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Association of SUMO4 Met55Val Variation with Increased Insulin Resistance in Newly Diagnosed Type 2 Diabetes in a Chinese Population*

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Summary: SUMO4 Met55Val variation was shown to be related to type 2 diabetes susceptibility and the vascular complications in Asian people. To further examine the related mechanisms, this study was designed to evaluate the association of SUMO4 Met55Val polymorphism with insulin resistance and β cell function in newly diagnosed type 2 diabetic patients in a Chinese population. Four hundred and twenty seven newly diagnosed type 2 diabetic patients were selected for SUMO4 Met55Val polymorphism genotype analysis. All subjects underwent a 75-g oral glucose tolerance test (OGTT) to estimate the insulin sensitivity and β cell function. Anthropometrics and a metabolic profile were used for phenotyping analysis. The results showed that the SUMO4 Met55Val polymorphism was associated with higher insulin resistance (P<0.001) and lower insulin sensitivity (P<0.001). Patients with GG genotype had higher levels of plasma glucose, insulin and C peptide. Insulin sensitivity index (ISI) was closely correlated with body mass index (BMI) in patients with GG genotype in comparison to the counterparts with AG or AA genotype (r= –0.504 *vs.* r= –0.430 *vs.* r= –0.340). Multiple regression linear analysis showed that SUMO4 Met55Val polymorphism was an independent determinant for insulin sensitivity (P=0.001), which, along with triglyceride, BMI and sex, could account for 20.1% of the variation in ISI. The result remained the same after adjusting for BMI and sex. No association was found between SUMO4 Met55Val polymorphism and β cell function (all P>0.05). It was concluded that SUMO4 Met55Val variant was associated with increased insulin resistance in Chinese patients with newly diagnosed type 2 diabetes.
Key words: SUMO4; type 2 diabetes; insulin resistance

SUMO4 is a novel protein belonging to the small ubiquitin-related modifier family. The conjugation of SUMO4 to IκBα was found to suppress the nuclear factor-κB (NF-κB) transcriptional activity. The novel polymorphism of SUMO4 Met55Val which is derived from an amino acid mutation in the conserved met55 residue can activate the NF-κB and lead to the over-expression of NF-κB dependent gene products[1]. A study by Zou *et al* showed that SUMO4 Met55Val polymorphism was implicated in some diseases which are closely related to inflammation process[2]. This variant was also revealed to be associated with type 1 diabetes, an autoimmune related disease, in Asian populations[3–5].

Recently, the relationship between SUMO4 Met55Val polymorphism and type 2 diabetes has been a subject of interest in the endocrinological community. Studies by Noso[6] and Shimada[7] reported the contribution of the SUMO4 Met55Val polymorphism to type 2 diabetes susceptibility in a Japanese population. Lin *et al*[8] found the type 2 diabetic patients with GG genotype might have worse glycemic control. Furthermore, SUMO4 Met55Val variation was shown to be related to diabetic complications, such as diabetic nephropathy[9] and coronary heart diseases[7].

Insulin resistance is an important pathogenesis of type 2 diabetes, and inflammation is supposed to exert considerable influence on the development of insulin resistance[10–12]. TNFα, one of the downstream factors of NF-κB, can induce insulin resistance in experimental animals[13]. The activated IKKβ/NF-κB can lead to insulin resistance by activating the transcription of many cytokines[14]. Researchers have noted the different insulin resistance traits are associated with SUMO4 Met55Val variations in healthy people[6]. However, as hypoglycemia medications have always been administered to diabetic patients before the investigation, it's difficult to evaluate the association of this polymorphism with insulin resistance in diabetic patients.

In the present study, the relationship between SUMO5 Met55Val variant and insulin resistance and β cell function was examined in patients with newly diagnosed type 2 diabetes.

