Association of a 27-bp Repeat Polymorphism in Intron 4 of Endothelial Constitutive Nitric Oxide Synthase Gene With Serum Uric Acid Levels in Chinese Subjects With Type 2 Diabetes

Yau-Jiunn Lee, Dao-Ming Chang, and Jack C.R. Tsai

Nitric oxide (NO) was found to modulate uric acid production through its influence on xanthine oxidase activity, and a close circadian relationship of serum uric acid (SUA) and NO was reported. Studies also revealed that serum NO activity could be determined by endothelial constitutive nitric oxide synthase gene (ecNOS) polymorphism. This study was designed to investigate whether SUA could be influenced by a 27-bp repeat polymorphism in intron 4 of ecNOS gene. A total of 398 nondiabetic subjects and 800 patients with type 2 diabetes were studied. The ecNOS gene intron 4 polymorphism was determined by polymerase chain reaction (PCR). The mean SUA level of patients having type 2 diabetes was significantly lower than that of control subjects (6.1 ± 1.8 mg/dL *v* 6.6 ± 1.8 mg/dL, $P < .001$); and the mean SUA level of diabetic patients with ecNOS ab/aa genotypes was lower than that of patients with bb genotype (5.7 ± 1.6 mg/dL *v* 6.2 ± 1.8 mg/dL, $P = .008$). When subgrouped by gender, the SUA of female diabetic subjects was found to be significantly associated with ecNOS genotype. Using Pearson's correlation analysis and multiple linear regression analysis, ecNOS genotype was noticed to be an independent factor in contributing to SUA variability in female diabetic patients. Our results suggest that SUA levels may be associated with NO activity and can be genetically predetermined.

THE ROLE OF serum uric acid (SUA) as an independent risk factor of cardiovascular disease has been cause for debate for nearly 50 years. However, recent medical reports present ever increasing evidence that supports the theory.[1,2] Even though precise mechanisms remain uncertain, altered SUA levels are associated with metabolic derangement (ie, hypertension, insulin resistance, dyslipidemia, obesity, aging, renal disease, and menopause),[3-7] suggesting an epiphenomenon, or a regulation mechanism of uric acid production. Interestingly, almost all of the factors correlated to increased SUA levels have been shown to either reduce vascular nitric oxide (NO) production and/or increase NO destruction.[8]

Researchers generally agree that vascular NO activity may possibly affect enzymes involved in uric acid metabolism.[9-11] Yet, vascular NO activity may be regulated by xanthine oxidase, the rate-limiting enzyme of uric acid production,[12] through the generation of superoxide anion radicle (O_2^-). Recently, Mazzali et al reported increased blood pressure readings in rats whose uric acid levels had been altered by means of a crystal-independent mechanism, and with the stimulation of renin-angiotensin system and the inhibition of neuronal NO synthase.[13] Other studies have reported a close circadian relationship of SUA and NO in healthy male subjects,[14] and the close link between SUA and vascular NO activity in individuals with cardiovascular disease.[15] These facts suggest a close relationship between SUA levels and NO activity. Moreover, studies also indicate that serum NO activity may be genetically linked to endothelial constitutive nitric oxide synthase gene (ecNOS) polymorphism.[16] This report tests the hypothesis that SUA is genetically predetermined by ecNOS genotypes, and that it is specifically linked to ecNOS gene intron 4 polymorphism.

MATERIALS AND METHODS

Subjects

The target study group included 800 outpatients diagnosed with type 2 diabetes who regularly attended the Diabetic Clinic of Pingtung Christian Hospital from February 1998 to December 2001; diagnosis was based on World Health Organization (WHO) criteria.[17] Three hundred ninety-eighty subjects showing no clinical signs of diabetes were randomly recruited to participate as control group subjects. The definition of nondiabetic is subjects who have fasting plasma glucose level lower than 126 mg/dL and no family history (including parents, siblings, and children) of type 2 diabetes. All participants provided comprehensive medical history, including smoking behavior; all were of Han Chinese ethnic origins without any known ancestors of other ethnic backgrounds, and all lived within the same geographic region at the time of study.[18] This study was approved by the human research ethics committee of our hospital, and informed consent was obtained from each patient.

