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# Association of genetic variants in *Sirt1* and *Nrf2* gene with metabolic syndrome risk in a Chinese Han population

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## Abstract

Metabolic syndrome (MetS) is a complex of interrelated risk factors including central adiposity, raised blood pressure, hyperglycemia, elevated triglyceride levels and low high-density lipoprotein. Association studies have reported several genetic variants in *Sirt1* gene and *Nrf2* gene (*Sirt1* rs7895833 A>G, *Sirt1* rs2273773 C>T and *Nrf2* rs6721961 C>A ) with contributions to T2DM susceptibility as well as some glycemic and metabolic traits. However, little is known about contributions of these three single nucleotide polymorphisms (SNPs) to above phenotypes in Chinese. Our study recruited 141 individuals with MetS and 549 individuals without MetS to investigate association between three SNPs of *Sirt1*, *Nrf2* gene and MetS risk in a Chinese Han population using PCR-CTPP method. The result showed that the AA genotype of *Sirt1* rs7895833

was 2.41 times higher in MetS group than those of AG genotype（P=0.038）and 1.94 times higher than those of GG genotype（P=0.016）. he serum level of low-density lipoprotein cholesterol and HOMA-IR were significantly higher (P<0.05) in AA genotype of *Sirt1* rs7895833 compared with AG and GG genotype in general population. And the serum level of total cholesterol in AA genotype was lower (P=0.033) than other two genotypes. However, the genotype frequency of *Sirt1* rs2273773 and *Nrf2* rs6721961 in MetS group were not significantly different from that in the control subjects, and those two genetic variants were not correlated with metabolic traits. These results underscore the contributions of SNPs of *Sirt1* rs7895833 to MetS susceptibility in Chinese as well as glycemic and metabolic traits.

**Key words:** Metabolic syndrome; Polymorphism; *Sirt1*; *Nrf2*

## 1. Introduction

Metabolic syndrome (MetS) is a clustering of risk factors, such as central obesity, dyslipidemia, elevated blood pressure (BP), and abnormal glucose metabolism. MetS has been associated with type 2 diabetes mellitus (T2DM), cardiovascular disease (CVD) and a plethora of cancers [1]. According to data released by a joint interim statement of the International Diabetes Federation (IDF); American Heart Association; and International Association for the Study of Obesity in 2009, the worldwide prevalence of MetS is about 20% [2], which has become a serious global public health problem. Based on the 2010 China Noncommunicable Disease Surveillance, the estimated prevalence of metabolic syndrome was 33.9%, which indicates that metabolic syndrome affects approximately 454 million adults in China [3]. Insulin resistance (IR) and central obesity are widely believed to be the vital features of the MetS. On the basis of family and twins studies, the genetics of MetS appears to stand out as a potential causative factor [4]. Researches on the genetics factors of MetS currently focus on candidate genes related to lipid, glucose and energy metabolism.

Sirtuin 1 (*Sirt1*) is a longevity gene that protects cells against oxidative and genotoxic stress by deacetylating a large number of substrates such as p53 and forkhead transcription factors (FOXOs) [5]. Recently studies have also revealed that *Sirt1* plays an important role in glucose homeostasis and fat metabolism [6,7]. Although previous researches have showed that some of the *Sirt1* SNPs are associated with glucose tolerance, obesity, body fat and blood pressure [8-10], there is almost no study related

*Sirt1* gene SNPs and MetS in a Chinese Han population.

Furthermore, *Sirt1* is involved in the biological processes of oxidative stress and inflammation by activating transcriptional activity of downstream genes, including nuclear-related factor 2 [11]. The nuclear factor erythroid 2 related factor 2 (*Nrf2*) is a member of the cap 'n' collar family of transcription factors, which plays a crucial role in regulating the expression of antioxidant genes. Many SNPs in the *Nrf2* gene are predicted to affect risk of newly diagnosed T2DM, increased blood pressure and cardiovascular mortality [12-14]. However, there is no report on *Nrf2* SNPs in Chinese MetS patients.

