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Polymorphisms in lipid metabolism related miRNA binding sites and risk of metabolic syndrome

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ABSTRACT

MicroRNAs (miRNAs) regulate posttranscriptional gene expression usually by binding to 3′-untranslated regions (3′UTRs) of target message RNAs (mRNAs). Previous studies have demonstrated that SNPs within miRNA target sites could modulate miRNA–mRNA interaction to affect the regulation of target genes and the individual's diseases. So far, little is known about the relationship of miRNA binding site polymorphisms with the risk of metabolic syndrome (MetS) in the general population. Therefore, we conducted a case–control study in Chinese Han population to evaluate the association between SNPs within miRNA binding sites and risk of MetS. 8 SNPs in miRNA binding sites with a minor allele frequency (MAF) of ≥0.05 in the Chinese Han population were selected by bioinformatics software. TaqMan ®assay was performed to test the genotypes in MetS patients (n = 1026) and normal controls (n = 1032). We found rs5750146 (adjusted odds ratio (OR) = 1.24 for GA/AA, P = 0.023, compared with GG), rs5999924 (adjusted OR = 1.22 for AT/TT, P = 0.038, compared with AA) in the *APOL6* 3′UTR were identified to correlate with MetS in the total sample and females. Rs11724758 (adjusted OR = 0.65 for AA, P = 0.002, compared with GG) in the *FABP2* 3′UTR was found to correlate with MetS in the total sample and males. Correlations between *FABP2* rs11724758 polymorphisms and components of MetS reveal that high-density lipoprotein cholesterol (HDL-c) levels are significantly higher in *FABP2* rs11724758 AA genotype carrier compared with noncarriers, whereas triglycerides (TG) and fasting plasma glucose (FG) were to be significantly lower in the AA genotype carrier. These findings indicate that these three polymorphisms which located at the predicted miRNAs binding sites were identified to contribute to susceptibility to MetS in the Chinese Han population.

1. Introduction

Metabolic syndrome (MetS) is a common, multicomponent condition characterized by insulin resistance, dyslipidemia, abdominal obesity, and hypertension that is associated with an increased risk of type 2 diabetes mellitus (T2DM), cardiovascular diseases, and atherosclerosis (Moller and Kaufman, 2005). Although the clinical definition of MetS may vary between defining agencies, a major step toward a consensus and a global definition has been proposed (Eckel et al., 2010). The existence of MetS is indisputable and understanding its etiology is extremely important (de Luca and Olefsky, 2008). It is well known that the development of MetS is affected by genetic factors, environmental factors and lifestyle changes (Miyamoto et al., 2009). However, the etiology of MetS remains largely unclear and genetic inheritance has been demonstrated to be a major risk factor for MetS (Pollex and Hegele, 2006).

Previously studies have reported that small non-coding microRNAs (miRNAs) are important components of complex gene regulatory networks (Aravin et al., 2007; Reinhart and Bartel, 2002). Accumulating evidence has revealed that miRNAs are involved in a number of biological processes, including cell differentiation, proliferation and apoptosis that commonly occur in a variety of diseases, such as cancer, diabetes, schizophrenia and obesity (Coyle, 2009; Ortega et al., 2010; Satzger et al., 2010; Yang and Kaye, 2009). miRNAs are endogenous 19–22 nt RNAs which function as components of the miRNA-induced silencing

Abbreviations: MetS, Metabolic Syndrome; miRNA, microRNA; 3'UTRs, 3'untranslated regions; T2DM, Type 2 diabetes mellitus; mRISC, miRNA-induced silencing effector complex; WC, waist circumference; TG, Triglyceride; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL-C, High density lipoprotein cholesterin; LDL-C, Low density lipoprotein cholesterin; TC, Total cholesterol; FPG, Fasting plasma glucose; OR, Odds Ratio; CI, Confidence Interval; PCR, Polymerase Chain Reaction; SNP, Single Nucleotide Polymorphism; MAF, minor allele frequency; BMI, Body Mass Index; LDA, Linkage Disequilibrium Analysis; IDF, International Diabetes Federation; NHLBI, National Heart, Lung and Blood Institute; AHA, American Heart Association; NCBI, National Center of Biotechnology Information.

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effector complex (mRISC). The microRNA sequence directs the sequence-specific binding of the mRISC complex to one or more target mRNAs, repressing their translation to protein (Bartel, 2009). This binding can be affected by single nucleotide polymorphisms (SNPs) that can reside in miRNA target sites and eliminate an existing binding site, create an erroneous binding site or affect binding affinity. A number of studies have confirmed that SNPs in 3′ untranslated regions (3′UTRs) targeted by miRNAs alter the strength of miRNA binding and affect regulation of target genes and the individual's diseases (Brendle et al., 2008; Kontorovich et al., 2010; Lei et al., 2011; Wang et al., 2008; Zhao et al., 2011). To date, however, genetic studies have not attempted to identify SNPs in miRNA binding sites which correlate with MetS risk.

Previous studies have identified numerous miRNAs differentially expressed in MetS (Esau et al., 2004; He et al., 2007; Keller et al., 2011; Kloting et al., 2009; Ortega et al., 2010; Wang et al., 2002; Wilfred et al., 2007; Xie et al., 2009) (Table 1). To investigate the potential impact of these miRNAs-related SNPs in individuals with MetS, a panel of 8 SNPs in the miRNA binding sites was selected and an association study in MetS cases and controls was conducted.

