

Original Article

Research in etiology of MONW based on epigenetics and constitution Identification of traditional Chinese and medicine theory: design and protocol

Jinjin Wang¹, Jianfeng Zhang², Xueqin Xu¹, Lanying Pei¹, Chunyang Sun¹, Xiaohui Liu¹, Ying Liu¹, Guoli Yan¹

¹Discipline of Public Health and Preventive Medicine, Center of Preventive Medicine Research and Assessment, Henan University of Traditional Chinese Medicine, Zhengzhou 450008, People's Republic of China; ²Henan Armed Police Corps Hospital, Zhengzhou 450000, People's Republic of China

Received September 4, 2016; Accepted September 29, 2016; Epub January 1, 2017; Published January 15, 2017

Abstract: Background: We aimed in this study to evaluate the constitution status of Traditional Chinese Medicine (TCM) in metabolic obesity normal weight (MONW) and the genetic effectors of this phenotype from single nucleotide polymorphisms (SNP) and DNA methylation modification. Methods: One to one matched case-control study was conducted in our research. We conducted the standardized Constitution in Chinese Medicine Questionnaires (CCMQ) questionnaire, testing anthropometric measurements, genotyping the six gene polymorphism loci (including *FTO* rs1421085, rs9939609, rs1121980, *PPP1R3B* rs2126259, rs9987269 and *GCKR* rs780094) by the TaqMan probe, and using pyrosequencing to detect DNA methylation level of *FTO*, *PPP1R3B* and *GCKR* gene. Discussion: The results of this research will provide convinced evidence for etiology of MONW from the constitution status of TCM, genetic effects, and interactions among them and behavior risk factors laying the foundations of individualized prevention and the Precision Medicine.

Keywords: Constitution status of TCM, MONW, Chinese, genetic effect

Introduction

In recent years, as aging and westernized life-style is gradually increasing in Chinese population, metabolic abnormalities have been more and more frequent. The independent abnormal metabolic index is rare, so for a variety of different combinations of mutually abnormal metabolic markers by people have been gradually more widespread concerned. The metabolic obesity normal weight (MONW) is the one. MONW is a specific phenotype combining obesity with metabolic which first proposed in 1981, which BIM within the normal range, whereas there are several metabolic risk factors clustering [1]. The central link of this subtype is insulin resistance, which prone to type 2 diabetes, hypertension, hypertriglyceridemia [2, 3], and could be corrected through controlling of diet and exercise [2, 4]. The meta-analysis showed that the overall prevalence of MONW was 19.98%, and European population had the highest MONW prevalence [5]. It is

noteworthy that with the recent trend of rising prevalence of childhood obesity, MONW also have been spreading to children and adolescents [6, 7].

The danger of MONW is more serious than the opposite metabolic phenotype-metabolically healthy obese (MHO). A research conducted by Korea showed that the risk of cardiovascular disease and all-cause risk ratio of MONW group is higher than MHO group [8], and the impacts on atherosclerosis were stronger than MHO group [9]. Thus, metabolic factors may play more important role than the degree of obesity for chronic non-communicable disease, such as cancer, cardiovascular disease [10, 11]. Because of the characteristics of normal weight, MONW is often more likely to be overlooked. To carry out the research about the etiology has an important significance for early identification and intervention on MONW.

Chinese medicine believes that physical constitution of the human life process is congenital

and acquired based on the formation of the structure, physiological and psychological aspects of a comprehensive state of relatively stable natural qualities and the development process human personality in adaption for the natural and social environment. The constitution status differences resulted in disorder variability. The relationship between the disease and different physical phenotypes reveals the inner nature of the development of the disease. For the particular metabolic phenotype-MONW, the physical identification could reveal the etiology and pathogenesis from the inherent nature of the disease, and provide approaches to protect the health population.

MONW is the result of environmental factors and genetic factors working together. It is important to confirm susceptibility loci of MONW combined with environmental factors for reducing the incidence of MONW. Currently, the research on MONW mainly focused on its impact on a variety of chronic non-communicable disease, whereas, the manuscript about genetic background is rare. Based on the research reported in 2014 which did cluster analysis of fasting insulin-associated genetic variants [12], combined with Chinese database, we selected *FTO*, *GCKR*, and *PPP1R3B* genes to evaluate their relationships with MONW in Chinese. But only finding the cause from the DNA sequence was one-sided. In many cases, although there was no change in DNA sequence, it could change the phenotype of the organism occurs, which means the same genotype, different phenotypes as epigenetic phenomenon.

To sum up, we aimed in this study: (1) to identify the constitution status of Traditional Chinese Medicine in MONW providing the evidences on physical nursing and physical intervention from the perspective of physical science to; (2) to evaluate the genetic effectors of this phenotype from single nucleotide polymorphisms and DNA methylation modification; (3) to analyze the interactions among susceptibility genes, physical identification and behavioral risk factors providing the basis of individualized prevention and the Precision Medicine.

