The status of gut microbiota, metagenome and microbiome research in Malaysia

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Human gut microbiota is defined as the community of microorganisms that reside in the human gastrointestinal (GI) tract.[1] The GI tract presents one of the biggest interfaces within the human body (250 – 400 m^2) where host cells, microorganisms and antigens interact and regulate the functioning of the human host.[2] Previous estimates suggested that the number of gut microbiota outnumber human cells by 10:1,[3] however, this has been updated to approximately 1:1 in the recent calculation.[4] Notwithstanding the revision, the estimated number of gut microbiota in different parts of the GI tract is significant (10^3-10^{11}).[4] Bacteria community in the gut provides essential functions and services, ranging from immunity, digestion to enzymatic regulation.[5,6] Due to its importance, the human microbiome project was established by the United States National Institutes of Health (NIH) about a decade ago to improve the understanding of the human microbiota in health and diseases.[7] With coordinated efforts and improvement in sequencing techniques and bioinformatics tools, the linkages of gut microbial dysbiosis and various diseases have been established.[8,9] For instance, obesity was found to be linked with the imbalance in the taxa affiliated with *Firmicutes* (i.e. *Christensenella*) and *Bacteriodetes*.[6,10] Specifically, lower prevalence of *Firmicutes* is associated with low butyrate-production and higher risk for obesity, colorectal cancer, irritable bowel disease and Crohn's disease.[9,11,12] In addition, low microbial diversity was found to be a consistent pattern for medical conditions such as irritable bowel syndrome, psoriatic arthritis, and diabetes.[8] Further, the changes in the gut microbial composition may also affect brain functions and behaviour.[13]

Cataloguing of the gut microbial taxonomic signatures is commonly carried out based on next generation sequencing of 16S rRNA genes. As such, it is also known as 16S rRNA gene microbiota analysis.[1] As the target gene is amplified using universal primers before sequencing, the method is sensitive with low DNA concentration requirement. Nevertheless, it is noteworthy that there are no truly "universal" 16S rDNA primers.[14,15] The sequences will then be aligned, filtered, binned, and classified based on their taxonomic assignment before statistical comparison and interpretation.[16] On the other hand, metagenome and microbiome refers to the "collection of genes and genomes from the members of microbiota" and "entire habitat including all the genes and genomes of the residing microorganisms (i.e. virus, fungus, bacteria etc) and their environment" respectively.[1] The former can be assessed using whole genome sequencing technology while the latter provides a systems biology view of the gut through the integration of omics such as metagenomics, metabonomics and metaproteomics.

Malaysia as an ideal laboratory for gut microbial research

High inter-individual variation is a common feature of gut microbiome.[17,18] Indeed, little overlap in microbiome was observed even in twins[19] and the majority of the heritable taxa originated from a single phylum, *Firmicutes*.[20] This is further complicated by confounding factors such as age, diet, genetics and health status. For instance, the progression from infant to elderly is associated with the increase in *Bacteroides* and *Eubacterium* but reduction in *Bifidobacterium* in the gut. Separately, differences in the abundance of gut bacterial taxa such as *Prevotella* and *Bacteriodes* were found when comparing the gut microbial composition between subjects from different geographical locations (i.e. Amazonas of Venezuela, rural Malawi and US metropolitan).[22] Such differences are likely to be attributed to the variation in the lifestyle and diet.[9,23,24]. While the contribution of genetics, geographical locations and diets to the development of gut microbiota is well recognised, the current view of "core microbiome" and its association to health status are skewed towards the western populations.[25-27]

Malaysia is a developing country with a population size of approximately 32 million. The demographics are made up of multiple ethnic groups including Malay, Chinese, Indian, and aborigines. This provides a diverse genetic pool that represents at least three of the most populous countries in the world (i.e. China, India and

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Indonesia). Further, the ethnics groups are distinct culturally with each practicing different lifestyles and diets. A microbiome study in Malaysia will therefore cover a wide range of confounders and determinants for the development of microbiota in the GI tract; at the same time, increase the coverage of the Asian cohort in the GenBank database.

Malaysia is also home to tropical diseases such as malaria, dengue, leishmaniasis, schistosomiasis, and soil transmitted helminths (STHs) which are prevalent among the lower income populations such as the aborigines.[28] Apart from selected STHs, the interplay between these diseases and gut microbiome modulation is largely unknown. With a relatively more modernised and comprehensive healthcare system (Malaysia ranked 49 based on 2010 WHO Health Care System Performance Rankings) than the majority of the tropical diseases endemic countries, Malaysia possesses the clinical capacity and infrastructure to research the role of gut microbiome in the prevention and prognosis of tropical diseases.

Current status of gut microbiome research in Malaysia and challenges

Gut microbiota and metagenome research in Malaysia are still in their infancy despite the promise. A non-exhaustive search using GenBank with Boolean Search String [(Malaysia) and ((Human Gut Microbiota) or (Human Gut Microbiome))] returned only 6 hits for Bioproject. Among them, 50% are related to colorectal cancer. Using the same search string at PubMed, 50 research results were returned and only approximately 18% (9 hits) are original studies on human gut microbiota/metagenome. Further, >90% of the papers were published within the past five years. The main subjects of the papers are colorectal cancer, helminthic infections and *Helicobacter pylori* infection.[29-31] The surprising lack of studies allude to a great opportunity for gut microbiota/microbiome related research in Malaysia.

It is noteworthy that one of the main hurdles for gut microbial research in Malaysia is the lack of public interest to provide stool samples. Stool is generally regarded as unhygienic and personal. The hassle of transferring stool samples into sterile containers, and the need to store stools in refrigerated conditions before sample submission further deter potential volunteers. In addition, despite the slight reduction in sequencing cost over the last few years, large scale commercial sequencing cost remains high at RM450 to RM3000 per sample depending on the sequencing platform (Illumina HiSeq, MiSeq or PacBio, personal experience). The cost is prohibitive for medium to large scale sequencing study (e.g. n >500) as the ceiling of "service" budget for most of the research grants in Malaysia is generally around RM60k – RM100k (estimated based on total funding quantum at RM150k – RM250k). Finally, bioinformatics researchers who specialise in gut microbiome related sequencing analyses are rare in Malaysia.

The way forward for gut microbiota, metagenomics and microbiome studies in Malaysia

With the limited resources available, there is a need for a more coordinated and concerted effort in gut microbial research. The establishment of working groups such as "The Malaysia Working Group on Gastrointestinal Health (MYGiH)"[32] is a good start to provide consultation for the standardisation of methodology and to coordinate multi-centre-based systematic sampling. These will facilitate better sampling coverage and the ease of inter-laboratories comparison.

Currently, understanding of the workings of gut microbiome is highly skewed to bacteria and relatively little is known about the taxonomy and functions of archaea, viruses and fungi in the gut. This is especially true for the Asian cohort. A comprehensive research into these biotas in the gut is therefore warranted.

In addition, the utilisation of other omics such as metaproteomics, metatranscriptomics and metabonomics is essential to provide a systems biology view of the host-gut microbiome interaction.[33] The integration of different biological aspects is expected to provide insight into the complex dynamics of the body systems and to facilitate the modelling of these complex relationships.[34]

Keywords: Malaysia, gut microbiota, gut microbiome

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