Our study aimed to investigate the relationship between *Sirt1* rs7895833 A>G in the promoter region, *Sirt1* rs2273773 C>T in exon 5 silent mutation and *Nrf2* rs6721961 C>A in the promoter region and metabolic syndrome risk in a Chinese Han population using polymerase chain reaction with confronting two-pair primers (PCR-CTPP) technique.

## 2. Materials and Methods

### 2.1 Study Subjects

Our study was conducted from March to May 2010 in the Caihe community of Hangzhou, Zhejiang province, China. The study group consisted of 690 eligible Han Chinese adults aged 40–65 years old. The Medical Ethics Committee of Sir Run Run Shaw Hospital affiliated to School of Medicine, Zhejiang University approved this study. Written informed consent was obtained from all participants. The following

subjects were excluded according to: 1) impaired liver or renal function, 2) malignant tumors, 3) cardiovascular or peripheral vascular disease, 4) acute infectious disease or chronic inflammatory disease, 5) pregnancy, 6) thyroid disease and with glucocorticoid treatment, 7) incomplete clinical data.

### 2.2 Measurements

Face-to-face interviews were conducted among the subjects by trained medical staffs using a standardized questionnaire to collect the information about their general social demographic characteristics. All participants submitted to at the local community health care centers at 7:00-8:00 am following an overnight fast. Subjects without a validated history of diabetes mellitus (DM) received a 75 g oral glucose tolerance test (OGTT), whereas a 100 g carbohydrate (steamed bread meal) test was conducted on subjects with DM [15]. Fasting plasma glucose (FPG), 2-hour postprandial blood glucose (2hPG), triglyceride (TG), total cholesterol (TC), low density lipoprotein-cholesterol (LDL-C), high density lipoprotein-cholesterol (HDL-C), insulin, uric acid, creatinine, urea nitrogen, urine albumin-to-creatinine ratio (UACR), uric acid (UA), serum creatinine (CREA), and the serum urea nitrogen (BUN) were assayed with an autoanalyzer (Aeroset, Chicago, IL, USA). Glycosylated hemoglobin $A_1c$ ($HbA_1c$) was measured by ion-exchange high-performance liquid chromatography (Hemoglobin Testing System; Bio-Rad, Hercules, CA, USA). Serum insulin levels were measured with radioimmunoassay using the insulin detection kit (Beijing North Institute of Biological Technology, China). Insulin sensitivity was assessed by homeostasis model

assessment for insulin resistance (HOMA-IR) based on fasting glucose and insulin measurements as follows: [insulin (μIU/ml) × fasting blood glucose (mg/dl)/18]/22.5 [16]. Body mass index (BMI) was calculated by dividing body weight by height squared. Waist circumference (WC) was measured at the horizontal plane between the inferior costal margin and the iliac crest on midaxillary line. Hip circumference was measured at the widest point of the hips, and the waist-to-hip ratio (WHR) was calculated and recorded for each patient. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured in triplicate using a mercury sphygmomanometer, and the average of the three measurements was recorded. Body fat percentage (Fat%) was measured by a bioelectrical impedance analysis system (BIA) (TBF-300, Tanita Co, Japan). MRI scans were performed at the level of umbilicus between L4 and L5 with the subject in the supine position. Abdominal visceral fat area (VFA) and abdominal subcutaneous fat area (SFA) were calculated using SliceOmatic software (version 4.2).

**2.3 Definition of MetS**

MetS was defined according to standards generated by Guidelines for the Prevention and Treatment of Type 2 Diabetes in China [17]. Individuals with three or more of the following abnormalities were considered as having MetS: central obesity (WC > 90 cm for men and >85 cm for women); hypertriglyceridemia (≥1.70 mmol/L); low HDL-C (<1.04 mmol/L); elevated BP (≥130/85 mmHg or current treatment for hypertension); and hyperglycemia (FPG ≥ 6.1 mmol/L or 2 h postprandial glucose (2 h PG)≥7.8 mmol/L).