All patients underwent complete physical examinations and routine biochemical analyses of blood and urine, as well as an assessment for the presence and extent of macrovascular or microvascular diabetic complications. The anthropometric parameters required to calculate body mass index (BMI) and waist-to-hip ratio (WHR) were measured. Seated blood pressure, plasma biochemical parameters, and urinary microalbumin were measured after overnight fasting. Plasma triglycerides, total, low-density lipoprotein (LDL)- and high-density lipoprotein (HDL)-cholesterol, uric acid, creatinine, and glucose were determined by standard commercial methods on a parallel-multichannel analyzer (Hitachi 7170A, Tokyo, Japan). Urinary albumin concentrations were measured by immunoturbidmetry (Beckman Instruments, Galway, Ireland). Persons showing urinary tract infection, urolithiasis, and other renal disorders, and those taking allopurinol, diuretics, or angiotensin II type 1 receptor antagonist were excluded from the control group.

From the Department of Clinical Research, Pingtung Christian Hospital, Pingtung, Taiwan.

Submitted January 28, 2003; accepted April 22, 2003.

Supported by a grant from National Science Council of Taiwan (91-2314-B-475-001).

Address reprint requests to Yau-Jiunn Lee, MD, Department of Clinical Research, Pingtung Christian Hospital, No. 60 Da-Lien Rd, Pingtung 90000, Taiwan.

0026-0495/03/5211-0045$30.00/0

doi:10.1016/S0026-0495(03)00258-0

Endothelial Nitric Oxide Synthase Gene Polymorphism

Genomic DNA was prepared from peripheral blood using standard techniques. A 27-bp repeat sequence in intron 4 of ecNOS gene was determined by polymerase chain reaction (PCR) analysis using primers and protocol detailed by Wang et al.[19] PCR reactions were performed in a volume of 25 μL containing 200 ng of DNA, 1.5 mmol/L $MgCl_2$, 200 μmol/L dinucleotide triphosphates mix, 1 μmol of each primer, and 0.8 U Amplitag polymerase (Takara, Shiga, Japan) in a buffer supplied by the manufacturer. After denaturation at 94°C for 10 minutes, PCR temperature cycling (at 94°C for 40 seconds, 64°C for 40 seconds, and 72°C for 50 seconds) was repeated for 40 cycles followed by extension at 72°C for 5 minutes. The primers used were 5′-AGGCCCTATGGTAGTGCCTTT-3′ and 5′-TCTCTTAGTGCTGTGGTCAC-3′. The PCR product was directly separated by electrophorersis in 5% nondenaturing polyacrylamide gel and visualized in UV light after ethidium bromide staining for 30 minutes.

Statistical Analysis

Data are presented as the mean ± SD. Statistical analysis was calculated using the Statistical Package for Social Science software (SPSS for Windows, version 7.5.1, 1996, SPSS Inc, Chicago IL); differences in genotype distribution and allele frequencies among the group members were assessed according to Pearson chi-square test. Due to the limited number of individuals possessing the aa genotype, tests to determine relevance between subjects with and without the a variant (bb *v* ba and aa) were made. Prior to statistical analysis, fasting level plasma glucose and serum triglycerides were logarithmically transformed to achieve normal distribution. One-way analysis of variance (ANOVA) was used for testing the differences of 3 ecNOS genotypes and by post hoc analysis using Dunnett's 2-tailed test; unpaired *t* test was used to compared the differences between patients with different alleles (with or without a allele). Correlation analysis was made using Pearson's correlation coefficients. A multiple linear regression model was used to study the relationship of SUA and to assess the influence of independent variables (ie, sex, age, diabetes duration, hemoglobin A_{1c} [HbA_{1c}], serum total cholesterol, serum LDL-cholesterol, serum HDL-cholesterol, triglycerides, BMI, WHR, ecNOS genotypes, and presence of hypertension or albuminuria). Appropriateness of regression models was judged using the Durbin-Watson statistic and partial plots of the residuals. Differences with a *P* value less than .05 were considered significant and relevant to statistical interpretation.

