobacteria*, *Spirochaetes*, SR1, *Synergistetes*, *Tenericutes* and TM7.

Oral Microbiome and Biomarkers

The oral microbiome provides an ideal source for biomarker discoveries due to low inter- and intra- biological variations, in contrast to other tumour biomarkers originating from the host [56]. Recently, culture-independent techniques have revolutionized the knowledge of the gut and oral microbiota. These techniques are based on sequence divergences of the small subunit ribosomal ribonucleic acid (16S rRNA) and can demonstrate the microbial diversity of the gut and oral microbiota, providing qualitative as well as quantitative information on bacterial species and changes in the gut and oral microbiota in health and disease [57].

Gaining insight into the structure, organization, and function of microbial communities has been proposed as one of the major research challenges of the current decade [58], and it will be enabled by both experimental and computational metagenomic analyses. Progress made in high throughput technologies has contributed to developing new fields such as metagenomics, which allows qualifying and quantifying microbial ecosystem composition and functionality with unprecedented resolution. The association of the gut microbiota with human health and disease has been widely discussed [59].

The advent of next-generation sequencing (NGS) technologies and their applications on human populations have enabled clinical diagnostic and microbiological applications through the comparison of microbial communities [60, 61].

.**Salivary biomarkers of potential diagnostic value**

To date, salivary biomarkers of potential diagnostic value has been identified and validated for both oral and systemic diseases/disorders viz. Oral cancer [62]. With advances in detection and quantification methods in genomics, proteomics and metabolomics, saliva has emerged as a good source of samples for detection of disease biomarkers [63].

Owing to the rapid progress made in salivary studies, researchers have proposed the concept of salivaomics. Salivaomics encompasses genomics, transcriptomics, proteomics, metabonomics and microRNA (miRNA) analysis. Salivaomics Knowledge Base (SKB) that can systematically manage the data of research related to salivaomics was built by Wong [64]. It has collected a large amount of information related to salivaomics, pharmacoproteomics, pharmacogenomics and similar fields. It has been well recognized that salivary biomarkers can be exploited for the early diagnosis of some oral and systemic diseases [61].

Saliva is now considered a potential pool of biological markers that range from changes in biochemicals, DNA, RNA and proteins to the microbiota structure. It is relatively safe to collect saliva and minimizes the risk of virus spread. Hence, saliva provides a new, non-invasive and simple way to help in the diagnosis of disease, and it is expected to become a substitute for serum or urine tests in disease diagnosis.

The advantages of saliva as a diagnostic fluid are that it is simple to collect, convenient to store, essentially non-invasive and contains high-quality DNA. Thus, we conclude that saliva is a perfect substitute for blood. The research in salivaomics has an important role in identifying biomarkers of diseases and potential drug targets. Salivaomics also has the potential to diagnose diseases in their early stages.

In Table II a list of oral microbial biomarkers investigated for selected diseases and disorders are given. However, research on saliva and its applications for the diagnosis of disease is still in its early stages and the progress of these studies is limited by the lack of efficient and useful methods and techniques [61].

TABLE II. Oral microbial biomarkers investigated for selected oral and systemic diseases

Disease	**Oral microbial biomarkers investigated**	**Research Group & Year**	**Reference**
Dental caries	*Streptococcus mutans*, *Lactobacillus spp.*	Larmas, 1975; Jordan *et al* 1987	[65] [66]
Periodontal disease	*Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Tannerella forsythia*, *Campylobacter rectus*, *Treponema denticola*	Kumar *et al*, 2005 Sakamoto, *et al.*, 2000 Paster & Dewhirst, 2009, Paju *et al*, 2012;	[67] [68] [69] [70]
Oral cancer	*Capnocytophaga gingivalis*, *Prevotella melaninogenica*, *Streptococcus mitis*	Mager *et al*, 2005	[71]
Obesity	*Selenomonas noxia*	Goodson *et al*, 2009	[72]
Crohn's disease	*Fusobacteria, Firmicutes*	Docktor *et al*, 2012	[73]
Pancreatic cancer	*Neisseria elongata*, *Streptococcus mitis*	Farrell *et al*, 2012	[74]
Chronic pancreatitis	*Granulicatella adiacens*, *Streptococcus mitis*	Farrell *et al*, 2012	[74]

Oral Microbiome and Biomarker Development: Challenges

One area of marked focus is the ability to use changes in microbial community signatures, e.g. dysbiosis, to interrogate potential biomarkers associated with risk, onset or exacerbation of disease as well as for prediction of treatment success [75]. Recent metagenomic assays have shown that human microbial communities can be used as biomarkers for host factors such as lifestyle disorders like obesity [76] and disease/disorder like in Crohn's disease [77] in colitis [78], cardiovascular diseases [79] and Autism Spectrum Disorders [24].