132

133 **2.4 Genotyping of *Sirt1* and *Nrf2* Gene SNPs**

134 Three tagging SNPs, namely, *Sirt1* rs2273773, *Sirt1* rs7895833 and *Nrf2* rs6721961
135 were selected from the HapMap database that covered 100% of the common variations
136 of the *Sirt1* and *Nrf2* Gene in Chinese. The genotyping of these SNPs was performed
137 using polymerase chain reaction with confronting two-pair primers (PCR-CTPP) assay
138 [18]. Briefly, 25-μl total PCR mixtures containing 100–200 ng DNA, 10.0 pmol of each
139 primers, 10μl of GoTaq Green Master Mix, and 10μl of Water-DEPC treated in the
140 supplied reaction buffer were prepared. PCR was performed with the primers as shown
141 in **Table 1**, with the initial denature at 95 °C for 10 min.; 35 cycles of 95 °C for 1 min.,
142 63 °C for 1 min., and 72 °C for 1 min, and additionally, the final step at 72 °C for 5 min.
143 PCR products were visualized on a 2 % agarose gel with Gel-Red staining and
144 genotyped. Three genotypes for each polymorphism were defined by three distinct
145 banding patterns; for **rs7895833 A>G polymorphism**: 320, 241 bp for AA genotype;
146 320, 241, and 136 bp for AG genotype; and 320, 136 bp for GG genotype; for
147 **rs2273773 C>T polymorphism**: 314, 228 bp for CC genotype; 314, 228, 135 bp for
148 CT genotype; and 314, 135 bp for TT genotype; for **rs6721961 C>A polymorphism**:
149 282, 113 bp for CC genotype, 282, 205, 113 bp for CA genotype, and 282, 205 bp for
150 AA genotype (**Figure. 1**) [9,19]. Meanwhile, PCR products were sequenced by
151 Hangzhou Qingke Xinye Biotechnology Company Ltd. The results of gene sequencing
152 were consistent with those obtained by gel imaging.

153

### 2.5 Statistical Analysis

Categorical variables were presented as frequency and percentage. All the continuous variables were tested for normal distribution and normally distributed variables were expressed as mean ± standard deviation (SD). Variables with a skewed distribution were presented as median value (interquartile range) [M(IQR)]. The Hardy-Weinberg equilibrium was tested by a goodness-of-fit $\chi 2$ test in non-MetS group. Differences of baseline characteristics of between participants with and without Mets were analyzed by t-test for normally distributed variables and Mann-Whitney *U* test for skewed distributed variables. Pearson's correlation test was also applied to determine the relation between the risk of MetS and other parameters. The binary logistic regression without or with age, sex, smoking and drinking as covariables was used to calculate crude odds ratio (OR) or adjusted OR as well as 95% confidence interval (95% CI) to determine association between each SNP and the risk of MetS. Both the additive genetic model and dominant model were estimated in above analyses. The linear regression analysis with adjustment to age, sex, smoking and drinking was performed to test association between each SNP and the metabolic traits. Results for the linear regression analysis are presented as regression coefficient ± standard error (SE). All statistical analyses were performed with SPSS 22.0 (IBM, Armonk, NY, USA) and significance was defined as a P value of $<0.05$.

## 3. Results

### 3.1 Anthropometric and metabolic characteristics of of study subjects

The anthropometric and metabolic characteristics of the studied 690 subjects at baseline were presented in Table 2. Totally, 141/690 (20.4%) of individuals met criteria for MetS. As expected, participants with MetS had a greater number of adverse risk factors than participants without MetS, including higher body BMI, WHR, Fat%, SFA, VFA, HOMA-IR, HbA1c, TC, UA, UACR, and MetS defning parameters (higher WC, blood glucose, TG, BP, and lower HDL-C) ($P < 0.05$ for all). However, there were no statistically significant difference in LDL-C, CREA, and BUN between the study groups ($p>0.05$). In addition, no significant differences was observed in age, sex, smoking and drinking between the cases and controls, thus the four variables were further adjusted in the logistic and linear regression model to control possible confounding effects among main effects of each SNP on the traits or disease.