1 MATERIALS AND METHODS

1.1 Study Population

Four hundred and twenty seven patients with newly diagnosed type 2 diabetic were selected from the Outpa-

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tient/Inpatient Department of Endocrinology at Zhongnan Hospital of Wuhan University between 2006 and 2009. Blood samples were collected for SUMO4 Met55Val polymorphism analysis. The type 2 diabetes diagnosis was based on the 2003 American Diabetes Association criteria. All patients underwent a complete physical examination and laboratory tests. Patients' height and weight were measured in the absence of shoes and in light outdoor clothes. The ratio of weight (kg) to the square of height (m^2) was defined as body mass index (BMI). Patients with type 1 diabetes, diabetic ketoacidosis, hyperosmolar nonketotic diabetic coma, tumor, infection, rheumatic diseases, and severe liver or renal diseases were excluded from the study. This study was approved by the Institutional Ethics Committee of Wuhan University, and informed consents were obtained from all subjects.

1.2 Laboratory Analysis

All subjects underwent a 75-g oral glucose tolerance test (OGTT), and venous blood samples were obtained at 0, 30, 60, 120, and 180 min for determination of plasma glucose, insulin, and C peptide. Other biochemical indexes were measured at a fasting state. Glucose levels were determined by a glucose oxidase method, glycosylated hemoglobin by an ion-exchange HPLC procedure, and triglyceride and cholesterol levels by enzymatic methods. Serum C peptide was measured by radioimmunoassay. Areas under the curve (AUC) for plasma glucose, insulin and C peptide were calculated by trapezoidal rule. Insulin resistance was estimated by homeostasis model assessment (HOMA) according to the following formula[15]: HOMA = fasting insulin (uU/mL) × fasting glucose (mmol/L)/22.5. Insulin sensitivity index (ISI) was calculated from glucose and insulin values during the OGTT as described by Matsuda and DeFronzo (10 000/square root of [fasting glucose×fasting insulin]×[mean glucose×mean insulin during OGTT])[16]. B cell function was assessed as HOMA-β ([20×fasting insulin]/[fasting glucose–3.5])[17], corrected incremental insulin response (CIR) (100×plasma insulin at 30 min/[plasma glucose at 30 min×(plasma glucose at 30 min–3.89)])[18] and insulinogenic index ([plasma insulin at 30 min－fasting insulin]/[plasma glucose at 30 min －fasting glucose])[18].

1.3 Genotype Analysis

Genomic DNA was prepared from peripheral blood by using standard techniques. Genotyping of the A/G polymorphism was performed by restriction fragment-length polymorphism analyses. PCR was carried out with a final reaction volume of 25 μL using specific oligonucleotides designed according to the published sequences of the human SUMO4 gene, resulting in a predicted 107-pb fragment. Each PCR contained 50–100 ng genomic DNA, 1 μmol/L of each primer (forward, 5'-ATTGTGAACCACGGGGATTGTTA-3' and reverse, 5'-CTTCATCTTCCATTTCCAACTG-3'), 2 mmol/L $MgCl_2$, 50 mmol/L KCl, 20 mmol/L Tris-HCl, 0.2 mmol/L dNTPs, and 1 U Taq polymerase (Invitrogen, USA). The reaction conditions were as follows: an initial denaturation step at 94°C for 5 min, followed by 94°C for 30 s, annealing at 54°C, and extension at 72°C for 20 s. Thirty five cycles were run, with a final additional extension step at 72°C for 10 min. Ten mL of PCR product was then incubated at 37°C with 5 U *Mse* Ⅰ restriction enzyme. The product of the enzymatic digestion was analyzed on a 1.5% agarose gel stained with ethidium bromide and visualized by optical densitometry. The G allele was identified as an uncut 107 bp fragment and the A allele as a doublet of 86 and 21 bp bands, respectively. Patients were classified into groups of AA, AG and GG genotypes according to the presence of the alleles. All amplification reactions were performed in duplicate.

