Association between insulin receptor gene polymorphism and the metabolic syndrome in Han and Yi Chinese

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Insulin resistance has been a possible underlying pathophysiologic defect inducing the metabolic syndrome (MS). However association studies regarding insulin receptor gene in different ethnic groups are scarce in literature. Here we conduct an association study between MS and genetic polymorphism of the INSR gene in Yi and Han Chinese. In a cross-sectional study, 3,436 Yi and Han people were investigated. Ethnicity-specific case-control studies were designed, with MS patients diagnosed as cases and non-MS people as controls matched on gender and age. Polymerase chain reaction-restriction fragment length polymorphism was used to detect the genotypes of the exon 8 of the INSR gene. Data were analyzed using one-way analysis of variance, chi-square test, and logistic regression where appropriate. Systolic blood pressure (SBP) was significantly higher in MS patients with the N2N2/N2N3 genotypes than that in those with the N3N3 genotype of both ethnic population ($p<0.05$). Frequency of the N2 allele was significantly higher in MS patients than that in controls of ethnic Han ($p=0.020$). Multivariable logistic regression analysis showed that the NsiI polymorphism of the exon 8 of the INSR was an independent predictor for MS in Han people adjusted for total cholesterol, sex, physical activity, educational level, family income, alcohol intake and smoking (OR=2.55, 95% CI: 1.31-4.94, $p=0.006$). The results indicated that NsiI polymorphism of the INSR gene is associated with SBP in these two different ethnic groups, and significantly associate with MS in Han Chinese. These findings contribute to our better understanding on the genetic basis of MS.

Key Words: metabolic syndrome (MS), insulin receptor (INSR) gene, NsiI Polymorphism, ethnic Yi, China

INTRODUCTION
The metabolic syndrome (MS) is now well recognized as a growing public health problem for low- and middle-income countries, with suggested ethnic predisposition in Asians, and characterized cluster of risk factors including central obesity, hypertension, glucose intolerance, hyperinsulinemia, low serum level of high-density lipoprotein-cholesterol (HDL-C) and high serum level of triglycerides. People with MS are at increased risk for type 2 diabetes (T2DM) and cardiovascular disease. More evidences have shown that insulin resistance (IR) is an underlying pathophysiologic defect inducing development of MS, and Asians have a tendency to suffer from IR, but its etiology is still unclear and may be related to complex interactions between genetic, metabolic and environmental factors. Most epidemiological studies have demonstrated that some lifestyles and behavior, including sedentary activities, smoking, alcohol consumption and unhealthy dietary intakes are associated with MS in adults. So, there is an urgent need to conduct studies regarding genetic and environmental factors related to IR in Asians. Insulin resistance is partly due to different mutations in the insulin receptor (INSR) gene. The INSR gene exon 8 and exon 17 are known to be associated with hypertension in Caucasian and Chinese populations. Frequency of the N2 allele of the NsiI restriction fragment length polymorphism (RFLP) in the exon 8 of the INSR gene is higher in hypertensive patients as compared with normotensive individuals in Caucasian populations, whereas frequency of the N2 allele is higher in Chinese hypertensive patients, which may attribute to ethnic difference. In addition, some studies suggest the NsiI
RFLP of the INSR gene correlated with diabetes as a susceptible gene. But no association was found in other studies.\textsuperscript{26-29} Aim of the current study is to investigate the possible role of exon 8 variants of the INSR gene in development of MS, in Yi and Han Chinese populations.

**MATERIALS AND METHODS**

**Subjects and design**

Our study based on a cross-sectional design was conducted among Yi and Han people living in Xichang city and Butuo, Zhaojue, Jinyang, Puge and Xide counties in Sichuan province. Study participants including Yi and Han populations were stratified by their living areas, and adults over 20 years of age were randomly selected from 15 communities surveyed during 2007 to 2008. The study was approved by the local ethics committee and informed consent was obtained from the study subjects.

The survey included face-to-face interviews with trained interviewers who were fluent in Yi or Chinese languages, anthropometric measurements on the spot and blood biochemical examinations. Data collected included demographic information such as gender, age, educational level and occupation, and lifestyles such as smoking, alcohol consumption, as well as family income, family medical and personal medical history such as diagnosis and treatment for hypertension, diabetes and dyslipidemia.

Blood pressure was measured on the right arm in a sitting position using a standard mercury sphygmomanometer with reading to the nearest 2 mmHg and the mean of three consecutive readings was used for analysis. The second measurement was taken after a 10-minute rest.

Standard techniques for body measurements were used for all the participants wearing light clothing and no shoes, weight by a battery-operated electronic scale and height by a portable stadiometer, with reading to the nearest 0.1 kilogram and 0.1 centimeter, respectively. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared (kg/m\(^2\)).