Methods and design

Design

One to one matched case-control study was conducted in our research. We conducted

questionnaire, testing anthropometric measurements, genotyping the six gene polymorphism loci (including *FTO* rs1421085, rs99-39609, rs1121980, *PPP1R3B* rs2126259, rs9987269 and *GCKR* rs780094) by the TaqMan probe, and using pyrosequencing to detect DNA methylation level of *FTO*, *PPP1R3B* and *GCKR* gene. Data on demographic and anthropometric characteristics were collected by interviewer-administered questionnaire. Anthropometric measurements included body weight, body height, body mass index (BMI), waist circumference (WC), and blood pressure. An electronic sphygmomanometer was used to measure blood pressure. We obtained informed consent from all participants, and the study was approved by the Ethics Committees of the First Affiliated Hospital of Henan University of TCM.

Estimating of sample size

For association of genetic susceptibility, sample size calculation involved use of power for genetic association analyses (PGA) package (<http://dceg.cancer.gov/bb/tools/pga>), which $\alpha = 0.05$ and power = 90%, the main outcome of these gene based on our meta-analysis of association between these SNPs and other metabolic disorders, the lowest MAF of six SNPs (0.078 from NCBI), and the prevalence of MONW in China 11.25%. Each group needs at least 300 objects.

For physical identification of Constitution, based on the Chinese general population database, the Inherited Special Constitution was the lowest rates of nine kings of physical identification exposure about 0.034 in Henan province, the Inherited Special Constitution exposure of MONW (5.87%) and control (3.83%), the sample size in this study can be estimated initially according to the one to one matched case-control study's formula:

$$m = [z_{\alpha}/2 + z_{\beta} \sqrt{p(1-p)}]^2 / (p - 1/2)^2$$

$$p = OR / (1 + OR)$$

Each group needs at least 696 objects.

Combination of these two aspects, considering the dropout rate, we decided ultimately to include 770 objects in each group.

Etiology of MONW

Table 1. SNPs in our study

Gene	SNP	Allele	Ancestral allele	MAF in CHB	MAF in CEU
FTO	rs9939609	A/T	A	0.183	0.460
	rs1421085	C/T	T	0.171	0.460
	rs1121980	C/T	T	0.793	0.518
PPP1R3B	rs9987269	C/G	G	-	-
	rs2126259	A/G	G	0.024	0.088
GCKR	rs10885122	G/T	T	0.078	0.100

Patients

The cases in this study were from Henan Province in China, and recruited from four hospitals in Henan province (including the first, second and third affiliated hospital of Henan University of TCM and Henan Province People's Armed Police Corps Hospital).

Inclusion criteria

The diagnostic criteria of MONW currently did not reached general consensus, for which mainly determined by obesity and metabolic syndrome diagnostic criteria. The common criteria include Wildman standard, Karelis standard, ATPIII and the HOMA index. Because our study conducted in Chinese Han population, combining with the Han population characteristic, we finally adapted the Wildman standard [13] for BMI between 18.5-23 kg/m² [14] and satisfying the following two:

- Systolic/diastolic blood pressure \geq 130/85 mmHg or use of any antihypertensive drugs;
- TG \geq mmol/L or use of any lipid-lowering drugs;
- Fasting plasma glucose \geq 5.6 mmol/L or use of any hypoglycemic agents;
- HOMA-IR $>$ 5.13;
- hs-CRP $>$ 0.1 mg/l;
- HDL-C \geq 1.0/1.3 mmol/l (male/female).

Exclusion criteria

Patients with any of the following conditions will be excluded: pregnant, handicapped, or mentally disturbed; had obesity caused by disease or were taking certain drugs; and had cancer or were unable or unwilling to participate.

Controls

The controls were from the same district matching cases according to the same sex, the age difference $<$ 5 years.

Constitution identification of traditional Chinese medicine

The scores of 9 different basic Constitutional types (including Balanced Constitution, Qi-deficient Constitution, Yang-deficient Constitution, Yin-deficient Constitution, Phlegm-dampness Constitution, Damp-heat Constitution, Stagnant Blood Constitution, Stagnant Qi Constitution, Inherited Special Constitution) on MONW were according to the standardized Constitution in Chinese Medicine Questionnaires (CCMQ) reported in 2009, which adopted by ministerial-level appraisal, approved by the Chinese Medical Association, recognized as the industry standard. In our study, according to the Chinese Medical Association Standards, the investigations were undergoing by the investigator owing the Chinese Medicine bachelor degree and with a uniform training.