1.4 Statistical Analysis

Statistical analysis was performed by using SPSS for Windows, version 11.5. Genotype and allele frequencies were compared with the Hardy-Weinberg equilibrium model. Results were expressed as $\bar{x}\pm s$. Clinical and laboratory data from subjects with AA, AG, and GG genotype were compared by using ANOVA test. The non-normal variables were log-transformed to approximate a normal distribution. However, for clarity, results were expressed as untransformed values. To examine the main effect of the SUMO4 Met55Val variant, three genotypes (AA, AG, and GG) were tested separately, followed by pooling of the AG and GG groups. Student's *t* test or Mann-Whitney *u* test was employed for comparisons between two groups. Multivariate analysis was performed with a backward stepwise option to assess the influence of multiple variables on insulin sensitivity. The probability of entering or removing was set at 0.10. A $P<0.05$ was considered statistically significant.

2 RESULTS

2.1 Clinical Characteristics of Different Genotypes

Clinical characteristics of the subjects with different SUMO4 genotypes were shown in table 1. The proportions of subjects with AA, AG and GG genotypes were 45.7%, 43.3% and 11%, respectively, which were highly concordant with the Hardy-Weinberg equilibrium. There was no significant difference among the three groups in gender, age, body mass index, total cholesterol, HDL cholesterol, LDL cholesterol and HbA1c levels. Weight ($P=0.009$) and triglyceride ($P=0.036$) were significantly higher in AG and GG genotypes groups. The results from the pooled AG and GG genotypes were comparable.

2.2 Association of SUMO4 Met55Val Polymorphism with Insulin Resistance and β Cell Function

Data of plasma glucose levels, insulin levels and C peptide levels from OGTT were shown in fig. 1. Plasma glucose at all time points and fasting plasma insulin were significantly higher in AG and GG genotype than in AA genotype (all $P<0.05$). The same trend was observed in glucose AUC during OGTT test ($P<0.05$). Plasma insulin concentrations at 0.5, 1, 2, 3 h and plasma C peptide concentrations at all time points were not significantly different among three genotypes. The plasma insulin AUC ($P=0.202$) and plasma C peptide AUC ($P=0.579$) were higher in GG genotype, but the difference did not achieve statistical significance. Peak insulin secretion time was postponed in GG (2.21±0.66 h, $P=0.751$ *vs.* AA genotype) and AG (2.19±0.65 h, $P=0.800$ *vs.* AA genotype) genotypes compared with the AA (2.17±0.65 h) genotype without any statistical significance. The insulin peak secretion to basal secretion ratio was higher in the AA (6.87±5.53) genotype than in GG (5.03±3.95, $P=0.026$ *vs.* AA genotype) and AG (5.11±3.30, $P=0.035$ *vs*. AA genotype) genotypes.

Table 1 Anthropometric and biochemical variables of all subjects stratified by SUMO4 genotypes

Variables	AA (*n*=195)	AG (*n*=185)	GG (*n*=47)	*P*	$P_{AA\ vs\ AG/GG}$
M/F	121/74	113/72	27/20	0.844	0.765
Age (years)	56.32±11.85	55.7±11.99	58.26±11.99	0.500	0.974
Weight (kg)	66.51±11.57	71.03±9.55	67.98±10.86	0.009	0.046
BMI (kg/m^2)	24.89±3.79	25.75±3.20	26.16±3.18	0.120	0.198
TC (mmol/L)	4.81±1.04	5.09±1.16	5.04±0.98	0.211	0.782
TG (mmol/L)	1.85±1.14	2.62±2.95	2.73±2.04	0.036	0.002
HDL-c (mmol/L)	1.26±0.61	1.14±0.41	1.12±0.28	0.482	0.230
LDL-c (mmol/L)	2.89±0.81	3.84±0.89	3.32±2.39	0.521	0.165
HbA1c	8.64±2.42	9.04±2.10	9.12±2.41	0.341	0.360

M, male; F, female; BMI, body mass index; TC, total cholesterol; TG, triglyceride; HDL-c, high density lipoprotein cholesterol; LDL-c, low density lipoprotein cholesterol; HbA1c, hemoglobin A1c