Metagenomic analyses additionally present their own specific issues, including sequencing errors, chimeric reads [80, 81] and complex underlying biology; many microbial communities have been found to show remarkably high inter-subject variability. Key factors in successful microbiome-based biomarker identification and validation include stringent sampling protocols, high quality metagenomic data with sequencing depth adequate to identify all key players, and the ability to identify bacteria of interest. When properly employed, these factors can also result in the successful linking of metabolic and functional profiles to human or animal phenotypic data.

Conclusion

Identifying the most biologically informative features differentiating two or more phenotypes can be challenging in any genomics dataset, and this is particularly true for metagenomic biomarkers [82]. Although the microbiome represents an attractive target for the development of personalized treatment approaches, standardization of methods to develop reliable and reproducible microbiome-based diagnostic and therapeutic strategies remains a challenge. The strong effort by the scientific community, as well as collaboration with rapidly emerging biotech companies, provides an optimistic outlook for developing microbiome-dependent and microbiome-targeted diagnostics and therapeutics [38]. Biomarkers will not replace clinical assessments, which characterize the extent of specific deficits, but could potentially change intervention/treatment goals and methods [83]. Microbiome information, which may be one component of a larger data profile for individuals in this era of personalized medicine, may be used for risk prediction and/or diagnosis of disorder/disease. It could be used for disorder/disease progression and for stratification of subjects in clinical drug trials.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this work.

Acknowledgement: The authors express their thanks to the unknown reviewer for the valuable suggestions.

Reference

1. Strimbu K, Tavel JA. What are biomarkers? 2010. Curr Opin HIV AIDS; 5: 463–466
2. Mayeux, Richard, 2004. Biomarkers: Potential Uses and Limitations. NeuroRx. Apr; 1(2): 182–188.
3. Yerys BE, Pennington BF. 2011. How do we establish a biological marker for a behaviorally defined disorder? Autism as a test case. Autism Res; 4: 239–241.
4. Hulka BS. 1990. Overview of biological markers. In: Biological markers in epidemiology (Hulka BS, Griffith JD, Wilcosky TC, eds), pp 3–15. New York: Oxford University Press.

5. Naylor S. 2003. Biomarkers: current perspectives and future prospects. Expert Rev Mol Diagn 3: 525–529.

6. Chaplin, A.V. & Rebrikov, Denis & Boldyreva, M.N. 2017. The human microbiome. Bulletin of Russian State Medical University. 6. 5-13.
7. Dewhirst, F. E., Chen, T., Izard, J., Paster, B. J., Tanner, A. C., Yu, W. H., Lakshmanan, A., … Wade, W. G. 2010. The human oral microbiome. *Journal of bacteriology*, *192*(19), 5002-17.
8. Rajpoot M, Sharma AK, Sharma A, Gupta GK. 2018. Understanding the microbiome: Emerging biomarkers for exploiting the microbiota for personalized medicine against cancer. Semin Cancer Biol. Oct; 52(Pt 1): 1-8.
9. Marchesi, J.R., Adams, D.H., Fava, F. *et al*. 2016. The gut microbiota and host health: a new clinical frontier. *Gut*; 65: 330–339
10. Scotti, Elena , Boué, Stéphanie, Sasso, Giuseppe Lo *et al.* 2017. Exploring the microbiome in health and disease: Implications for toxicology. Toxicology Research and application. Vol 1: 1-37.
11. Turnbaugh PJ, Ley RE, Hamady M, Fraser-Liggett CM, Knight R, Gordon JI 2007. The human microbiome project: a strategy to understand the microbial components of the human genetic and metabolic landscape and how they contribute to normal physiology and predisposition to disease. Nature 449: 804–810.
12. Zaura E, Keijser BJ, Huse SM, Crielaard W 2009. Defining the healthy ‘‘core microbiome’’ of oral microbial communities. BMC Microbiol 259: 12.
13. Sonnenburg JL. and Fischbach MA 2011. Community health care: therapeutic opportunities in the human microbiome. Sci Transl Med 3: 1–5.
14. Li J, Butcher J, Mack D, *et al*. 2015. Functional impacts of the intestinal microbiome in the pathogenesis of inflammatory bowel disease. Inflammatory Bowel Diseases; 21(1): 139–153.
15. Tilg H, Moschen AR. Microbiota and diabetes: an evolving relationship. Gut. 2014; 63: 1513–21.
16. Qin J, Li Y, Cai Z, *et al.* 2012. A metagenome-wide association study of gut microbiota in type 2 diabetes. Nature. 490(7418): 55–6.
17. Joossens M, Huys G, Cnockaert M, *et al.* 2011. Dysbiosis of the faecal microbiota in patients with Crohn’s disease and their unaffected relatives. Gut; 60: 631–7.
18. Hedin C, McCarthy N, Louis P, *et al.* 2014. Altered intestinal microbiota and blood T cell phenotype are shared by patients with Crohn’s disease and their unaffected siblings. Gut; 63 (10): 1578–86.
19. Arslan N. 2014. Obesity, fatty liver disease and intestinal microbiota. World Journal of Gastroenterology; 20(44): 16452–63.
20. Sobhani I, Tap J, Roudot-Thoraval F, *et al.* 2011. Microbial dysbiosis in colorectal cancer (CRC) patients. PLoS One; 6(1): e16393
21. Naseribafrouei A, Hestad K, Avershina E, Sekelja M, Linløkken A, Wilson R, et al. 2014.Correlation between the human fecal microbiota and depression. Neurogastroenterol Motil.; 26: 1155–1162.
22. Smythies LE, Smythies JR. 2014. Microbiota, the immune system, black moods and the brain-melancholia updated. Front Hum Neurosci.; 8: 720.
23. Macfabe DF. 2012. Short-chain fatty acid fermentation products of the gut microbiome: implications in autism spectrum disorders. Microb Ecol Health Dis.; 23, Article: 19260.
24. Li, Qinrui *et al*. (2017). The Gut Microbiota and Autism Spectrum Disorders, Front Cell Neurosci. 11: 120.