SNP selection, genotyping and DNA methylation modification

Based on the article published in 2014 about the genetic evidence for MONW linking insulin resistance, hypertension, coronary artery disease and type 2 diabetes [12], we selected FTO, PPP1R3B and GCKR genes to evaluate. Genotyping plans to use of TaqMan Probe and use of pyrosequencing to detect DNA methylation level of genes. **Table 1** listed SNPs selected in our study.

Statistical analysis

The data will be analyzed by SPSS 21.0 statistical software package. The physical identifications of TCM on MONW were evaluated by logistic regression analyses and cluster analysis. The relationships between genes and MONW were calculated by logistic regression analyses. The associations between genes and quantitative traits FPG and lipid metabolism indicators such as level of triglycerides (TG), total cholesterol (TC), high-density lipoprotein-cholesterol (HDL-C), and low-density lipoprotein-cholesterol (LDL-C) were evaluated by linear regression. The interactions among genes and behavior risk factors were analyzed by multifactor dimensionality reduction (MDR) and multiplicative logistic regression after adjusting for sex, age, and BMI. All tests were two-sided and were considered statistically significant with at $P \leq 0.05$.

Discussions

The population could be divided into four subtypes when considering metabolic phenotypes with obesity: metabolically healthy with normal weight (MHNW), metabolically obese but normal weight (MONW), metabolically healthy obese (MHO), and metabolically abnormal obese (MAO). As compared with MHO, MONW subtype may have higher visceral fat, lipid metabolisms, and it could be ignored because of lower lean body mass. In contrast to MHO and MHNW, MONW owed high risk of chronic non-communicable diseases, such as type 2 diabetes and cardiovascular diseases. Despite many studies, the mechanisms of MONW remain uncertain.

According to the theory of Traditional Chinese Medicine (TCM), the constitution status of people can be divided into nine types, including Balanced Constitution, Qi-deficient Constitution, Yang-deficient Constitution, Yin-deficient Constitution, Phlegm-dampness Constitution, Damp-heat Constitution, Stagnant Blood Constitution, Stagnant Qi Constitution, and Inherited Special Constitution. The identification of constitution status of MONW is helpful to figure out which constitution is significant for it, and for these people who own this significant constitution, early effective lifestyle intervention will be taken to prevent the occurrence of MONW. The Constitution in Chinese Medicine Questionnaire has been translated into English, and for all global people, it is feasible to identify the constitution status.

Epigenetics is defined as: there are some genetic regulators besides the nuclear genome sequence including DNA methylation, non-coding RNA regulation, histone modification and so on. Any part of the exception will affect chromatin structure and gene expressing leading to more complex syndrome of the diseases. Different from the classical genetics, epigenetics mainly focus on the maintain mechanisms of these epigenetic code, and how to determine cell phenotype and ontogeny. In epigenetics, DNA methylation is an important epigenetic modifications form, which has an important role in maintaining the normal function of cell and embryo development. Therefore, DNA methylation levels of *FTO*, *PPP1R3B*, and *GCKR* genes were estimated in our study.

We did evidence-based research on the major databases (including EMBASE, Pubmed, Cochrane for English, and CNKI, VIP and Wanfang database for Chinese) to collect the information about the associations between *FTO*, *PPP1R3B*, *GCKR* genes and MONW through meta-analysis providing the evidences for the estimation of the sample size and the statistical power of the research.

Henan province is a populous in China. The sample size can be fully guaranteed based on the actively cooperation of the three affiliated hospitals of our institution and the People's Armed Police Corps Hospital of Henan Province. The members of the group have a reasonable structure incorporated into the Chinese medical diagnosis, the basic theory of Traditional Chinese Medicine, as well as a number of researchers specializing in prevention and laboratory.

In conclusion, the purpose of this research is to provide convinced evidence for etiology of MONW from the constitution status of TCM, genetic effects, and interactions among them and behavior risk factors laying the foundations of individualized prevention and the Precision Medicine.

Acknowledgements

This study was supported by the Doctoral Research Fund of Henan Chinese Medicine (NO. BSJJ2014-18). We sincerely thank for all of the authors for their work in this study. We obtained informed consent from all participants, and the study was approved by the Ethics Committees of the First Affiliated Hospital of Henan University of TCM.

Disclosure of conflict of interest

None.

Authors' contribution

J. Wang and G. Yan produced the design of the study. X. Liu, J. Zhang contributed to collected samples. J. Wang, J. Zhang, L. Pei, Y. Liu, and G. Yan conducted to search database. J. Wang, X. Liu, X. Xu, and C. Sun conducted to lab, data extraction and statistical analyses. All authors approved the final manuscript.

Abbreviations

SNP, single nucleotide polymorphism; TCM, Traditional Chinese Medicine; MHNW, metabolically healthy with normal weight; MONW, metabolically obese but normal weight; MHO, metabolically healthy obese; MAO, metabolically abnormal obese; BMI, body mass index; WC, waist circumference; MAF, Minor allele frequency; CHO, Han Chinese in Beijing, China; CEU, Utah residents with Northern and Western European ancestry from the CEPH collection.