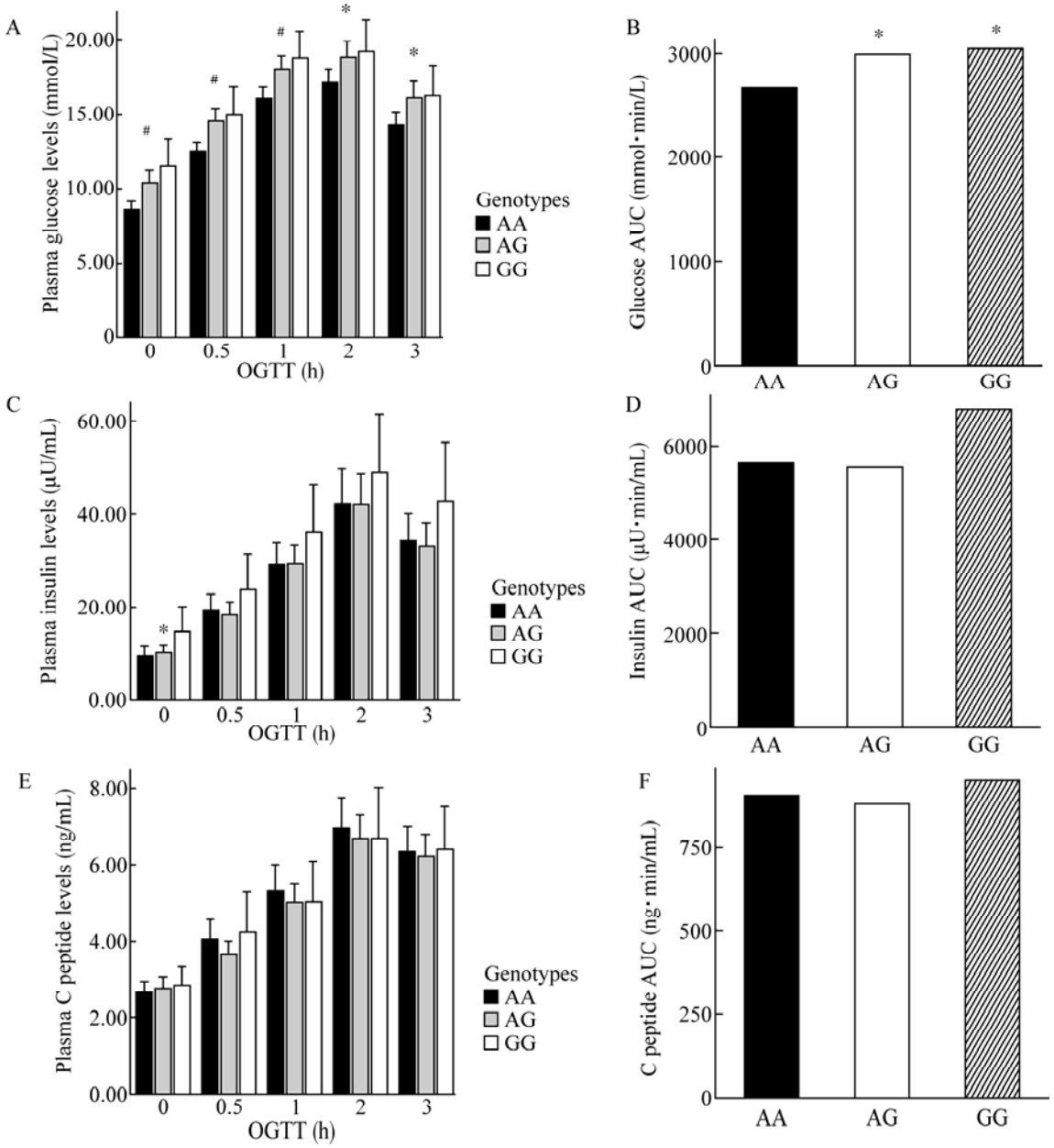

Fig. 1 Comparison of different indexes from OGTT test

A: Plasma glucose at different time points during OGTT in different genotypes; B: Glucose AUC during OGTT in different genotypes; C: Plasma insulin levels at different time points during OGTT in different genotypes; D: Insulin AUC during OGTT in different genotypes; E: Plasma C peptide at different time points during OGTT in different genotypes; F: C peptide AUC during OGTT in different genotypes

$^*P$<0.05, $^\#P$<0.01 as compared with AA genotype

Insulin resistance defined as HOMA-IR (P<0.001) and insulin sensitivity index (P<0.001) were significantly different from each other among genotypes (table 2), which was not the case for β cell function expressed as HOMA-β (P=0.860), CIR (P=0.171) and insulinogenic index (P=0.478) (table 2), suggesting insulin resistance but not β cell function was associated with SUMO4 Met55Val polymorphism.

