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Original Article

Intestinal gutsfeature and role of ApoE and glucose metabolism in cerebral infarction patients

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Abstract: Cerebral infarction has currently become No. 1 disabling disease and No. 2 fatal disease in China, seriously affecting patient's quality of life. Cerebral infarction is considered to be closely related to carbohydrate metabolism in the body while human intestines are the main organ for the digestion of food. This study was designed to observe changes in the composition of intestinal flora in phylum level and carbohydrate metabolism using high-throughput sequencing technology in patients with cerebral infarction, followed by analysis of the corresponding characteristics, in order to explore the potential new mechanism of pathogenesis involved in cerebral infarction. 10 fecal samples from patients with cerebral infarction and 10 samples from healthy volunteers as control were selected to determine the abundance of intestinal flora in phylum level using sequencing technology for characteristic analysis. When compared with healthy group, the flora composition in phylum level was low similarity ($P<0.05$), *Firmicutes* and *Proteobacteria* in CI patients were significantlyincreased while *Actinobacteria*, *Bacteriodetes*, *Cyanobacteria* and *Verrucomicrobia*significantly decreased ($P<0.05$) in patients with cerebral infarction ($P<0.05$). The level of ApoE and glucose in CI patients significantly was increased ($P<0.05$). There was an altered intestinal guts feature in cerebral infarction patients and it may be the potential relationship between metabolism of ApoE and glucose and cerebral infarction.

Keywords: Cerebral infarction, intestinal guts, flora feature analysis

Introduction

Cerebral infarction (CI), also known ascommon disease in the elderly, severely affecting patient's quality of life [1, 2]. In recent years, due to irrational life style or environmental factors in personal life, the age for initial onset of cerebrovascular diseases progressively becomes younger [3, 4]. Cerebrovascular diseases have currently become No. 1 disabling disease and No. 2 fatal disease in China. Despite great improvement in the treatment of cerebral infarction in recent years, the therapeutic efficacy is still unsatisfactory in the majority of patients; the mortality and disability rate are still high while the prognosis is poor [5, 6]. Therefore, exploring the pathogenesis of cerebral infarction and actively preventing cerebral infarction are considered to be the most cost-effective method for the control of cerebral infarction.

In the past few years, the complex intestinal microbial ecosystem has been extensively studied. In our opinions, the diet structure can affect intestinal flora; in contrast, changing the composition of intestinal flora can also affect human carbohydrate metabolism, while alteration of carbohydrate metabolism is considered to be one of the potential causes of cerebral infarction. Accordingly, we analyzed the intestinal flora with sequencing analysis in healthy subjects and patients with cerebral ischemia or cerebral infarction in order to examine the relationship between cerebral infarction or ischemia and intestinal flora. The purpose of our preliminary analysis was to determine whether intestinal flora can be used as a biomarker