25. Li J, Zhao F, Wang Y, Chen J, Tao J, Tian G, et al. . Gut microbiota dysbiosis contributes to the development of hypertension. Microbiome (2017) 5:14.
26. Qin N, Yang F, Li A, *et al*. 2014. Alterations of the human gut microbiome in liver cirrhosis. Nature; **513**:59–64.
27. Yu LX and Schwabe RF. 2017. The gut microbiome and liver cancer: mechanisms and clinical translation. Nat Rev Gastroenterol Hepatol; **14**.
28. Kong., H. H. *et al*. 2012. Temporal shifts in the skin microbiome associated with disease flares and treatment in children with atopic dermatitis. *Genome Res.* **22**, 850–859
29. Atkinson A J *et* al 2001. NCI-FDA Biomarkers Definitions Working Group; Biomarkers and surrogate endpoints: preferred definitions and conceptual framework; Clin. Pharmaco. Ther. 69 89–95
30. Scott KP, Gratz SW, Sheridan PO, Flint HJ. Duncan SH. 2012. The influence of diet on the gut microbiota. Pharmacol Res. 69:52–60.
31. Jenkinson HF and Lamont RJ. 2005. Oral microbial communities in sickness and in health. Trends Microbiol.; 13(12): 589–95.
32. O'Dwyer DN, Dickson RP, Moore BB. 2016. The lung microbiome, immunity, and the pathogenesis of chronic lung disease. *J Immunol*; **196**: 4839–4847.
33. Budden KF, Gellatly SL, Wood DL, Cooper MA, Morrison M, Hugenholtz P *et al*. 2017. Emerging pathogenic links between microbiota and the gut-lung axis. *Nat Rev Microbiol*; **15**: 55–63.
34. Hamady M, Fraser-Liggett CM, Turnbaugh PJ, Ley RE, Knight R, Gordon JI: The Human Microbiome Project. Nature. 2007, 449: 804-810.
35. Golub TR: Molecular classification of cancer: class discovery and class prediction by gene expression monitoring. Science. 1999, 286: 531-537.
36. Petricoin EF, Ardekani AM, Hitt BA, Levine PJ, Fusaro VA, Steinberg SM, Mills GB, Simone C, Fishman DA, Kohn EC, Liotta LA. 2002. Use of proteomic patterns in serum to identify ovarian cancer GLOSSARY. Lancet, 359: 572-577.
37. Wei X and Li K-C. 2010. Exploring the within- and between-class correlation distributions for tumor classification. Proc Natl Acad Sci USA., 107: 6737-6742.
38. Kashyap, Purna C., Chia, N., Nelson, H., Segal, E. And Elinav, E. 2017. Microbiome at the Frontier of Personalized Medicine. Mayo Clinic Proceedings , Volume 92 , Issue 12 , 1855 – 1864
39. Gross L. 2007. Human Gut Hosts a Dynamically Evolving Microbial Ecosystem, PLoS Biol; 5(7): e199
40. Avila M., Ojcius D.M. and Yilmaz O. 2009. The oral microbiota: living with a permanent guest, DNA Cell Biol.; 28: 405–411.
41. Curtis M.A., Zenobia C. andDarveau R.P. 2011. The relationship of the oral microbiotia to periodontal health and disease, Cell Host Microbe.; 10: 302–306.
42. Sachdeo, A., Haffajee, A. D., and Socransky, S. S. 2008. Biofilms in the edentulous oral cavity. *J. Prosthodont.* 17, 348–356.
43. Bradshaw, D., and Marsh, P. (1998). Analysis of pH–driven disruption of oral microbial communities *in vitro*. *Caries Res.* 32, 456–462.
44. Wu, J., Peters, B. A., Dominianni, C., Zhang, Y., Pei, Z., Yang, L., et al. 2016. Cigarette smoking and the oral microbiome in a large study of American adults. *ISME J.* 10, 2435–2446.
45. Cani PD. Delzenne NM. 2009. Interplay between obesity and associated metabolic disorders: new insights into the gut microbiota. Curr Opin Pharmacol.; 9: 737–743.
46. Everard A. Cani PD. Diabetes, obesity and gut microbiota. Best Pract Res Clin Gastroenterol. 2013;27:73–83.