RESULTS

Table 1 presents the clinical characteristics of the study subjects. Compared to the control group, diabetic patients exhibit significantly higher levels of BMI, WHR, fasting level plasma glucose, blood pressure, total cholesterol, and triglycerides, and lower levels of HDL-cholesterol and uric acid.

The ecNOS 27-bp repeat polymorphism of intron 4 distributions within the study groups are presented in Tables 1 and 2. The genotype frequency distributions of this polymorphism were in Hardy-Weinberg equilibrium in the study groups. There were no differences in ecNOS genotype distribution and allele frequency between patients with type 2 diabetes mellitus and control subjects. Table 1 shows the clinical biochemical variables and anthropometric parameters of different ecNOS genotypes within the study groups. Diabetic subjects with the ecNOS a allele had a significantly lower SUA level (bb: ab/aa = 6.2 ± 1.8 mg/dL *v* 5.7 ± 1.6 mg/dL, *P* = .008), while there were no significant differences in other variables in diabetic subjects with different ecNOS genotypes. There was a marginal statistical significance (*P* = .052) between the uric acid levels of subjects with or without ecNOS a allele in the whole population studied. No statistically significant differences were found in the variables between the control subjects with different ecNOS genotypes (Table 1).

When subgrouped by gender, data concerning female diabetics showed significant links between ecNOS genotypes and

Table 1. Clinical Characteristics of Control Subjects and Patients With Type 2 Diabetes With Different ecNOS Genotypes

	Nondiabetic Subjects			Type 2 Diabetic Subjects			All Subjects			*P* Value		
Parameter	Total	bb	ab/aa	Total	bb	ab/aa	Total	bb	ab/aa	DM *v* Control	Type 2 DM bb *v* ab/aa	All Subjects bb *v* ab/aa
No.	398	340	57/1	800	674	112/14	1,198	1,014	169/15	.089		
Age (yr)	61.8 ± 12.5	61.4 ± 12.7	64.0 ± 11.0	61.7 ± 10.7	61.7 ± 10.5	62.8 ± 9.9	61.7 ± 11.2	61.6 ± 11.3	63.1 ± 10.2	.96	.292	.11
Gender (M/F)	283/115	243/87	40/18	365/435	309/365	56/70	648/550	552/452	96/88			
BMI	24.6 ± 3.7	24.7 ± 3.8	24.3 ± 3.3	25.4 ± 3.6	25.4 ± 3.5	25.5 ± 3.5	25.2 ± 3.7	25.2 ± 3.6	25.2 ± 3.8	<.001	.819	.376
WHR	0.93 ± 0.07	0.93 ± 0.07	0.93 ± 0.07	0.96 ± 0.07	0.96 ± 0.07	0.95 ± 0.07	0.96 ± 0.07	0.96 ± 0.07	0.95 ± 0.07	<.001	.166	.222
Fasting glucose (mg/dL)	99 ± 12	99 ± 13	101 ± 12	185 ± 69	185 ± 70	184 ± 77	164 ± 70	165 ± 70	162 ± 75	<.001	.993	.599
Systolic BP (mm Hg)	138 ± 19	138 ± 19	137 ± 20	143 ± 21	142 ± 21	144 ± 20	141 ± 20	141 ± 20	142 ± 20	<.001	.377	.489
Diastolic BP (mm Hg)	83 ± 12	83 ± 12	81 ± 12	87 ± 13	87 ± 13	87 ± 13	86 ± 13	86 ± 13	86 ± 13	<.001	.755	.928
Total cholesterol (mg/dL)	194 ± 40	196 ± 41	185 ± 36	200 ± 44	198 ± 42	200 ± 42	198 ± 43	197 ± 42	195 ± 41	.044	.566	.65
Triglyceride (mg/dL)	139 ± 90	142 ± 94	120 ± 59	177 ± 144	169 ± 133	191 ± 167	167 ± 133	160 ± 122	169 ± 147	<.001	.115	.389
HDL-cholesterol (mg/dL)	46 ± 13	46 ± 13	47 ± 11	44 ± 19	44 ± 20	45 ± 15	46 ± 15	45 ± 16	46 ± 12	.009	.587	.478
LDL-cholesterol (mg/dL)	122 ± 35	123 ± 35	112 ± 32	122 ± 35	121 ± 33	125 ± 32	122 ± 35	122 ± 34	121 ± 33	.754	.289	.908
Creatinine (mg/dL)	1.2 ± 0.4	1.2 ± 0.4	1.2 ± 0.3	1.2 ± 0.7	1.2 ± 0.8	1.1 ± 0.5	1.2 ± 0.7	1.2 ± 0.7	1.1 ± 0.5	.762	.401	.526
Uric acid (mg/dL)	6.6 ± 1.8	6.6 ± 1.8	6.8 ± 1.3	6.1 ± 1.8	6.2 ± 1.8	5.7 ± 1.6	6.2 ± 1.8	6.3 ± 1.8	6.0 ± 1.6	<.001	.008	.052