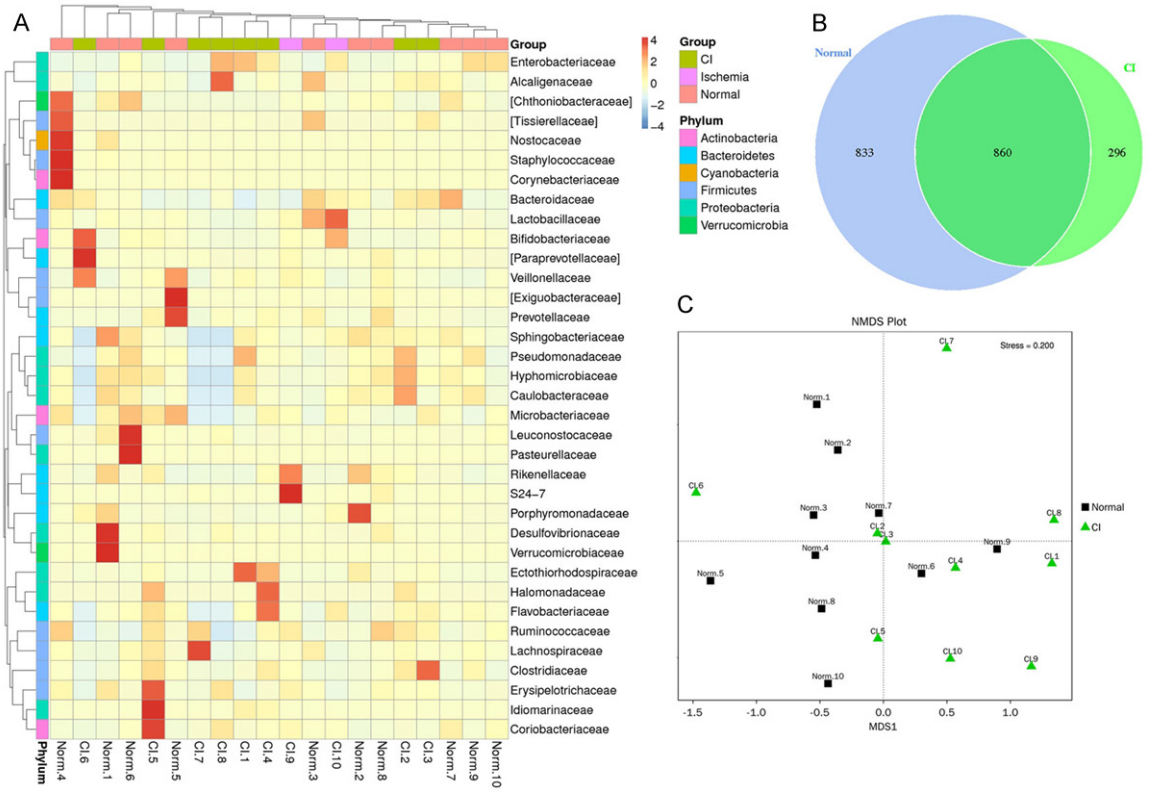

Figure 1. The intestinal gut similarity analysis in Normal group and CI group. The heatmap analysis (A) and the MDS analysis (B) showed that there was low similarity in community composition in phylum level. (C) The sequencing analysis results showed that there were 833 and 296 key OTUs in Normal group and CI group.

for the diagnosis of cerebral infarction and ischemia.

Materials and methods

General data

This study selected patients admitted to this hospital in May-October of 2015, including 10 patients diagnosed of cerebral infarction with head CT scanning (CI group); 6 patients were males and 4 patients were females. Ten healthy volunteers, all of them age was 53-82 years with a mean age of 64 years were selected for the normal group (Norm group). None of the study subjects received antibiotics within 1 week before sample collection. This study was approved by Medical Ethics Committee of Tianjin Huanhu Hospital. Prior to initiation of this study, the subjects in healthy control group not only received physical examination to confirm the lack of metabolic diseases, cardiovascular diseases, malignant tumors and other diseases but also signed the inform consent forms.

Intestinal gut DNA sequencing

Feces were split at 95°C for 10 min and 4 μl of RNaseA was added, incubated at 70°C for 30 min. The bacteria genomic DNA was amplified with primers specific for the V4 hypervariable regions of the 16S rDNA gene and sequenced by Illumina MiSeq platform.

Serum glucose and ApoE detection

5 ml venous blood with empty stomach was collect and Centrifuge with 300 g for 10 minutes. The level of ApoE was measured by ELISA assay and level of glucose was measured by Beckman AU5800 analyser.

Statistical analysis

The cluster heatmap and non-metric dimensional scaling (NMDS) ordination of Bray-Curtis dissimilarity were analyzed using the pheatmap package in R software (Version 2.15.3) using euclidean distance by hclust method. Differential abundance of phylum was analyzed using

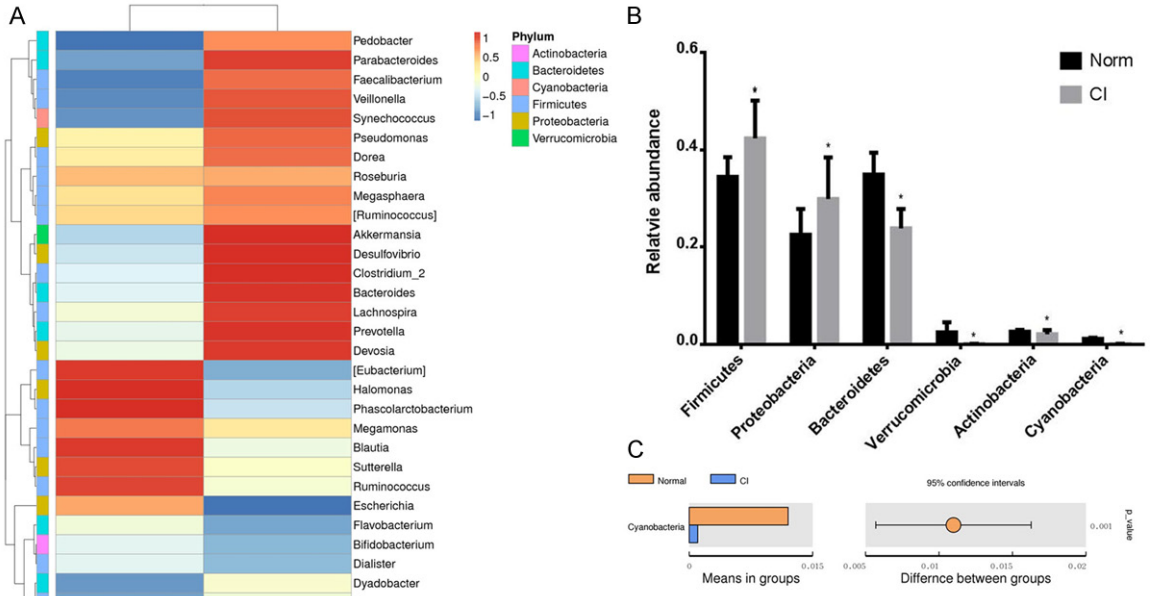

Figure 2. The abundance of intestinal gut analysis in Normal group and CI group. A: The abundance similarity analysis in Normal group and CI group. B: The relative abundance analysis in CI group compared with Normal group. C: The phylum abundance of *Cyanobacteria* was most charged. *, compared with Norm group, P<0.05.