Address correspondence to: Guoli Yan, Discipline of Public Health and Preventive Medicine, Center of Preventive Medicine Research and Assessment, Henan University of Traditional Chinese Medicine, No. 1 Jinshui Road, Zhengdong New District, Zhengzhou 450056, Henan, People's Republic of China. Tel: 0086-(0)371-67002510; Fax: 0086-(0)371-67002510; E-mail: 13937187109@163.com

References

- [1] Ruderman NB, Schneider SH, Berchtold P. The “metabolically-obese”, normal-weight individual. *Am J Clin Nutr* 1981; 34: 1617-1621.
- [2] Aung K, Lorenzo C, Hinojosa MA, Haffner SM. Risk of developing diabetes and cardiovascular disease in metabolically unhealthy normal-weight and metabolically healthy obese individuals. *J Clin Endocrinol Metab* 2014; 99: 462-468.
- [3] Kwon BJ, Kim DW, Her SH, Kim DB, Jang SW, Cho EJ, Ihm SH, Kim HY, Youn HJ, Seung KB, Kim JH, Rho TH. Metabolically obese status with normal weight is associated with both the prevalence and severity of angiographic coronary artery disease. *Metabolism* 2013; 62: 952-960.
- [4] Ruderman N, Chisholm D, Pi-Sunyer X, Schneider S. The metabolically obese, normal-weight individual revisited. *Diabetes* 1998; 47: 699-713.
- [5] Wang B, Zhuang R, Luo X, Yin L, Pang C, Feng T, You H, Zhai Y, Ren Y, Zhang L, Li L, Zhao J, Hu D. Prevalence of Metabolically Healthy Obese and Metabolically Obese but Normal Weight in Adults Worldwide: A Meta-Analysis. *Horm Metab Res* 2015; 47: 839-845.
- [6] Ding WQ, Yan YK, Zhang MX, Cheng H, Zhao XY, Hou DQ, Mi J. Hypertension outcomes in metabolically unhealthy normal-weight and metabolically healthy obese children and adolescents. *J Hum Hypertens* 2015; 29: 548-554.
- [7] Guerrero-Romero F, Rodriguez-Moran M. Metabolically obese normal-weight children. *World J Clin Pediatr* 2012; 1: 37-39.
- [8] Choi KM, Cho HJ, Choi HY, Yang SJ, Yoo HJ, Seo JA, Kim SG, Baik SH, Choi DS, Kim NH. Higher mortality in metabolically obese normal-weight people than in metabolically healthy obese subjects in elderly Koreans. *Clin Endocrinol (Oxf)* 2013; 79: 364-370.
- [9] Yoo HJ, Hwang SY, Hong HC, Choi HY, Seo JA, Kim SG, Kim NH, Choi DS, Baik SH, Choi KM. Association of metabolically abnormal but normal weight (MANW) and metabolically healthy but obese (MHO) individuals with arterial stiffness and carotid atherosclerosis. *Atherosclerosis* 2014; 234: 218-223.
- [10] Gao B, Zhang L, Zhao M; China National Survey of Chronic Kidney Disease Working Group Investigator. Underweight but metabolically abnormal phenotype: Metabolic features and its association with cardiovascular disease. *Eur J Intern Med* 2016; 29: 46-51.
- [11] Oh CM, Jun JK, Suh M. Risk of cancer mortality according to the metabolic health status and degree of obesity. *Asian Pac J Cancer Prev* 2014; 15: 10027-10031.
- [12] Yaghoobkar H, Scott RA, White CC, Zhang W, Speliotes E, Munroe PB, Ehret GB, Bis JC, Fox CS, Walker M, Borecki IB, Knowles JW, Yerges-Armstrong L, Ohlsson C, Perry JR, Chambers JC, Kooner JS, Franceschini N, Langenberg C, Hivert MF, Dastani Z, Richards JB, Semple RK, Frayling TM. Genetic evidence for a normal-weight “metabolically obese” phenotype linking insulin resistance, hypertension, coronary artery disease, and type 2 diabetes. *Diabetes* 2014; 63: 4369-4377.
- [13] Wildman RP, Muntner P, Reynolds K, McGinn AP, Rajpathak S, Wylie-Rosett J, Sowers MR. The obese without cardiometabolic risk factor clustering and the normal weight with cardiometabolic risk factor clustering: prevalence and correlates of 2 phenotypes among the US population (NHANES 1999-2004). *Arch Intern Med* 2008; 168: 1617-1624.
- [14] WHO Expert Consultation. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. *Lancet* 2004; 363: 157-163.