NOTE. Data are expressed as means ± SD. Comparisons were performed by unpaired *t* test or chi-square test.

Abbreviations: DM, diabetes mellitus; BMI, body mass index; WHR, waist-to-hip ratio; BP, blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein

Table 2. Clinical Characteristics of Subjects With Type 2 Diabetes With Different ecNOS Genotypes

Parameter	All						Female						Male					
	Total	bb	ab	aa	*P*-1*	*P*-2†	Total	bb	ab	aa	*P*-1*	*P*-2†	Total	bb	ab	aa	*P*-1*	*P*-2†
No.	800	674	112	14			435	365	62	8			365	309	50	6		
Age (yr)	61.7 ± 10.7	61.7 ± 10.5	60.1 ± 9.7	68.6 ± 11.0	.02	NS	62.2 ± 10	61.9 ± 10	63.3 ± 9.6	72.4 ± 9.9	.005	NS	61.4 ± 10.9	61.5 ± 11.1	60.5 ± 9.3	63.7 ± 11.2	NS	NS
BMI	25.4 ± 3.6	25.4 ± 3.5	25.4 ± 3.4	25.9 ± 3.9	NS	NS	25.8 ± 3.8	25.9 ± 3.8	25.2 ± 3.7	27.2 ± 3.6	NS	NS	25.0 ± 3.0	24.9 ± 2.9	25.8 ± 3.0	23.8 ± 3.8	NS	NS
WHR	0.96 ± 0.07	0.96 ± 0.07	0.96 ± 0.07	0.95 ± 0.05	NS	NS	0.97 ± 0.07	0.97 ± 0.07	0.96 ± 0.08	0.93 ± 0.05	NS	NS	0.95 ± 0.06	0.96 ± 0.06	0.94 ± 0.05	0.97 ± 0.05	NS	NS
Age of DM onset (yr)	52.5 ± 10.9	52.1 ± 10.9	54.1 ± 10.2	54.6 ± 13.6	NS	NS	52.6 ± 10.6	52.1 ± 10.5	54.4 ± 10.0	58.8 ± 13.8	NS	.04	52.3 ± 11.2	52.1 ± 11.3	53.8 ± 10.6	49.0 ± 12.4	NS	NS
Known diabetes duration (yr)	7.1 ± 6.5	7.2 ± 6.6	6.6 ± 6.3	7.1 ± 5.8	NS	NS	7.3 ± 6.8	7.4 ± 6.8	7.0 ± 7.0	6.8 ± 6.7	NS	NS	6.9 ± 6.1	7.0 ± 6.2	5.8 ± 5.0	7.7 ± 5.0	NS	NS
HbA_{1C} (%)	8.4 ± 2.2	8.4 ± 2.2	8.4 ± 2.2	8.4 ± 2.0	NS	NS	8.6 ± 2.2	8.6 ± 2.2	8.7 ± 2.2	7.5 ± 1.6	NS	NS	8.2 ± 2.2	8.2 ± 2.3	8.1 ± 2.1	9.7 ± 1.8	NS	NS
