Brief Report

SUMO4 M55V Variant Is Associated With Diabetic Nephropathy in Type 2 Diabetes

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OBJECTIVE—*SUMO4* mRNA was recently found to be mainly expressed in the kidney, and the methionine-to-valine substitution at codon 55 (M55V) variant of *SUMO4* may induce higher nuclear factor-κB (NF-κB) activity. Because NF-κB is known to mediate the development of diabetic nephropathy, we examined the association between the *SUMO4* M55V variant and the severity of diabetic nephropathy.

RESEARCH DESIGN AND METHODS—We recruited a total of 430 patients with type 2 diabetes. The M55V (rs237025, 163A→G) polymorphism of *SUMO4* was genotyped by real-time PCR, and urine albumin concentration was measured by radioimmunoassay.

RESULTS—The frequencies of *SUMO4* AA, GA, and GG were 52.6, 40.7, and 6.7%, respectively, in the normoalbuminuric group; 45.5, 47.3, and 7.1% in the microalbuminuric group; and 36.9, 46.2, and 16.9% in the macroalbuminuric group. We detected a significant linear trend for *SUMO4* genotype between the macroalbuminuric and normoalbuminuric groups. The mean urine albumin–to–creatinine ratio (42.3 ± 108.82 mg/mmol) in the GG group was significantly higher than in the AA (14.9 ± 51.49 mg/mmol) and GA (17.0 ± 43.74 mg/mmol) groups. Multivariate logistic regression analysis showed the *SUMO4* M55V variant to be independently associated with the severity of diabetic nephropathy.

CONCLUSIONS—This study indicates that the *SUMO4* gene M55V variant is associated with severity of diabetic nephropathy in patients with type 2 diabetes. ***Diabetes*** **56:1177–1180, 2007**

The pathogenesis of diabetic nephropathy, a leading cause of end-stage renal disease (1,2), appears to be multifactorial. Several epidemiological (3,4) and familial (4–6) studies have suggested that genetic susceptibility plays an important role in the development and progression of diabetic nephropathy.

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ACR, albumin-to-creatinine ratio; NF-κB, nuclear factor-κB.

Nuclear factor-κB (NF-κB), a transcription factor widely distributed in most cell types, including renal cells (7), can be activated by various molecules, such as high glucose and cytokines (8,9). High glucose is the main determinant of the development and progression of diabetic nephropathy, and some studies have demonstrated that high glucose levels can rapidly activate NF-κB in renal cells (10–12). Therefore, high-glucose–induced NF-κB activation may be involved in diabetic nephropathy.

The *SUMO4* gene, encoding small ubiquitin-like modifier 4, is a posttranscriptional modifier recently identified as a novel member of the SUMO family (13,14). Additionally, SUMO expression has been found to be mainly expressed in the kidneys and immune system (14). *SUMO4* can modify immune response through the putative substrate, inhibitor IκBα, a suppressor of NF-κB, and then regulate the activation of NF-κB (13). A common single nucleotide polymorphism encoding a methionine-to-valine substitution at codon 55 (M55V) has been recently identified (13,14). This M55V substitution can result in higher NF-κB transcriptional activity and greater expression of interleukin 12B (13). These findings prompted us to investigate the association of the *SUMO4* M55V variant with severity of diabetic nephropathy in type 2 diabetic patients.

RESEARCH DESIGN AND METHODS

A total of 430 patients with type 2 diabetes attending the Outpatient Department of Endocrinology and Metabolism at Kaohsiung Medical University Hospital were included in this study. Type 2 diabetes was defined based on 2003 American Diabetes Association criteria. Participants were >30 years old and did not need insulin injections, at least during the 1st year. Patients with known active infection, congestive heart failure, malignancy, gout, genitourinary tract infection, liver disease, and other systemic diseases were excluded. All patients underwent physical examination and laboratory tests. BMI was calculated as the weight in kilograms divided by the square of height in meters. Hypertension was defined as blood pressure >140/90 mmHg or use of antihypertensive medication. Hyperlipidemia was defined as plasma LDL cholesterol >2.6 mmol/l, plasma triglycerides >1.7 mmol/l, or use of antihyperlipidemic medication. Patients' renal status was assessed by measuring urinary albumin-to-creatinine ratio (ACR) for three consecutive urine collections. Severity of renal involvement was classified into *1*) normoalbuminuria (ACR <2.3 mg/mmol), *2*) microalbuminuria (at least two or more ACR values between 2.3 and 22.3 mg/mmol), and *3*) macroalbuminuria (ACR >22.3 mg/mmol). The Kaohsiung Medical University ethics committee approved this protocol, and all patients signed an informed consent form.