47. Duran-Pinedo AE, Frias-Lopez J. 2015. Beyond microbial community composition: functional activities of the oral microbiome in health and disease. Microbes Infect.; 17(7): 505–16.
48. Evrensel, A. and Ceylan, M. E. 2015. The Gut-Brain Axis: The missing Link in Depression. Clinical Psychopharmacology and Neuroscience;13(3):239-244
49. Bienenstock J, Kunze W and Forsythe P. 2015. Microbiota and the gut-brain axis. Nutr Rev.Aug;73 Suppl 1:28-31.
50. Pflughoeft, K. J. & Versalovic, J. 2012. Human microbiome in health and disease. Annual Review of Pathology: Mechanisms of Disease7, 99-122.
51. Preidis, G. A. & Versalovic, J. 2009. Targeting the human microbiome with antibiotics, probiotics, and prebiotics: gastroenterology enters the metagenomics era. Gastroenterology136, 2015-2031.
52. Al-Asmakh, M., Anuar, F., Zadjali, F., Rafter, J. & Pettersson, S. 2012. Gut microbial communities modulating brain development and function. Gut microbes3, 366-373.
53. Vogelmann R and Amieva MR. 2007. The role of bacterial pathogens in cancer. Curr Opin Microbiol.; 10: 76-81
54. Musso G, Gambino R. Cassader M. 2010. Obesity, diabetes, and gut microbiota: the hygiene hypothesis expanded? Diabetes Care.;33: 2277–2284.
55. Danser MM, Gómez SM, Van der Weijden GA. 2003. Tongue coating and tongue brushing: a literature review. Int J Dent Hyg.; 1: 151-8
56. Lim, Yenkai & Totsika, Makrina & Morrison, Mark & Punyadeera, Chamindie. 2017. Oral Microbiome: A New Biomarker Reservoir for Oral and Oropharyngeal Cancers. Theranostics. 7. 4313-4321.
57. Abe K, Takahashi A, Fujita M, Imaizumi H, Hayashi M, *et al.* 2018. Dysbiosis of oral microbiota and its association with salivary immunological biomarkers in autoimmune liver disease. PLOS ONE 13(7): e0198757.
58. 2020 visions. Nature. 2010, 463: 26-32.
59. Walsh, C. J., Guinane, C. M., O'Toole, P. W. & Cotter, P. D. 2014. Beneficial modulation of the gut microbiota. FEBS Letters 588, 4120-4130.
60. Zhang L, Henson BS, Camargo PM, Wong DT. 2009. The clinical value of salivary biomarkers for periodontal disease. Periodontology 2000., 51: 25-37.
61. Zhang L, Farrell JJ, Zhou H, Elashoff D, Akin D, Park NH, Chia D, Wong DT. 2010. Salivary transcriptomic biomarkers for detection of resectable pancreatic cancer. Gastroenterology, 138: 949-957.
62. Lim, Y. *et al*. 2016. Salivary DNA methylation panel to diagnose HPV-positive and HPV-negative head and neck cancers. *BMC Cancer* **16**, 1–12.
63. Farah, R. et al 2018. Salivary biomarkers for the diagnosis and monitoring of neurological diseases. Biomedical Journal. Volume 41, Issue 2, pp. 63-87.
64. Wong, D. T. 2015. Salivary extracellular noncoding RNA: emerging biomarkers for molecular diagnostics. *Clin Ther* **37**, 540–551.
65. Larmas M. 1975. A new dipslide technique for counting of salivary lactobacilli. Proc. Finn. Dent. Soc. 71:31–35.
66. Jordan HV, Laraway R, Snirch R, Marmel M. 1987. A simplified diagnostic system for cultural detection and enumeration of Streptococcus mutans. J. Dent. Res. 66:57–61.
67. Kumar PS, Griffen AL, Moeschberger ML, Leys EJ. 2005. Identification of candidate periodontal pathogens and beneficial species by quantitative 16S clonal analysis. J. Clin. Microbiol. 43: 3944 –3955.