Fasting glucose (mg/dL)	185 ± 69	185 ± 70	184 ± 78	185 ± 78	NS	NS	185 ± 68	185 ± 67	190 ± 72	162 ± 81	NS	NS	184 ± 74	184 ± 72	177 ± 83	218 ± 69	NS	NS
Systolic BP (mm Hg)	143 ± 21	142 ± 21	144 ± 21	144 ± 15	NS	NS	144 ± 22	144 ± 22	146 ± 22	143 ± 20	NS	NS	140 ± 20	140 ± 20	140 ± 19	146 ± 8	NS	NS
Diastolic BP (mm Hg)	87 ± 13	87 ± 13	88 ± 13	86 ± 13	NS	NS	88 ± 13	88 ± 13	88 ± 14	85 ± 12	NS	NS	87 ± 13	86 ± 13	87 ± 12	89 ± 15	NS	NS
Total cholesterol (mg/dL)	200 ± 44	198 ± 42	199 ± 43	206 ± 41	NS	NS	202 ± 42	202 ± 42	202 ± 42	201 ± 40	NS	NS	193 ± 43	192 ± 42	195 ± 44	213 ± 44	NS	NS
Triglyceride (mg/dL)	177 ± 144	169 ± 133	187 ± 142	232 ± 308	NS	NS	164 ± 104	161 ± 98	187 ± 137	143 ± 77	NS	NS	183 ± 171	179 ± 164	183 ± 149	350 ± 457	NS	NS
HDL-cholesterol (mg/dL)	46 ± 13	46 ± 13	46 ± 11	49 ± 11	NS	NS	50 ± 14	50 ± 14	48 ± 12	51 ± 13	NS	NS	42 ± 11	41 ± 11	45 ± 10	46 ± 7	NS	NS
LDL-cholesterol (mg/dL)	122 ± 35	121 ± 33	124 ± 32	126 ± 33	NS	NS	125 ± 34	124 ± 34	126 ± 32	135 ± 39	NS	NS	118 ± 32	117 ± 32	121 ± 33	113 ± 20	NS	NS
Creatinine (mg/dL)	1.2 ± 0.7	1.2 ± 0.8	1.1 ± 0.5	1.1 ± 0.3	NS	NS	1.1 ± 0.8	1.1 ± 0.9	1.0 ± 0.5	1.0 ± 0.4	NS	NS	1.3 ± 0.7	1.3 ± 0.8	1.2 ± 0.4	1.1 ± 0.2	NS	NS
Uric acid (mg/dL)	6.1 ± 1.8	6.2 ± 1.8	5.9 ± 1.7	5.2 ± 1.5	.044	.008	5.7 ± 1.7	5.8 ± 1.8	5.4 ± 1.5	4.6 ± 1.5	.041	.02	6.5 ± 1.7	6.6 ± 1.7	6.3 ± 1.7	5.9 ± 1.0	NS	NS

NOTE. Data are expressed as means ± SD. Comparisons were performed by ANOVA for continuous variables or chi-square test for categorical variables.
Abbreviation: NS, not significant.
*bb *v* aa group by ANOVA and post hoc analysis by Dunnett 2 tailed test.
†bb *v* ab/aa group by unpaired *t* test.