Laboratory analysis. Urine albumin concentration was measured by radioimmunoassay. The sensitivity limit for albumin measurement was 0.3 μg/ml. Intra- and interassay coefficients of variation were <5 and <7%, respectively. A1C was measured in whole blood using ion exchange high-performance liquid chromatography by the Bio-Rad Variant Hemoglobin Testing System (Bio-Rad Laboratories, Hercules, CA). Urine and blood creatinine, uric acid, triglyceride, total cholesterol, and LDL and HDL cholesterol levels were assayed using a biochemistry automatic analyzer (Beckman-Coulter, Fullerton, CA).

TABLE 1
Clinical and biochemical characteristics of type 2 diabetic patients according to their albuminuric status

	Normoalbuminuria	Microalbuminuria	Macroalbuminuria	*P*
n	253	112	65	
Sex (M/F)	131/122	42/70	34/31	0.052
Age (years)	58.4 ± 10.75	60.0 ± 12.78	61.9 ± 11.94	0.069
BMI (kg/m^2)	25.0 ± 3.57	25.3 ± 3.72	25.3 ± 3.48	0.654
Diabetes duration (years)	7.6 ± 6.17	9.0 ± 6.11	12.1 ± 8.37	<0.001*†
Systolic blood pressure (mmHg)	129.3 ± 15.13	132.0 ± 12.78	136.8 ± 14.13	0.001*
Diastolic blood pressure (mmHg)	80.2 ± 8.07	80.1 ± 7.36	80.9 ± 6.59	0.787
A1C (%)	7.50 ± 1.65	8.46 ± 2.00	8.30 ± 1.80	<0.001*‡
Plasma total cholesterol (mmol/l)	5.6 ± 1.12	5.6 ± 1.19	6.3 ± 1.70	<0.001*†
Plasma triglycerides (mmol/l)	2.0 ± 1.61	2.1 ± 1.67	2.7 ± 2.26	0.011*†
Plasma HDL cholesterol (mmol/l)	1.0 ± 0.30	1.0 ± 0.54	1.0 ± 0.29	0.995
Plasma LDL cholesterol (mmol/l)	3.7 ± 1.01	3.6 ± 1.09	4.1 ± 1.44	0.045†
Plasma uric acid (μmol/l)	345.0 ± 104.7	327.1 ± 99.93	416.4 ± 116.0	<0.001*†

Data are means ± SD. Comparisons were performed by ANOVA for continuous variables followed by Tukey's test and χ^2 test for categoric variables. *Macroalbuminuria vs. normoalbuminuria; †macroalbuminuria vs. microalbuminuria; ‡microalbuminuria vs. normoalbuminuria.

Genotyping. Genomic DNA was extracted from peripheral blood samples. *SUMO4* M55V variant was determined by allele-specific real-time PCR using Applied Biosystems 7900 real-time PCR system. The TaqMan probes were labeled one at the 5′ end with the fluorescent dye 6-carboxyfluorescein (FAM) and the other at the 5′ end with the fluorescent 50-flourescein (VIC). The following primers and probes were used: forward primer, 5′-GCCCACCAAA TCGGAA CTG- 3′ (corresponding to nucleotides 94201 through 94220 of *SUMO4* exon 1 DNA sequences; GenBank accession no. AL031133); reverse primer, 5′-GGCAGACACCAC TTAGTAAACTAATGAAA- 3′ (nucleotides 94287 through 94259 of *SUMO4* exon 1 DNA sequences; GenBank accession no. AL031133); probe 1 was 5′(FAM)-ATCTGCTTCATTGACAAT-(MGB)-3′ and probe 2 was 5′-(VIC)-ATCTGCTTCACTGACAAT-(MGB)-3′ (the C/T polymorphism is the antisense strand). The reaction mixture contained 2.5 μl of the 2X TaqMan Universal PCR MasterMix, 0.125 μl 40X SNP genotyping assay mix, 1 μl of DNA, and nuclease-free water up to 1.375 μl. Cycling times and temperatures were as follows: initial denaturation was carried out for 10 min at 95°C, followed by 40 cycles of denaturation at 92°C for 15 s and combined primer annealing/extension at 60°C for 1 min. Data were displayed using Sequence Detection System (Applied Biosystems).

Statistical analysis. All data were presented as means ± SD. Statistical analysis was performed using SPSS for Windows, version 8.1 (SPSS, Chicago, IL). Genotype and allele frequencies were compared with the Hardy-Weinberg equilibrium model using the Pearson χ^2 test. Pearson's χ^2 test was also used to assess the association of M55V variant with patients' renal status. Allelic and genotypic associations of the M55V variant found significant were evaluated by computing odds ratios and 95% CIs. Tests of linear trend for *SUMO4* genotypes were performed by assigning an ordinal variable to the genotypes in the logistic model. Comparison of patients stratified by albuminuric status or genotype groups was performed by one-way ANOVA for continuous variables and χ^2 test for discrete variables. Post hoc comparisons were made using Tukey's test. The independent relationships between diabetic nephropathy and continuous (duration of diabetes, A1C, and BMI) and categorical (hypertension, hyperlipidemia, *SUMO4* genotype, and smoking) parameters were tested by multivariate logistic regression. $P < 0.05$ was considered significant.