Blood was collected after a minimum 10-hour fasting early in the morning on the spot from each participant at the time of interview and processed at 37°C for 4 to 5 hours before electrophoresis on a 2% agarose gel and visualization by ethidium bromide staining. Specimen with the N\(_1\) or A allele lacking the restriction site resulted in 324 bp long product, but the PCR product containing the mutant G or N\(_2\) allele was cleaved into 239-bp and 85-bp long fragments.

**Detection of the polymorphism**

Genomic DNA was extracted from peripheral blood by a standard method, and genotypes for NsiI RFLP in exon 8 of the INSR gene were determined with PCR amplification, which was performed in a thermocycler with 35 cycles at 95°C, 55°C and 72°C for 1 minute each. Amplification was carried out in a final volume of 50 μl containing 1 μg DNA extracted, 200 μM of each dNTP, 1 U of Taq polymerase, 5 μl of reaction buffer (500 mM KCl, 14 mM MgCl\(_2\), 10 mM Tris-HCl at pH 8.3), 0.2 mM of each primer (5'-CGG TCT TGT AAG GGT AAC TG-3', 5'-GAA TTC ACA TTC CCA AGA CA-3'). The 324-base pair (bp) amplification product was then digested with 2.5 U NsiI (Promega Corporation, Madison, WI, USA) at 37°C for 4 to 5 hours after electrophoresis on a 2% agarose gel and visualization by ethidium bromide staining.

**Definition of the metabolic syndrome**

MS was assessed based on the criteria set by the CDS under the CMA in 2004 with presence of three or more of the following:\textsuperscript{20}

1. Overweight or obesity defined as BMI equal to or more than 25.0 kg/m\(^2\)
2. High blood pressure defined as systolic blood pressure equal to or more than 140 mmHg and/or diastolic blood pressure equal to or more than 90 mmHg
3. Hypertriglyceridemia defined as serum level of triglycerides equal to or more than 1.7 mmol/L, or low HDL-C defined as serum level of HDL-C equal to or less than 0.9 mmol/L in men and less than 1.0 mmol/L in women
4. Impaired fasting glucose defined as plasma level of glucose equal to or more than 6.1 mmol/L.

Participants who received antihypertensive or hypoglycemic medications were also regarded as those with high blood pressure or high (impaired) fasting glucose.

**Statistical analysis**

Differences in the mean between two genotype groups and also between two ethnic groups were tested by student t-test. Multivariable logistic regression analysis was performed to evaluate associated factors of risk for MS with odds ratio (OR) and its 95% confidence interval (CI). Goodness of fit chi-square test was used to detect whether the genotypic distribution was in Hardy-Weinberg equilibrium. Difference in allele and genotype distributions between MS case and controls was detected by \(\chi^2\) test. \(P\)-value less than 0.05 were considered as statistical significant. All data analyses were performed using SAS 9.1 software (SAS Institute Inc, Cary, NC).

**RESULTS**

The most relevant clinical characteristics of all MS patients and controls in two ethnic groups are summarized in Table 1. As expected, all five components of MS were more prevalent in patients with MS than those in controls in both ethnic groups. MS groups had higher systolic blood pressure, diastolic blood pressure, fasting plasma glucose, triglyceride, total cholesterol, BMI, as well as...
lower HDL-C, compared to those in control group (p<0.001). No clinical characteristic differences were found between the two ethnic patient groups and also when compared between the two ethnic control groups.

Table 2 shows major clinical parameters of MS patients according to genotype, non-carriers with the N1N1 genotype and carriers with the N1N2+N2N2 genotype. Systolic blood pressure was significantly higher in carriers than that in non-carriers in MS patients among these two ethnic populations (p<0.05). However, no significant difference in diastolic blood pressure, serum levels of total cholesterol and triglyceride were found between the varied genotype groups in MS patients. Compared to Yi carriers, serum levels of triglyceride was higher in Han carriers in MS patients, there were no apparent differences in other clinical characteristics between the two ethnic patient groups with the same genotypes.

Frequencies of all the genotypes were compatible with Hardy-Weinberg equilibrium. Frequencies of the genotypes and NsiI RFLP allele in the two groups are shown in Table 3 and Table 4. Frequency of the NsiI RFLP N2 allele accumulated in Han group with MS (23.3%) as compared to 14.4 percent in controls, suggesting significance of the NsiI RFLP N2 allele as a probable independent risk factor for MS. After adjustment for sex, difference in total cholesterol, physical activity, educational level, family income, alcohol consumption, and smoking, there was a statistically significant association between NsiI RFLP and MS (N1N2+N2N2 vs. N1N1: OR=1.71, 95% CI: 1.07~2.36, p=0.048). With additional adjustment for ethnicity, the association became insignificant. Further separately analyses showed that the N1N2+N2N2 genotype carriers had an increased risk for developing MS than the non-carriers in the Han participants (OR=2.25, 95% CI: 1.25~4.05, p=0.006) without adjustment. The association became stronger (OR=2.55, 95% CI: 1.31~4.94, p=0.006) after adjustment for difference in total cholesterol, sex, physical activity, educational level, family income, alcohol consumption, and smoking between the carriers and non-carriers.