Table 2 Comparison of insulin sensitivity and β cell function

Variables	AA (n=195)	AG (n=185)	GG (n=47)	P
Insulin sensitivity index				
ISI	100.39±64.91	84.07±48.77	66.67±45.81	<0.001
HOMA IR	2.70±1.98	3.26±2.01	4.13±2.86	<0.001
β Cell function index				
HOMA-β	37.88±16.66	33.41±14.73	41.73±24.01	0.860
CIR	18.26±7.93	17.38±9.78	16.17±6.55	0.171
Insulinogenic index	2.07±1.92	1.94±1.69	1.73±1.69	0.478

ISI, insulin sensitivity index; CIR, corrected incremental insulin response

Considering the close relationship between insulin resistance and BMI, correlation analysis was performed. As shown in fig. 2, the slopes of the correlation line were significantly steeper in AG and GG genotypes than in AA genotype (r=–0.504 in GG genotype, r=–0.430 in AG genotype, r=–0.340 in AA genotype), suggesting that the carriers of the Val allele were more insulin resistant at a lower BMI.

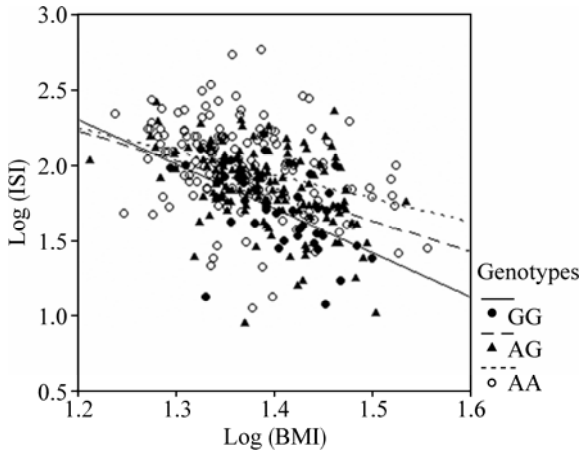

Fig. 2 The effect of genetic variants in the SUMO4 gene on the correlation between obesity and insulin resistance

2.3 Independent Correlation Factors for Insulin Resistance

Because insulin resistance is known to be affected by multiple independent factors, a multiple regression linear analysis was performed with the ISI as the dependent variable. The results revealed that SUMO4 Met55Val polymorphism was an independent determinant for insulin sensitivity (P=0.001), which, along with triglyceride, BMI and sex, could account for 20.1% of the variation in ISI (table 3). And no influence of total cholesterol, HDL cholesterol, LDL cholesterol, sex and age on HOMA-IR was observed. The correlation between SUMO4 Met55Val polymorphism and insulin sensitivity remained significant after adjusting for BMI (P=0.002) and sex (P=0.001).

Table 3 Stepwise regression analysis of the estimated indices for insulin sensitivity

Dependent variable	Covariate entered	Covariate removed	Adjusted r^2	P
ISI	TG		0.201	0.040
	BMI			0.003
	SUMO4 Met55Val Genotype			0.004
	Sex			0.009
		TC		0.681
		HDL-C		0.595
		LDL-C		0.353
		Age		0.189