RESULTS

Table 1 shows that macroalbuminuric patients had a longer duration of diabetes and higher total cholesterol, triglyceride, and uric acid concentrations than either the normoalbuminuric or microalbuminuric subjects. These patients also had higher systolic blood pressure than those with normoalbuminuria and higher LDL cholesterol than those with microalbuminuria. The A1C value in both macroalbuminuric and microalbuminuric groups was higher than in normoalbuminuric patients.

The frequencies for AA, GA, and GG genotypes were significantly different in normoalbuminuric, microalbuminuric, and macroalbuminuric patients (Table 2). Additionally, the distribution of the G and A alleles was also significantly different among the three groups. After adjusting for A1C, we found a significant trend for *SUMO4* genotype between macroalbuminuric and normoalbuminuric groups. The odds ratio (95% CI) for GG and GA genotypes relative to the AA genotype were 3.263 (1.331–8.002) and 1.752 (0.953–3.222) between two groups.

As shown in Table 3, patients with a GG genotype were similar to patients with GA and AA genotypes with regard to age, duration of diabetes, BMI, lipid value, and creatinine value. However, they had significantly higher A1C than GA and AA genotypes and significantly higher dia-

TABLE 2
Genotype and allele frequencies of *SUMO4* polymorphism in Taiwanese type 2 diabetic patients stratified by albuminuric status

	Normo-albumin-uria	Micro-albumin-uria	Macro-albumin-uria	χ^2	*P*	Microalbuminuria vs. normoalbuminuria*	Macroalbuminuria vs. normoalbuminuria†	Macroalbuminuria vs. microalbuminuria‡
n	253	112	65					
Genotypes								
AA	133 (52.6)	51 (45.5)	24 (36.9)	10.506	0.033	1.00 (ref.)	1.00 (ref.)	1.00 (ref.)
GA	103 (40.7)	53 (47.3)	30 (46.2)			1.418 (0.879–2.285)	1.752 (0.953–3.222)	1.207 (0.623–2.338)
GG	17 (6.7)	8 (7.1)	11 (16.9)			0.977 (0.377–2.534)	3.263 (1.331–8.002)	3.002 (1.070–8.540)
Alleles								
A	369 (72.9)	155 (69.1)	78 (60)	8.321	0.016	1.00	1.00	1.00
G	137 (27.1)	69 (30.9)	52 (40)			1.164 (0.816–1.661)	1.795 (1.192–2.705)	1.511 (0.961–2.376)

Data are *n* (%) or adjusted odds ratio (95% CI). Odds ratios are adjusted for A1C level. *P for trend 0.390; †P for trend 0.006; ‡P for trend 0.068.

TABLE 3
Clinical and biochemical characteristics of type 2 diabetic patients classified by *SUMO4* M55V genotypes

	AA	GA	GG	*P*
n	208	186	36	
Sex (M/F)	106/102	100/86	17/19	0.732
Age (years)	59.2 ± 11.81	59.0 ± 11.21	61.4 ± 11.72	0.501
BMI (kg/m2)	25.4 ± 3.58	24.8 ± 3.66	25.1 ± 3.20	0.279
Diabetes duration (year)	8.2 ± 6.51	8.9 ± 6.97	10.2 ± 6.43	0.208
Systolic blood pressure (mmHg)	131.1 ± 13.56	130.0 ± 14.2	136.6 ± 17.72	0.046*
Diastolic blood pressure (mmHg)	80.0 ± 6.82	80.7 ± 8.32	80.4 ± 8.87	0.628
A1C (%)	7.7 ± 1.56	7.8 ± 1.58	8.5 ± 2.07	0.016*†
Plasma total cholesterol (mmol/l)	5.7 ± 1.33	5.8 ± 1.21	5.8 ± 1.16	0.937
Plasma triglycerides (mmol/l)	2.0 ± 1.56	2.2 ± 1.97	2.1 ± 1.61	0.617
Plasma HDL cholesterol (mmol/l)	1.0 ± 0.29	1.0 ± 0.47	1.0 ± 0.27	0.272
Plasma LDL cholesterol (mmol/l)	3.8 ± 1.13	3.8 ± 1.13	3.8 ± 1.07	0.912
Plasma uric acid (μmol/l)	356.9 ± 112.42	339.0 ± 102.31	392.6 ± 113.61	0.40*
Creatinine (μmol/l)	88.4 ± 30.94	85.7 ± 43.32	97.2 ± 33.59	0.156
ACR (mg/mmol)	14.9 ± 51.49	17.0 ± 43.74	42.3 ± 108.82	0.029*†

Data are means ± SD. Comparisons were performed by ANOVA for continuous variables followed by Tukey's test and by χ^2 test for categoric variables. *GG genotype vs. GA genotype; †GG genotype vs. AA genotype.

stolic blood pressure and uric acid levels than patients with the GA genotype. Finally, the GG genotype group had significantly higher urinary ACR (42.3 ± 108.82 mg/mmol) than either the AA group (14.9 ± 51.49 mg/mmol) or the GA group (17.0 ± 43.74 mg/mmol).