Distribution of the NsiI RFLP N2 allele did not differ between the two groups in Yi participants, with its frequency of 19.2 percent in MS patients and 23.3 percent in the controls. No significant difference was observed in frequency of the genotype distribution between the two groups in Yi participants.

**DISCUSSION**

Association of various genotypes of the INSR gene in MS and hypertensive patients has been examined in varied ethnic populations. But, prior to the present study, no investigation in the Yi population has been conducted in China. We report here, for the first time, an association between the INSR gene polymorphism and features of MS in Yi and Han populations living in southwestern China.

Previous studies have demonstrated an association between genetic polymorphisms in exon 8 of the INSR gene
and hypertension. There was a tendency towards increased prevalence of the N1 allele of the NsiI RFLP located in exon 8 in hypertensives, compared with that in normotensives in an Australian white population.\(^{19}\) Additionally, a weak association was observed between the INSR gene NsiI RFLP and diastolic blood pressure in a Hong Kong Chinese population.\(^{21}\) No association between the INSR gene exon 8 and hypertension was found in a Mexican population.\(^{20,22}\) However, it was notable that some studies in Chinese populations revealed that frequency of the N2 allele of the NsiI RFLP was higher in hypertensives than that in normotensives,\(^{24,25}\) which might be due to ethnic difference and different sampling methods. Current findings showed that people carrying the N1N1 or N1N2 genotype were found to have significantly higher mean SBP than those with the N2N2 genotype. By multivariate logistic regression analysis, the INSR gene exon 8 variant is associated with MS that varied in different ethnic populations. The N2 allele was also more frequent in MS patients among the Han participants, which may play a major role in development of IR. A strong association between the N1N1 or N2N2 genotype was found in the Han participants, revealing that the N1N1 or N2N2 genotype confers independent risk of MS.

Most studies showed that variation in the NsiI cutting site responsible for this RFLP does not cause alteration in amino acid sequence (GCG-GCA), which may not directly cause development of various components of MS, but is likely to be in linkage disequilibrium with a “causative” variant nearby in the genome. Moreover, knockout mice deficient in the INSR gene showed the IRSNR level and its early signal transfer decreased by over 95%; whereas weight, plasma levels of triglyceride and free fatty acid elevated remarkably, indicating a mutation of the INSR gene to be associated with lipid metabolism in type 2 diabetes patients.\(^{31}\) Mutation in the INSR probably affected transcription of AGT or influenced other pathways involved in the form of INSR or insulin metabolism, which might relate to IR status. However, underlying mechanisms are still unclear and cannot be easily explained by an impact on INSR expression, which maybe reflected by their potential interactions with other genes and environmental triggers. Additionally, it is recognized that they may be insufficient to control the different environmental influences among different ethnicities. However, the negative results in the Yi participants do not rule out potential involvement of the INSR gene. Future research is needed to establish its actual mechanism and functional role.

The current study involved Yi and Han populations living in the same geographical areas for a relatively long period of time, without significant difference in demography or dietary behavior. In addition, previous findings suggested that Yi people are an ethnically homogeneous population.\(^{32-34}\) Within an ethnic population, genetic factors may prompt individuals predisposed to development of various components of MS. However, in our study, no association between the genotype and alleles of the INSR gene exon 8 and MS was found in Yi participants, whereas significant difference was found in frequency of the N2 alleles of the INSR gene exon 8 in Han participants. Furthermore, ethnic difference persisted even after adjustment of socioeconomic status and lifestyle factors. This finding seemed to be helpful for better understand-

### Table 3. Relationship between MS and the NsiI RFLP allele in Yi and Han participants

<table>
<thead>
<tr>
<th>Alleles</th>
<th>Yi participants</th>
<th>Han participants</th>
<th>p-value</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N1</td>
<td>194 (0.808)</td>
<td>181 (0.767)</td>
<td>0.270</td>
<td>0.78 (0.50-1.51)</td>
</tr>
<tr>
<td>N2</td>
<td>46 (0.192)</td>
<td>55 (0.233)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N1</td>
<td>161 (0.767)*</td>
<td>178 (0.856)</td>
<td>0.020*</td>
<td>1.81 (1.09-2.98)</td>
</tr>
<tr>
<td>N2</td>
<td>49 (0.233)*</td>
<td>30 (0.144)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*p=0.020, between N1 and N2 alleles.