2. Materials and methods

2.1. Subjects

We studied 1026 MetS patients and 1032 MetS-free controls with their informed consent. All subjects were genetically unrelated ethnic Han Chinese and were from Nanjing and surrounding regions in southeast China. The patients were consecutively recruited between 2008 and 2010 from the MetS inpatient or outpatient departments of three Affiliated Hospitals of Nanjing Medical University (NJMU, The Affiliated Changzhou 2nd Hospital of NJMU, the 3rd Affiliated Hospital of NJMU and the Affiliated Nanjing 1st Hospital of NJMU), without any restrictions on age and gender. MetS-free controls were randomly selected from the physical examination center within the same geographical area and the period of the cases. Controls received routine annual health examinations. Cases and controls were frequency-matched according to age (±5 years), gender, and residential area (urban or rural areas).

Written informed consent was obtained from each participants followed by an interview and a structured questionnaire to collect information on demographic data and environmental exposure history. Physical measurements were performed by trained doctors. Physical activity levels were defined as walking or riding ≥15 min/day and/or lifting or carrying heavy objects at work daily and/or doing sports or physical exercise >2 h/week. Current cigarette smokers were defined as subjects reporting ≥1 cigarette/day. Total alcohol intake was expressed as the sum of millimeters of alcohol per week from wine, beer, cider, and spirits.

2.2. Definition of MetS

Definition of MetS was based on clinical criteria from a previous joint interim statement of the International Diabetes Federation (IDF); National Heart, Lung and Blood Institute (NHLBI); American Heart Association (AHA); World Heart Federation; International Atherosclerosis Society and International Association for the Study of Obesity (Alberti et al., 2009). In summ ary, a MetS case must meet any three of the following five conditions: 1) waist circumference (WC) ≥90 cm in males, ≥80 cm in females; 2) triglycerides (TG) ≥150 mg/dL(1.7 mmol/L) in both genders (whereas drug treatment for elevated triglycerides is an alternate indicator); 3) high-density lipoprotein cholesterol (HDL-C) <40 mg/dL(1.0 mmol/L) in males, <50 mg/dL(1.3 mmol/L) in females(whereas drug treatment for reduced HDL-C is an alternate indicator); 4) systolic blood pressure (SBP) ≥130 mm Hg or diastolic blood pressure (DBP) ≥85 mm Hg in both genders (whereas antihypertensive drug treatment in a patient with a history of hypertension is an alternate indicator); 5) fasting glucose ≥100 mg/dL in both gender (whereas drug treatment of elevated glucose is an alternate indicator).

2.3. Measurements

Weight, height, and WC were measured by trained personnel, and body mass index (BMI) was calculated. Blood pressure was measured in the individual's right arm following a 10-min rest with a standard sphygmomanometer of appropriate cuff size. Following an overnight fast, venous blood samples were collected and promptly centrifuged, and the serum was stored at −20 °C. All samples were assessed using the same assay. Fasting plasma glucose (FG) was measured in the laboratories in the three affiliated hospitals of NJMU by the glucose oxidase method. Total cholesterol (TC), HDL, low-density lipoprotein cholesterol (LDL), and TG were determined in the three affiliated hospitals by an enzymatic colorimetric method (Au5400; Olympus, Tokyo, Japan). Genomic DNA was extracted from peripheral blood by the use of proteinase K and phenol/chloroform. This study was approved by the Research Ethics Committee of Nanjing Medical University.

2.4. miRNA target SNP selection

Eight miRNAs were previously identified to be differentially expressed in MetS. Potential miRNA binding sites in genomic sequences for the list of miRNAs were assessed by bioinformatics data bank using 4 bioinformatics software packages. Patrocles (http://www.patrocles.org/Patrocles_miRNAs.htm) (Hiard et al., 2010) and PolymiRTS Database (http://compbio.uthsc.edu/miRSNP/) (Bao et al., 2007) directly

Table 1
MiRNAs with altered expression in MetS and SNPs chosen for genotyping.

miRNA	Function	Reference	SNP ID	Target gene	MAF	Allele	Chromosome	Algorithm
miR-103	Adipocyte differentiation	Wilfred et al. (2007); Xie et al. (2009)	rs1042094	*PPP3CA*	0.140	A > G	4q24	PolymiRTS Database & Patrocles
			rs741191	*MED15*	0.640	G > A	22q11.2	PolymiRTS Database & miRanda
miR-143	(pre)Adipocyte differentiation	Esau et al. (2004)	rs5750146	*APOL6*	0.171	G > A	22q12.3	PolymiRTS Database & targetscan
miR-132	Adipocyte proliferation and growth, insulin resistance	Kloting et al. (2009)	rs11724758	*FABP2*	0.622	A > G	4q28-q31	PolymiRTS Database & miRanda & targetscan
miR-29a	Glucose transport, amino acid metabolism, insulin resistance	He et al. (2007); Wang et al. (2002)	rs6710015	*XDH*	0.268	T > C	2p23.1	PolymiRTS Database & Patrocles & miRanda & targetscan
miR-21	Robust expression in human adipose tissue and positively correlated with BMI	Keller et al. (2011)	rs4597342	*ITGAM*	0.280	C > T	16p11.2	miRanda & targetscan
miR-34a	Robust expression in pre-adipocytes and positively correlated with BMI	Kloting et al. (2009); Ortega et al. (2010)	rs2292899	*ACSL1*	0.634	A > G	4q35	PolymiRTS Database & Patrocles & miRanda & targetscan
miR-24	Robust expression in the adipocyte cultures	Keller et al. (2011)	rs5999924	*APOL6*	0.892	T > A	22q12.3	Patrocles & targetscan