3 DISCUSSION

The present study showed that SUMO4 Met55Val polymorphism was associated with insulin resistance in Chinese patients with newly diagnosed type 2 diabetes. The evidence arose from following aspects: 1) Insulin resistance indexes including fasting insulin levels and HOMA-IR were higher in GG and AG genotypes, while ISI was lower in GG and AG genotype. 2) SUMO4 Met55Val polymorphism was an independent predictor of insulin resistance; the result remained the same after adjusting for BMI and sex. 3) Carriers of the G allele were more insulin resistant at a lower BMI. 4) The G allele of the SUMO4 gene was associated with elevated serum triglycerides, which is a typical component of the metabolic syndrome related to insulin resistance. 5) The peak insulin secretion time during OGTT test in GG and AG genotypes was postponed as compared with AA genotype. But the present study did not support the relationship between SUMO4 Met55Val polymorphism and β cell dysfunction in patients with newly diagnosed type 2 diabetes. Noso *et al*[3] indicated that healthy people with G allele were more insulin resistant with fasting insulin concentration, HOMA-IR, insulin AUC/BMI and insulin AUC/glucose AUC tending to be higher.

Underlying mechanisms may contribute to the correlation between SUMO4 and insulin resistance, and insulin resistance in turn may be related to the chronic inflammatory state in type 2 diabetes. The activation of IKKβ and NF-κB can lead to insulin resistance[19], which may be related to some downstream cytokines or the

interactions with NF-κB[20], and inactivation of NF-κB may result in insulin sensitization[21]. SUMO4 was known to have a putative NF-κB binding motif within its promoter[22], thus exerting a negative effect on NF-κB transcriptional activity[1]. SUMO4 Met55Val substitution was observed to be associated with a much higher NF-κB transcriptional activity and a strong cellular response to stimulation[1]. Noso *et al*[3] indicated that under the stimulation of lipopolysaccharide, the peripheral blood mononuclear cells from inpatients with GG genotype could secrete more TNFα than patients with the other two genotypes[6]. SUMO4 can also suppress STAT1 DNA binding activity via direct sumoylation[23], which suggested the possibility of SUMO4 modification in JAK/STAT pathway contributing to the development of inflammation and/or insulin resistance[24]. A recently published paper showed that SUMO4 can interact with GAPDH via its ubiquitin domain under conditions of stress and obesity, indicating that the interaction between SUMO4 and GAPDH may affect insulin sensitivity regulation[25]. Additionally, SUMO4 sumoylation may play a role in the regulation of intracellular stress[26].

It is necessary to point out that SUMO4 may be involved with glucose metabolism, which in turn rationalizes the relationship between SUMO4 and insulin resistance. In our study, we found that newly diagnosed type 2 diabetic patients with G allele had higher HbA1c although the differences were not statistically significant. But the glucose levels at each time point during OGTT test were significantly higher in patients with G allele. Noso *et al*[6] and Lin *et al*[8] indicated type 2 diabetic patients with G allele were associated with higher HbA1c levels. Experimental study had demonstrated that the sumoylation of SUMO4 can be induced in an oxidative dependent way, and the substrates of SUMO4 include many proteins involved in glucose metabolism[26].

Limitations exist in this study, however. Firstly, the sample size and the proportion of GG genotype in type 2 diabetic patients were relatively small, so the results might need to be further verified in future studies with a larger sample size. Secondly, SUMO4 Met55Val variation seemed to have distinctive implication among different ethics. The relationship of SUMO4 Met55Val polymorphism with type 1 diabetes was more obvious in Asian populations than in others. To date, the relationship between SUMO4 Met55Val polymorphism and type 2 diabetes and the related characteristics were only observed in Asian population, which should be further confirmed in other populations. Finally, insulin resistance can predate the onset of type 2 diabetes by 10 to 20 years. It's also important to evaluate the relationship between SUMO4 Met55Val polymorphism and insulin resistance in people with pre-diabetes and people with high risk for diabetes.

In summary, we in this study evaluated the effects of the SUMO4 Met55Val polymorphism on insulin resistance in newly diagnosed type 2 diabetic patients and found that this polymorphism is a strong and independent indicator for insulin resistance. To our knowledge, this is the first report of a positive correlation between this polymorphism and insulin resistance in a Chinese population. Further studies involving more subjects are warranted to confirm our observation in other ethnic groups as well as in other Chinese populations. Sumoylation is an important posttranslational modification in numerous cellular activities. Further studies on the underlying mechanisms for SUMO4 in the pathogenesis of type 2 diabetes can provide a new insight into diabetes research.

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