### Table 4. Association between MS and the NsiI RFLP in Han and Yi participants

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>MS</th>
<th>non-MS</th>
<th>Unadjusted OR (95% CI)</th>
<th>Adjusted OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yi and Han participants</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N1N1</td>
<td>139 (0.382)</td>
<td>148 (0.667)</td>
<td>1.71 (1.07-2.36)*</td>
<td>1.25 (0.96-2.54)*</td>
</tr>
<tr>
<td>N1N2+N2N2</td>
<td>86 (0.618)</td>
<td>74 (0.333)</td>
<td>p=0.048</td>
<td>p=0.282</td>
</tr>
<tr>
<td>Han participants</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N1N1</td>
<td>79 (0.658)</td>
<td>70 (0.593)</td>
<td>0.76 (0.65-1.48)</td>
<td>1.40 (0.90-2.37)*</td>
</tr>
<tr>
<td>N1N2+N2N2</td>
<td>41 (0.342)</td>
<td>48 (0.407)</td>
<td>p=0.299</td>
<td>p=0.609</td>
</tr>
</tbody>
</table>

1*Adjusted for total cholesterol, sex, physical activity, educational level, family income, alcohol consumption and smoking.
2*Adjusted for ethnicity, total cholesterol, sex, physical activity, educational level, family income, alcohol consumption and smoking.
3*p<0.05, between N1N1 and N1N2+N2N2 genotype.

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ing on genetic basis of MS. Also, it is important to further explore potential gene-gene or gene-environment interaction that will contribute to potential risk for MS.

One possible explanation was that a weak association might have existed between MS and the INSR gene exon 8 in Y1 participants. However, minor effect was not found maybe due to small sample size or interaction between N2 allele and some other gene(s) that definitely contributes to MS as well.

The results of our study should be interpreted in the context of limitations. Firstly, genetic association studies are subject to random variation due to sample size effect; further, studies with larger sample sizes are needed to elucidate the function of this polymorphism in the pathogenesis of MS. Secondly, our study was cross-sectionally designed rather than longitudinally, therefore we cannot affirm that some control subjects did not have MS after the investigation. To avoid this bias it is important to reproduce the data in prospective studies. Additionally, a possible explanation for the result of association was that the INSR gene exon 8 may not have real biological effects and that it is most likely in linkage disequilibrium with the responsible mutations within the insulin receptor or in neighboring genes. However, this assumption has to be confirmed in further studies.

In conclusion, the association between the INSR gene exon 8 variant and the metabolic syndrome is varied in different ethnic populations. Our results provided genetic evidence to support the hypothesis that the INSR gene exon 8 variant is associated with pathogenesis of MS in the Chinese population. People carrying the N2 allele of exon 8 are at higher risk for MS if they are ethnically Han. Further prospective and functional studies are required to elucidate this relationship more clearly. Increased prevalence of MS in Asia is improbable to be merely due to genetic factors. It should also be highlighted that future studies need to evaluate interaction between the INSR gene exon 8 variant and environmental factors such as diet or physical activity on MS.

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AUTHOR DISCLOSURES

No conflicts of interest.

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Original Article

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中国彝族和汉族的胰岛素受体基因与代谢综合征的关联

胰岛素阻抗是可能引起代谢综合征(MS)的病理生理基础之一。然而，有关不同种族胰岛素受体(INSR)基因与代谢综合征的关联研究并不多见。本文探讨了中国彝族和汉族 INSR 基因多态性与 MS 的关联性。本研究共调查彝族和汉族 3,436 人。按照种族的不同，分别设计两组病例对照研究。分别选取汉族和彝族的 MS 病例，根据病例数随机选择汉族和彝族非 MS 对照者，且与病例在年龄和性别上进行匹配。应用聚合酶链式反应-限制性片段长度多态性(PCR–RFLP)检测 INSR 基因第 8 外显子的基因型。采用单因素方差分析，卡方检验和逻辑斯回归对数据进行分析。研究结果显示，无论彝族或汉族，MS 患者 N1N2/N2N2 基因型携带者收缩压比 N1N1 基因型携带者高(p<0.05)。汉族人群 INSR 基因第 8 外显子 Nsil 的基因型和等位基因在病例组和对照组中的频数分布差异有显著性，MS 病例组 N2 等位基因频率显著高于对照组(p=0.020)。多因素逻辑斯回归分析结果显示，调整总胆固醇，性别，体力活动，教育程度，家庭收入，吸烟情况和饮酒情况后，INSR 基因第 8 外显子 Nsil 多态性是汉族人群 MS 的独立预测因子 (OR=2.55，95%CI: 1.31-4.94，p=0.006)。研究结果表明，INSR 基因第 8 外显子 Nsil 多态性与彝族和汉族人群的收缩压相关联，且与汉族人群的 MS 相关联。本研究对于理解 MS 发生和发展的遗传基础提供了一定的科学依据。

關鍵字：代谢综合征、胰岛素受体基因、Nsil 多态性、彝族、中国