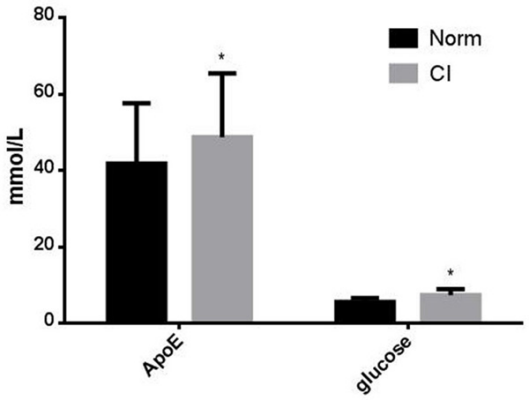

Figure 3. The ApoE and glucose level analysis in Normal group and CI group. *, compared with Norm group, P<0.05.

t-test in R software (Version 2.15.3) and visualized by in-house SVG scripts. Another data analysis was performed by SPSS 11.0 software and *t* test was applied in the intergroup analysis. The significant difference was as P<0.05.

Results

Intestinal gut similarity analysis

The heatmap analysis and the multidimensional scaling (MDS) analysis showed that there was low similarity in community composition in phylum level (**Figure 1A**, **1B**) and there were 833 and 296 key operational taxonomic units (OTUs) in Normal group and CI group (**Figure 1C**).

Heatmap and abundance of intestinal gut analysis

The abundance was low similarity in Normal group and CI group in heatmap analysis (**Figure 2A**) and the abundance analysis showed that the phylum abundance of *Firmicutes* and *Proteobacteria* was increased, and the phylum abundance of *Actinobacteria*, *Bacteriodetes*, *Cyanobacteria* and *Verrucomicrobia* was decreased in CI group compared with Normal group (P<0.05) (**Figure 2B**). Especially, the phylum abundance of *Cyanobacteria* was most charged (**Figure 2C**).

The ApoE and glucose level

The ELISA and biochemistry assay showed that there was an increased level of ApoE and glucose level in CI group compared with Normal group (P<0.05) (**Figure 3**), suggesting that there may be a potential relationship between intestinal guts and the metabolism of ApoE and glucose in cerebral infarction patient.

Discussion

Cerebral infarction is a localized ischemic necrosis of brain tissues or encephalomalacia due to cerebral blood supply disorder, ischemia and hypoxia [7]. Cerebral infarction is characterized by high incidence, high disability and high mortality, seriously threatening human life [8]. Consequently, research has been focusing on exploration of its mechanism and prediction and prevention of cerebral infarction. Ischemia is a transient short of blood supply and considered to reflect the initial symptom of cerebral infarction.

By examining the composition of intestinal flora in patients with cerebral infarction, this study found a significant increase in *Firmicutes* and *Proteobacteria*, and a significant decrease in *Actinobacteria*, *Bacteriodetes*, *Cyanobacteria* and *Verrucomicrobia* in cerebral infarction group. The composition of intestinal flora had low similarity between patients with cerebral infarction and healthy volunteers, which may be related to the course of cerebral infarction.

All of ApoE and glucose have been demonstrated was related with cerebral infarction. This study also shown that higher level of glucose and ApoE in cerebral infarction group, meanwhile, all of above guts had demonstrated was related with glucose and lipids metabolism [9-14], suggesting that there may be a potential relationship between intestinal guts and the metabolism of ApoE and glucose in cerebral infarction patient, which may play a very important role in cardiovascular and cerebrovascular diseases.

The findings provided a new basis for the diagnosis, stratification and pathogenesis in patients with cerebral infarction. Intestinal flora can promote the occurrence and development of diseases by regulating carbohydrate metabolism and other means. The development of a new generation of high-throughput sequencing technology characterized by its digital signal, high data throughput, high sequencing depth and high accuracy is able to quickly and comprehensively collect and analyze information such as composition, function, metabolism and related aspects to improve our understanding of intestinal flora. Identifying intestinal flora unique in patients with cerebral infarction can provide a new basis for the diagnosis and possible indication for the prevention with great economic and social significance.

Conclusions

There was an altered intestinal gut feature in cerebral infarction patients and it may be the potential relationship between metabolism of ApoE and glucose and cerebral infarction.

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Disclosure of conflict of interest

None.

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