Executive summary for ATF funded project (MSS 289 R)

A comparative study to investigate the gut microbiota of HIV patients with metabolic syndrome

Aim and Objectives

This project aims to characterize the gut microbiota in HIV positive patients with metabolic syndrome, and to correlate their microbiota profile with the clinical characteristics. These data can be clinically important for early detection and possible treatment of the disease via gut microbial modification.

Project design

HIV-infected (HIV+) and non-infected (HIV-) patients were recruited into this project from the Division of Infectious Diseases, Department of Medicine & Therapeutics at the Prince of Wales Hospital (PWH). Clinical data was collected from the subjects either through medical history taking or computer records. Stool samples were collected by the patients, and gut microbiota profiling was performed using 16S ribosomal RNA sequencing following DNA extraction. Bioinformatic analysis was performed to interpret the gut microbiota profile.

Target population

A total of 88 patients were recruited and their clinical data were retrieved. Patients were further classified into three groups: (1) HIV positive with metabolic syndrome (HIV+MetS+; Group A; n=30); (2) HIV positive without metabolic syndrome (HIV+MetS-; Group B; n=28); and (3) HIV negative controls with metabolic syndrome (HIV-MetS+; Group C; n=30). Metabolic syndrome was diagnosed in accordance with the criteria established by the National Cholesterol Education Program Adult Treatment Program III (NCEP-ATP III).

Main achievement

All three groups of patients showed similar clinical parameters, except those that are related to metabolic syndrome. Similarly, hypertension, diabetes and dyslipidemia were all commonly diagnosed on patients with metabolic syndrome with or without HIV. Regarding the parameters of HIV infection, no significant difference was observed except for the anti-retroviral treatment, where HIV patients with metabolic syndrome were more likely to be prescribed with Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs) than Protease Inhibitors (PIs).

Bioinformatic analysis was performed on the 16s rRNA sequence data. A trend towards a lower diversity, both richness (i.e. Sobs and Chao-1 indexes) and evenness (i.e. Shannon and Simpsons indexes), was observed among HIV infected patients (Groups A and B) when comparing to control group (Group C). Statistical significances in all alpha-diversity indices (i.e. Sobs, Chao-1, Shannon and Simpsons; p= 0.017, 0.0052, 0.003 and 0.0004 respectively) were observed when comparing the control group (Group C) to the HIV patients with metabolic syndrome (Group A). PERMANOVA test and PCA plot applied on beta-

diversity metric at the genus level showed a significant separation between control (Group C) and the groups of HIV patients (Groups A and B), suggesting significant differences in gut microbiota composition were found among the control and HIV patients, especially between control and HIV patients with metabolic syndrome; while only small differences in gut microbiota composition were observed between the two groups of HIV-infected patients with or without metabolic syndrome.

Wilcoxon test showed that several bacterial species were significantly elevated in Group A compared to Group C patients. But more importantly, certain bacteria which are not shown to be typical commensal gut bacteria, such as *Neisseria subflava*, were present in significantly higher numbers in metabolic syndrome patients, regardless of whether the comparison group were HIV-infected or HIV-free. Also, three other bacterial species: *Acinetobacter gullouiae*, *Bacteroides caccae*, and *Ruminococcus gnavus*, showed significant differences in Group C vs Group B group. With the fact that these bacteria are not typical commensal, these prompt the role of these bacteria as a potential microbial signature which could be used for the detection and risk stratification of metabolic syndrome.

Conclusions

This study is the first to investigate the role of gut microbiota in HIV positive patients with metabolic syndrome in Hong Kong. The study has provided data on microbial changes among these HIV positive patients, and identified bacterial signature, such as *Neisseria subflava, Acinetobacter gullouiae, Bacteroides caccae*, and *Ruminococcus gnavus* that may indicate the presence of metabolic syndrome. This gives opportunities for these signatures to be used as diagnostic or prognostic biomarkers for disease classification or risk stratification.

资助项目执行摘要 (MSS 289r)

研究 HIV 及代谢综合征患者的肠道微生物群

目的和目标

代谢综合征在 HIV 感染患者十分普遍。本项目旨在研究 HIV 及代谢综合征患者的肠道微生物群，并将其与临床特征相关联。这些数据对早期发现和通过肠道微生物修饰治疗该病具有重要的临床意义。

项目设计

本研究从威尔斯亲王医院内科及治疗学系传染病科招募 HIV 感染(HIV+)及非感染(HIV-)病人。患者收集粪便样本，提取 DNA 后采用 16S 核糖体 RNA 测序进行肠道菌群分析。采用生物信息学分析方法对肠道菌群进行分析。

目标人群

本研究收集 88 例患者的临床资料。患者进一步分为三组:(1) HIV 阳性合并代谢综合征(HIV+MetS+;A 组;n = 30);(2) HIV 阳性/无代谢综合征(HIV+MetS-;B 组;n = 28);(3) 代谢综合征 HIV 阴性对照(HIV- mets +;C 组;n = 30)。代谢综合征的诊断符合国家胆固醇教育计划成人治疗计划 III (ncepi - atp III)制定的标准。

主要成就

除与代谢综合征相关外，三组患者均表现出相似的临床参数。同样，高血压、糖尿病和血脂异常都是伴有 HIV 的代谢综合征患者的常见诊断。在 HIV 感染参数方面，除抗逆转录病毒治疗外，无显著差异，代谢综合征 HIV 患者更可能使用非核苷逆转录酶抑制剂(NNRTIs)而非蛋白酶抑制剂(PIs)。

本研究对 16s rRNA 序列数据进行生物信息学分析。在所有 alpha-多样性指数中，HIV 阳性患者展現较低的物種多样性。PERMANOVA 测试和 PCA 区域应用于 beta-多样性度量中，HIV 阳性患者(A 组及 B 組)與對照人仕(C 組)表现出明显的分离,显著他們在肠道微生物群的組成有明顯的差异。Wilcoxon 检验显示，A 组与 C 组相比，有几种细菌的种类明显升高，這些包括了 Neisseria subflava 菌。此外，C 组与 B 组的 Acinetobacter gullouiae、 Bacteroides caccae 及 Ruminococcus gnavus 等 3 种细菌也有显著性差异。由于这些细菌不是典型的共生菌，这些提示了这些细菌作为一种潜在的微生物特征的作用，可以用于检测和代谢综合征的风险分层。

结论

本研究是首次在香港研究肠道微生物群在 HIV 阳性代谢综合征患者中的作用。该研究提供了这些 HIV 阳性患者的微生物变化数据，并确定了可能表明存在代谢综合征的细菌特征。这为这些特征被用作疾病分类或风险分层的诊断或预后生物标志物提供了机会。