JTCM
中医杂志
Journal of Traditional Chinese Medicine

Online Submissions: http://www.journaltcm.com
info@journaltcm.com

J Tradit Chin Med 2015 December 15; 35(6): 646-652
ISSN 0255-2922

CLINICAL STUDY

Differential expression of immune-related genes between healthy volunteers and type 2 diabetic patients with spleen-deficiency pattern

Chen Longhui, Yang Zemin, Chen Weiwen, Li Ruliu, Lin Chuanquan, Guan Lihua, Zhu Zhangzhi, Chen Ruifang, Li Saimei, Zhao Lingbo, Zeng Jinhao, Wang Jianhua

Chen Longhui, Chen Weiwen, Li Ruliu, Lin Chuanquan, Wang Jianhua, Pi-wei Institute, Guangzhou University of Chinese Medicine, Guangzhou 510405, China
Yang Zemin, School of Basic Courses, Guangdong Pharmaceutical University, Guangzhou 510006, China
Guan Lihua, Zhu Zhangzhi, Chen Ruifang, Li Saimei, Zeng Jinhao, the First Affiliated Hospital of Guangzhou University of Chinese Medicine, Guangzhou 510405, China
Zhao Lingbo, Clinical Medical College of Acupuncture, Moxibustion and Rehabilitation, Guangzhou University of Chinese Medicine, Guangzhou 510405, China
Supported by the Administration of Traditional Chinese Medicine of Guangdong Province of China (Study on the Relevance Between the Pi-Deficiency Syndrome and Gene Differential Expression Profile of Immunity and Metabolism in Type 2 Diabetic Mellitus, No. 20123001), Special Funds from the Central Finance of China in Support of the Development of Local Colleges and Universities [Collaborative Innovation Platform for the Prevention and Treatment of Significant and Refractory Pi-Wei Diseases, Educational Finance Grant No. 338 (2013)], the National Natural Science Foundation of China (the Mechanism Study of Salivary Alpha Amylase Activity Change in Pi-Deficiency Syndrome Patients Based on the AMY1 Copy Number Variation, N-Glycosylated Protein Level and β-Adrenergic Receptor Activation, No. 81102703), and the Science and Technology Planning Project of Guangdong Province of China (miRNA as Material Basis for the New Hypothesis; "Pi-Metabolism Relevance," and Study on the Molecular Mechanisms of Treating Metabolic Disorders Through Pi, No. 2013A032500005).
Correspondence to: Prof. Chen Weiwen, Pi-wei Institute, Guangzhou University of Chinese Medicine, Guangzhou 510405, China. chenww@gzucm.edu.cn
Telephone: +86-20-36585079
Accepted: February 12, 2015

Abstract

OBJECTIVE: To investigate the clinical differentiation of spleen-deficiency pattern (SDP), a group of symptoms and signs defined in terms of Traditional Chinese Medicine for its clinical practice.

METHODS: Peripheral venous blood (> 3 mL) was collected from each of six type 2 diabetes mellitus (T2DM)-SDP patients and six healthy volunteers. After the isolation of peripheral white blood cells (PWBCs), total RNA was extracted, and quality control was performed on all RNA samples. Microarray experiments were conducted using the Agilent human whole genome gene chip, and genes demonstrating differential expression were screened. Bioinformatics analysis was conducted on these genes using several online databases.

RESULTS: We screened a total of 175 differentially expressed genes (DEGs), of which 111 (63%) were down-regulated and 64 (37%) were up-regulated in T2DM-SDP patients compared with healthy controls. Among the 175 genes, 158 had biological function annotations: 46 (29%) were directly related to an individual's immune regulation or response, 25 (16%) were associated with substance and energy metabolism of PWBCs which could also indirectly influence immunity, and the remaining 87 (55%) were involved in a variety of PWBC biological processes that might eventually influence the immune function. Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis revealed that the DEGs were predominantly enriched in seven immune-related pathways. Hierarchical cluster analysis identified gene expression patterns that were distinguishable between the two study groups.

CONCLUSION: Our results suggest that T2DM-SDP patients experience significant hypoimmunity and/or immune dysfunctions, and possess a specific gene expression profile. These findings offer new insights into SDP and the clinical pattern differentiation of T2DM-SDP.

