Gut Microbiomes and Viromes in Patients with Metabolic Syndrome

NCT number:

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Background

Metabolic syndrome (MS) is an umbrella term that describes conditions such as high blood pressure, elevated blood concentrations of triglycerides, cholesterol and sugar, and excess deposition of body fat. Individuals afflicted with one or more of these MS components are predisposed to obesity, diabetes and various heart-related diseases compared to persons without such conditions.

There is mounting evidence that the human gut microbiome plays a vital role in health and wellbeing. In particular, human population association and mouse model studies have demonstrated that gut microorganisms can be reproducibly linked to diseases such as obesity and diabetes. There is, however, considerably less effort directed towards applying these notable findings to healthcare of the general public, which is complicated by the fact that gut microbiomes are heavily influenced by biogeography, ethnicity, lifestyle and diet.

Objective

To assess variation in the gut microbiomes of healthy and MS individuals specific to Hong Kong, and to explore gut microorganisms as predictive/diagnostic markers of MS and clinical outcomes associated with medical interventions.

Primary Objectives

1. Generate a reference healthy gut microbiome database representative of the general adult population in Hong Kong
2. Describe how the gut microbiomes of individuals with MS deviate from the healthy reference

Secondary Objectives

1. Assess viability of using gut microbiome as clinical markers for disease prediction and progression
2. Characterise changes in gut microbiome in response to medical treatment, supplementation with probiotics/prebiotics, changes to lifestyle and other interventions

Research Plan and Methodology

The proposed project will have two phases: *(1) Phase 1* - A cross-sectional gut microbiome survey in healthy and individuals suspected to have MS to assess variation between 'healthy' and 'diseased' gut microbial communities, followed by *(2) Phase 2* - A longitudinal observational study of MS and partial MS patients undergoing clinical monitoring or treatment according to the current practice of the Lek Yuen Health Centre to describe changes in the gut microbiome associated with drug intake, lifestyle modification, probiotics/prebiotics intake and other interventions.

(1) Phase 1: Cross-sectional survey

Study population: Potential study subjects will be invited from health talks, blogs, notices and advertisement through various public media channels, as well as patients referred to the Lek Yuen Health Centre for suspected or confirmed MS. They will be classified into three groups: (i) Metabolic Syndrome-Full, (ii) Metabolic Syndrome-Partial, and (iii) Controls (Non-Metabolic Syndrome) according to criteria modified from the International Diabetes Foundation:

1. Waist circumference ≥90 cm (M), ≥80 cm (F)
2. Blood pressure: ≥130/85 mm Hg
3. Triglycerides: ≥1.7 mmol/L
4. High-density lipoprotein cholesterol: <1.03 mmol/L (M), <1.29 mmol/L (F)
5. Fasting blood sugar: ≥5.6 mmol/L

(i) Metabolic Syndrome-Full: any three or more of the five criteria
(ii) Metabolic Syndrome-Partial: any one or two of the five criteria
(iii) Controls (Non-Metabolic Syndrome): none of the five criteria

Exclusion criteria

1. Age: <35 or >65 years
2. Major organ system impairment: such as heart failure, renal failure, and severe impairment of respiratory function.
3. On long-term regular immunosuppressive therapy.
4. Current or history of malignancy.
5. Current or history of major gastrointestinal diseases, including inflammatory bowel disease, major gastrointestinal surgery, malignancy.
6. Current medication for glucose or lipid control, such as metformin and statin.

Sample size:

Using published gut microbiome and clinical data collected from a Swedish cohort (Karlsson et al. Nature 498.7452 (2013): 99), we estimated that 731 subjects allowed for 80% power in detecting a 1% difference in gut microbiome community diversity at 95% confidence between individuals with no or one of the above MS conditions other than blood pressure. Since we are proposing an observational study and gut microbiomes are highly variable even within cohorts, we aim to collect more than the estimated number of samples, detailed below:

1. Metabolic Syndrome-Full: 500 (male:female = 1:1)
2. Metabolic Syndrome-Partial: 500 (male:female = 1:1)
3. Controls (Non-Metabolic Syndrome): 500 (male:female = 1:1)

The second phase of the study will involve participants from the full and partial metabolic syndrome cohorts, hence we expect to survey around 1000 subjects. Based on the above estimate of 731 subjects for 80% power, we expect that 1000 subjects will still be sufficient to detect differences in microbial community diversity between the cohorts in part 2 of this study.

Clinical data & specimen collection:

At enrolment:

- Patients will be asked to complete a questionnaire to record demographic information such as age, gender, diet, lifestyle, socioeconomic status, history of illnesses and medication use
- A series of measurements will be recorded from the study subjects, including waist circumference, blood pressure, height and weight.
- A stool sample will be collected for studying the gut microbiome.
- 10 ml of blood sample will be collected for sugar, triglycerides, cholesterol and lipid measurements.

- 10 ml of blood sample will be stored for future studies of metabolic profiles and host genetics.

(2) Phase 2: Longitudinal observational study

Study population:

Subjects from Phase 1 of the study recommended to undergo medical follow-up at the Lek Yuen Health Centre will be invited to participate in Phase 2 "Longitudinal observational study". These subjects will either be Metabolic Syndrome-Full or Metabolic Syndrome-Partial. This study will not involve any changes to the subjects' medical procedures- any drug prescriptions and/or intervention will be according to the examining doctor's discretion and we will only monitor changes in the gut microbiome composition associated with these clinical procedures.

At follow-up visits:

- Subjects belonging to the "Metabolic Syndrome-Full (N = 500)" and "Metabolic Syndrome-Partial (N = 500)" cohorts will be re-examined at regular intervals according to the current practice of the clinic which depends on the need for drug treatment. Patients started on new medication are usually followed-up at 4-6 weeks, those on regular medication at 3-6 months.
- For the purpose of this study, each subject will be followed up to 36 months.
- At each follow-up visit, detailed clinical history, medication and lifestyle changes will be recorded. The 5 parameters for metabolic syndrome will be reassessed at each follow-up visit.
- 10 ml of fasting blood will be collected for glucose, triglycerides, cholesterol, and lipid measurements.
- A stool sample will be collected for studying changes in the gut microbiome.
- A urine sample will be collected for measuring excreted concentrations of any medication consumed
- Depending on subjects' follow-up frequency, each subject will be providing a total of six to nine blood, urine and stool samples over the 36-month duration of this study.

Gut microbial community data generation and analyses:

The collected stool samples well be used for investigating the gut microbial community composition using small subunit 16S ribosomal RNA gene amplicon and bulk DNA shotgun sequencing. 16S amplicons will be PCR-amplified from extracted stool DNA and then sequenced to create microbial community profiles. In addition, metagenome sequencing will be performed to identify the genomic potential of the gut microbiome in a subset of samples chosen based on results

of the 16S microbial community profiles, demographic information and clinical measurements. These results will be correlated with answers provided in the questionnaire and clinical measurements to identify associations between the gut microbiome and health status of the study subjects.

Patient Confidentiality

All information collected in the study will be kept confidential. Patient data will be coded with specifically assigned sample numbers and kept free of personal identifiers. All digital copies will be stored on an encrypted hard drive.

Ethical Considerations

This is an observational study where patient management including all pharmaceutical and non-pharmaceutical interventions will be conducted according to the current practice of the Lek Yuen Health Centre. The stool collection procedures are non-invasive, and should not create pain or discomfort. Blood samples will be collected from participants, and could result in a small degree of pain or bruising. This will be minimised by assigning experienced staff to draw blood samples.

Ethics Compliance

All study procedures will be carried out in compliance with the Declaration of Helsinki.