68. Sakamoto M, Umeda M, Ishikawa I, Benno Y. 2000. Comparison of the oral bacterial flora in saliva from a healthy subject and two periodontitis patients by sequence analysis of 16S rDNA libraries. Microbiol. Immunol. 44: 643–652.
69. Paster BJ, Dewhirst FE. 2009. Molecular microbial diagnosis. Periodontol. 2000 51: 38 –44.
70. Paju S, Pussinen P, Suominen-Taipale L, Hyvönen M, Knuuttila M, Könönen E. 2009. Detection of multiple pathogenic species in saliva is associated with periodontal infection in adults. J. Clin. Microbiol. 47: 235–238.
71. Mager D, Haffajee A, Devlin P, Norris C, Posner M, Goodson J. 2005. The salivary microbiota as a diagnostic indicator of oral cancer: a descriptive, non-randomized study of cancer-free and oral squamous cell carcinoma subjects. J. Transl. Med. 3:27.
72. Goodson JM, Groppo D, Halem S, Carpino E. 2009. Is obesity an oral bacterial disease? J. Dent. Res. 88:519 –523.
73. Docktor MJ, Paster BJ, Abramowicz S, Ingram J, Wang YE, Correll M, Jiang H, Cotton SL, Kokaras AS, Bousvaros A. 2012. Alterations in diversity of the oral microbiome in pediatric inflammatory bowel disease. Inflamm. Bowel Dis. 18:935–942.
74. Farrell JJ ZL, Zhou H, Chia D, Elashoff D, Akin D, Paster BJ, Joshipura K, Wong DT. 2012. Variations of oral microbiota are associated with pancreatic diseases including pancreatic cancer. Gut 61:582– 588.
75. Dietart RR *et al.* 2015. Biomarkers for the 21st century: listening to the microbiome; Toxicol Sci, 144(2): 208-216
76. Turnbaugh PJ, Bäckhed F, Fulton L, Gordon JI. 2008. Diet-induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome. Cell Host Microbe, 3: 213-223.
77. Manichanh C, Rigottier-Gois L, Bonnaud E, Gloux K, Pelletier E, Frangeul L, Nalin R, Jarrin C, Chardon P, Marteau P, Roca J, Dore J. 2006. Reduced diversity of faecal microbiota in Crohn's disease revealed by a metagenomic approach. Gut, 55: 205-211.
78. Sokol H, Seksik P, Furet JP, Firmesse O, Nion-Larmurier I, Beaugerie L, Cosnes J, Corthier G, Marteau P, Doré J. 2009. Low counts of *Faecalibacterium prausnitzii* in colitis microbiota. Inflamm Bowel Dis.; 15: 1183-1189.
79. Ordovas JM and Mooser V: Metagenomics: the role of the microbiome in cardiovascular diseases. Curr Opin Lipidol. 2006, 17: 157-161.
80. Swan Ka, Curtis DE, McKusick KB, Voinov AV, Mapa Fa, Cancilla MR. 2002. High-throughput gene mapping in *Caenorhabditis elegans*. Genome Res., 12: 1100-1105.
81. Wooley JC and Ye Y. 2009. Metagenomics: facts and artifacts, and computational challenges. J Comput Sci Technol., 25: 71-81.
82. Segata, N. *et al* 2011. Metagenomic biomarker discovery and explanation. *Genome Biology* **12**: R60
83. Walsh P, Elsabbagh M, Bolton P, Singh I. 2011. In search of biomarkers for autism: scientific, social and ethical challenges. Nat Rev Neurosci; 12: 603–612.