Key words: Diabetes mellitus, Type 2; Immunity; Metabolism; Leukocytes; Oligonucleotide array sequence analysis; Spleen-deficiency pattern

INTRODUCTION

Traditional Chinese Medicine (TCM) characterizes the spleen as an organ of comprehensive function associated with a number of biological systems including the immune system. Spleen-deficiency pattern (SDP) is a group of symptoms and signs defined by TCM clinical practice in terms of its theory. SDP is one of the most commonly encountered patterns in TCM practice and often involves immune dysfunction as well as disorders of the digestive system.

The occurrence and development of SDP is believed to have its own genomic basis.[1] Indeed, we previously showed that the differential gene profile of SDP patients (those with chronic gastritis and ulcerative colitis) was mainly associated with pathways of substance and energy metabolism and immune regulation, and that gene expression tended to be down-regulated compared with healthy controls.[2-6] These findings were also determined in a previous gene array study on peripheral white blood cells (PWBCs) from chronic gastritis and ulcerative colitis patients with SDP.[7] However, gene array studies on SDP have certain limitations, including a restriction of disease entities and tissue sources.

SDP is recognized by TCM as one of the primary causes for the occurrence and development of type 2 diabetes mellitus (T2DM).[8,9] Thus, the present study aimed to investigate the differential gene profile of PWBCs in T2DM-SDP patients to provide new insights into SDP clinical pattern differentiation.

MATERIALS AND METHODS

Recruitment of participants

Patients were included in the study if they met the following criteria: (a) were clinically diagnosed with T2DM (see below), (b) met the TCM pattern identified as SDP (see below), (c) were aged between 35 and 70 years, and (d) had signed the informed consent form. Exclusion criteria were as follows: (a) diagnosed with other endocrine diseases in addition to T2DM, or suffering severe complications caused by T2DM, (b) having infectious or inflammatory diseases, (c) having psychiatric or serious somatic diseases, (d) having dyslipidemia, and (e) pregnant or breastfeeding.

The T2DM diagnostic standard according to the American Diabetes Association is as follows:[10] (a) a fasting plasma glucose level ≥ 126 mg/dL (7.0 mmol/L), or (b) a 2-h plasma glucose level ≥ 200 mg/dL (11.1 mmol/L) during a 75 g oral glucose tolerance test, or (c) a random plasma glucose level ≥ 200 mg/dL (11.1 mmol/L) combined with classic symptoms of hyperglycemia or hyperglycemic crisis. The diagnostic standard of SDP has been established in the Guiding Principle of Clinical Research on New Drugs of Chinese Medicine.[11] The dominant symptoms are: (a) a pale tongue with a thin-white coating, (b) poor appetite, (c) abdominal distension, and (d) loose stools or diarrhea. The secondary symptoms are: (a) thinness, (b) weakness, and (c) a weak pulse. Possession of a pale tongue with a thin-white coating is essential for a diagnosis of SDP, and this should be combined with two of the other three dominant symptoms, or combined with one of the others and at least two of the secondary symptoms. Pattern differentiation was performed by two of the authors (Chen Longhui and Zhu Zhangzhi), and disagreement was resolved by discussion with Chen Weiwen.

Healthy volunteers aged between 35 and 70 years were interviewed by one of the authors (Chen Ruifang). The volunteers were free of endocrine diseases including T2DM, infectious and inflammatory diseases, psychiatric and serious somatic diseases, and dyslipidemia. They also had no apparent TCM patterns, and a normal tongue and pulse. Those who did not sign the informed consent form were excluded from the study.

We finally included 12 participants, of whom six were categorized as T2DM-SDP patients and six as healthy volunteers, from the First Affiliated Hospital of Guangzhou University of Chinese Medicine from October 2013 to December 2013. In the T2DM-SDP patients (three women and three men), the average age was 58.7 years (range, 48-67 years). In the healthy volunteers (three women and three men), the average age was 59.5 years (range, 50-65 years). No significant differences were observed between the two study groups regarding sex and age.

RNA isolation and quality control

Venous blood (> 3 mL) was collected from each fasting participant in the morning. EDTA (Beyotime Institute of Biotechnology, Shanghai, China) was introduced for anticoagulation. After separation, PWBCs were added to an appropriate volume of TRIzol (Invitrogen, Carlsbad, CA, USA) to achieve cell lysis. Total RNA was then isolated using the mirVana™ RNA Isolation Kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. After quantification using a NanoDrop ND-2000 spectrophotometer (Thermo Scientific, Waltham, MA, USA), we used the Agilent Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA) to detect the RNA Integrity Number (RIN) for RNA quality control.[12]