Multivariate logistic regression analysis showed that the genotypes of GG and GA were independently and significantly associated with diabetic nephropathy (Table 4). A1C, duration of diabetes, and BMI were also independently associated with degree of albuminuria.

DISCUSSION

This study found a significant association between the *SUMO4* M55V variant and the severity of diabetic nephropathy in patients with type 2 diabetes. In particular, this study showed that *1*) macroalbuminuric patients had a higher frequency of G alleles than normoalbuminuric and microalbuminuric patients, *2*) patients with GG genotypes had significantly more severe albuminuria, and *3*) the genotypes of GG and GA were independently associated with diabetic nephropathy.

It is now firmly established that NF-κB is involved in renal diseases (7). Similarly, NF-κB is also reported (7–12) to play an important role in the development of diabetic nephropathy. High glucose is known to be able to rapidly activate NF-κB and cytokines in renal cells (7–9). Recently, SUMO (small ubiquitin modifier) mRNA was found to be mainly expressed in the kidneys and immune system (14). Further evidence indicates that SUMO4 may conjugate to IκBα and negatively regulates NF-κB transcriptional activity (13), as well as enhances MnSOD expression by repressing AP (activator protein)-1 and -2α transcriptional activity (15). The *SUMO4* M55V substitution was reported to result in more NF-κB transcriptional activity and more expression of interleukin 12β (13). In this study, a higher proportion of type 2 diabetic patients carrying the G allele had macroalbuminuria than those carrying the A allele. Multivariate logistic regression analysis also showed that the genotypes GG and GA are independently associated with albuminuria in our diabetic patients. These findings implicated that there might be a link between the development of diabetic nephropathy and *SUMO4* gene polymorphism in Taiwanese patients with type 2 diabetes.

TABLE 4
Multivariate logistic analysis of risk factors associated with diabetic nephropathy in type 2 diabetic patients

	P	Odds ratio (95% CI)
SUMO*	0.029	1.650 (1.053–2.586)
Diabetes duration (years)	0.001	1.063 (1.026–1.102)
Hypertension (yes/no)	0.150	1.401 (0.885–2.217)
Hyperlipidemia (yes/no)	0.273	1.424 (0.757–2.679)
A1C (%)	0.000	1.328 (1.166–1.512)
Smoke (yes/no)	0.624	1.154 (0.650–2.049)
BMI (kg/m^2)	0.036	1.072 (1.005–1.145)

*GG and GA vs. AA.

The present study found macroalbuminuric patients to have significantly higher levels of uric acid than either microalbuminuric or normoalbuminuric patients. Other studies have also reported serum uric acid level to be independently correlated with urinary ACR (16,17). Generally, elevation of uric acid level is recognized as a consequence of impaired renal function (18), although uric acid may be harmful to the kidneys (16). Notably, patients with *SUMO4* GG genotype had significantly higher uric acid levels and also higher ACR levels than patients with GA genotypes. The simultaneous increases of ACR and uric acid levels in patients with GG genotype provide additional evidence associating the *SUMO4* GG genotype with severity of diabetic nephropathy.

Some investigators indicate the NF-κB pathway is related to the development of type 1 diabetes (19), and other studies (13,20,21) have found a significant association of *SUMO4* M55V gene variant with type 1 diabetes in Asian populations. In contrast, several studies have found no such association in European subjects (22,23), and even an opposite association in British individuals was reported (14). The reasons for these discrepancies could be genetic heterogeneity and gene-environment interaction in different ethic populations. In studies from Asian populations, the frequencies of *SUMO4* AA, GA, and GG have been reported to be 49, 41, and 10% in control subjects and 39, 49, and 12%, respectively, in patients with type 1 diabetes (13,20,21). In this study, the frequencies of *SUMO4* AA, GA, and GG in our type 2 diabetic patients were 48, 44, and 8%, respectively, similar to the above-mentioned studies in

Asia (13,20,21) but different from Caucasian studies (13,22). Therefore, our study has some limitations. The sample size was small, and the validity to extrapolate the association between the *SUMO4* gene variant and severity of diabetic nephropathy to other ethic populations requires further confirmation.

In conclusion, this study indicates that M55V polymorphism of *SUMO4* gene is associated with kidney dysfunction in Taiwanese patients with type 2 diabetes.

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