### 3.3 Associations between the three SNPs and the risk of MetS

Results of association between each SNP and MetS risk are shown in Table 3. There was only a significant difference in genotype frequency of *Sirt1* rs7895833 between the cases and controls ($P = 0.015$). Compared to the GG genotype, AG heterozygote exerted an increased risk of MetS (adjusted OR=1.937，95%CI: 1.09-2.144, $P = 0.016$), and AA homozygote had a further increased risk (adjusted OR=2.414, 95%CI: 1.37-3.512, $P = 0.038$). In other words, the risk for MetS was increased by 2.41 times in AA genotype and 1.94 times in AG genotype compared with carriers of GG genotype. There was no significant difference between the carriers of A allele and G allele ($P=0.675$). However, for *Sirt1* rs2273773 and *Nrf2* rs6721961, no statistically significant

198 difference was observed between the groups in the genotypes and allele frequencies
199 ($P$ >0.05 for both).
200
201 **3.4 Associations between the three SNPs and anthropometric and metabolic**
202 **characteristics**
203 Results for association analyses of the three SNPs with anthropometric and metabolic
204 characteristics are shown in **Table 4**. For the rs7895833 of *Sirt1* gene, there was a
205 significantly positive correlation between the genotype of AA and AG and the level of
206 serum **LDL-C** under both the additive model (regression coefficient ± standard error:
207 **0.338±0.098； *P*=0.001**) and dominant model (**0.272±0.077； *P*<0.001**). The **LDL-C**
208 level gradually increased from GG genotype carriers, AG genotype ones to AA
209 genotype ones. And significant correlations were observed between the AA or AG
210 genotype and **HOMA-IR** (**0.023±0.011; *P*=0.035**) under the dominant model, as that
211 individuals with the AA or AG genotype had visibly higher concentrations of HOMA-
212 IR than those with GG genotype. Meanwhile, the SNP was also significantly correlated
213 with **TC** level under both the additive model (**-0.117±0.055; *P*=0.033**) and dominant
214 model (**-0.104±0.043; *P*=0.016**) as that individuals harboring the GG or AG genotype
215 exerted lower concentration of TC than those harboring the AA genotype. Furthermore,
216 no other significant correlation was observed between the *Sirt1* rs7895833 and
217 anthropometric and metabolic characteristics. For *Sirt1* rs2273773 and *Nrf2* rs6721961,
218 there was no significant difference between them and anthropometric and metabolic
219 characteristics (data not shown).

## 4. Discussion

Nowadays, metabolic syndrome has been a global public health problem and understanding its molecular backgrounds gains importance worldwide [20]. There is a considerable data about the relation of *Sirt1* and *Nrf2* gene and metabolic diseases. However, little is known about the association of genetic variants in *Sirt1* and *Nrf2* gene with metabolic syndrome risk in a Chinese Han population. Thus, we performed our experiments three candidate polymorphisms in *Sirt1* and *Nrf2* gene in a Chinese Han population.

Shimoyama et al. [9] reported that the A allele carriers of *Sirt1* rs7895833 had a high risk of obesity in 1,279 Japanese health checkup examinees. Zillikens et al. [21] reported carriers of the A allele for rs7895833 had a higher BMI and thereby carriers of this allele had an increased risk of obesity in Dutch Caucasian populations. Associated researches considers the obesity epidemic to be the main driver of the high prevalence of the MetS [1,22]. In our research, the risk for MetS was increased by 2.41 times in AA genotype and 1.94 times in AG genotype compared with carriers of GG genotype. Based on the previous studies, the A allele carriers in rs7895833 tend to be obese, and thereby at a high risk for MetS, which was consistent with the present study. Thus, it is conceivable to see that subjects carrying the rs7895833 AG and AA genotypes had higher levels of LDL-C and HOMA-IR than those carrying the GG genotype. The most puzzling aspect of findings in this study is that the rs7895833 showed a negative correlation with TC. It is possible that other factors disturbed the correlation between the rs7895833 and TC level, such as lipid-lowering drugs, sample selection and skewed

distribution of data. Furthermore, the SNP of rs7895833 is located in the promoter. In addition, its base sequence is TTGACT, which has been proved to be a W-box-like element of promoter [23]. Therefore, it is likely that these polymorphisms affect the activity of *Sirt1* gene through regulating promoter activity and thereby *Sirt1* expression. *Sirt1* deacetylates PGC-1α to promote its activity and interacts with PPAR-γ to repress its transcription [6,7,24,25]. Thus, gene polymorphism of *Sirt1* might affect the activities of PGC-1α and PPAR-γ, and consequently it might be related to risk factors of MetS. This study showed the findings so they could be, suggesting the rs7895833A genotypes to be a possible biomarker of increased MetS susceptibility.

Van den Berg SW et al. [26] reported that the CT genotype of *Sirt1* rs2273773 had a higher BMI than TT genotype. In another study, the frequencies of TT genotypes and alleles for rs2273773 were significantly higher in patients with CVD compared to control group [27]. Shimoyama et al. [19] also observed that the *Nrf2* rs6721961 polymorphism has been associated with blood pressure in Japanese subjects. Wang et al. [12] investigated *Nrf2* gene polymorphism (rs6721961) in a Chinese population and observed that individuals with the AA genotype had a significantly higher risk of developing T2DM, relative to those with the CC genotype. However, there was no significant difference in genotype and allele distributions between groups in the present study. We speculated that our non-significant finding was majorly due to environmental factor and the limited sample size.

There are some limitations in the current study. The gene expressions and activities were not determined. The studied sample size was relatively small. Based on a

retrospective design, bias such as information bias and selection bias could not be rule out. Despite these limitations, this is the first epidemiological study that associates the SNPs of *Sirt1* and *Nrf2* genes to MetS.

## 5. Conclusions

In conclusions, *Sirt1* rs7895833 is associated with metabolic syndrome risk in a Chinese Han population. The *Sirt1* variants rs7895833 has contributions to serum levels of LDL-C, HOMA-IR and TC. *Sirt1* rs7895833 might be a promising variant for determining metabolic diseases. Our conclusion should be confirmed by a replication study with larger sample size in Chinese.

## Conflict of Interests

The authors report no conflict of interests and declare no competing financial interests.

## Acknowledgements

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**Figure 1. the genotype for SNPs showing in gel. a** Representative gel showing the genotype for rs7895833 SNP of Sirt1 gene. b Representative gel showing the genotype for rs2273773 SNP of Sirt1 gene. c Representative gel showing the genotype for rs6721961 SNP of Nrf2 gene. The first lanes of each gel contain a 500 bp DNA ladder.

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# Figures

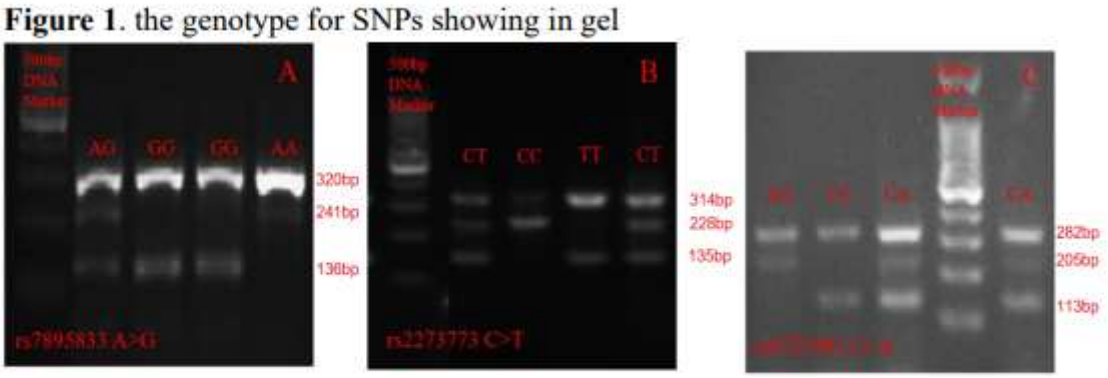


Fig.1 **a** Representative gel showing the genotype for rs7895833 SNP of *Sirt1* gene. **b** Representative gel showing the genotype for rs2273773 SNP of *Sirt1* gene. **c** Representative gel showing the genotype for rs6721961 SNP of *Nrf2* gene. The first lanes of each gel contain a 500 bp DNA ladder

**Figure 1**

The genotype for SNPs showing in gel

# Supplementary Files

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- Table.pdf