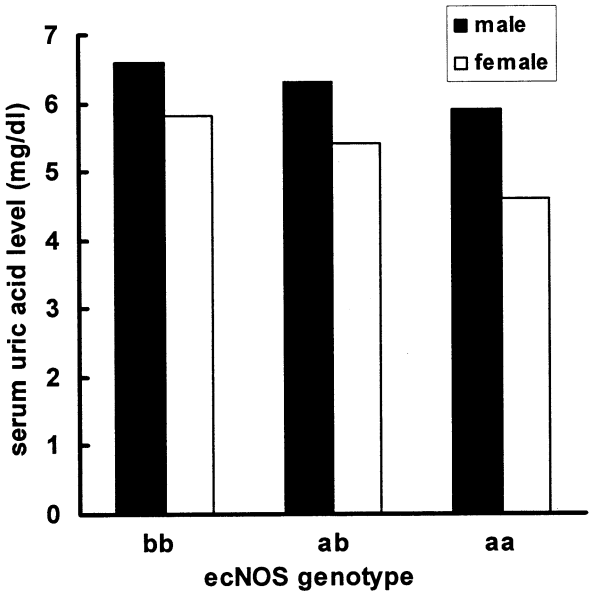

Fig 1. SUA levels in patients with type 2 diabetes. SUA levels were significantly lower in female patients with the aa genotype when compared to subjects with the bb genotype.

SUA (Fig 1 and Table 2). SUA levels for diabetic subjects exhibited univariate multicollinearity with several biochemical and physical factors (Table 3). In addition to age, gender, and BMI, SUA was closely correlated with serum creatinine, HDL-cholesterol, diastolic and systolic blood pressure, total cholesterol, known diabetic duration, triglycerides, and LDL-cholesterol. According to multiple linear stepwise regression analysis, the ecNOS genotype continued to remain a significant factor ($P = .005$; 95% confidence interval [CI], −.643∼−.115) with 7 variables present in patients with type 2 diabetes. In diabetic females, ecNOS genotype was found to be an independent contributing factor to SUA levels ($P = .003$; 95% CI, −.873∼−.186); this was not the case for male subjects. SUA levels in female control subjects did not differ statistically from that of male counterparts (6.4 ± 1.6 mg/dL *v* 6.7 ± 1.8 mg/dL, $P > .05$). An association between the ecNOS genotypes and SUA levels in female control subjects could not be made (results not shown).

DISCUSSION

Results show that SUA levels correlated with a 27-bp repeat polymorphism in intron 4 of the ecNOS gene in female subjects with type 2 diabetes. To the best of our knowledge, this is the first report to demonstrate that SUA levels may be genetically predetermined by ecNOS gene polymorphism. NO is known to interact with both the flavin prosthetic site and the molybdenum ion at the active site of xanthine oxidase, thereby interfering with its activity or the production of a desulfo-type inactive enzyme.[10] Subjects with the ecNOS intron 4 aa genotype were reported to have higher vascular NO activity,[16] thus suppressing uric acid production through the inhibition of xanthine oxidase. These facts may explain the relationship between SUA levels and ecNOS gene polymorphism as presented in this study.

SUA level is influenced by many factors, including food, aging, menopause, gender, obesity, dyslipidemia, insulin resistance state, blood pressure, renal function, sedentary lifestyle, and the intake of alcohol, diuretics, and/or drugs for hyperten-

Table 3. Correlation of Serum Uric Acid Level With Clinical Characteristics in Patients With Type 2 Diabetes

	All (N = 800)				Female (n = 435)				Male (n = 365)			
Variable	Coefficient	*P* Value*	*P* Value†	95% CI	Coefficient	*P* Value*	*P* Value†	95% CI	Coefficient	*P* Value*	*P* Value†	95% CI
Creatinine	0.103	.001	<.001	1.197∼1.721	0.28	<.001	<.001	1.224∼1.921	0.373	<.001	<.001	.929∼1.734
BMI	0.136	<.001	<.001	.041∼.110	0.182	<.001	<.001	.032∼.114	0.16	.001	<.001	.054∼.173
HDL-cholesterol	−0.213	<.001	<.001	−.028∼−.009	−0.160	<.001			−0.153	.002		
Diastolic BP	0.090	.005	<.001	.007∼.025	0.113	.010	.001	.008∼.032	0.091	.055		
Gender	−0.202	<.001	.004	−.622∼−.119	—	—			—	—		
ecNOS genotype	−0.092	.011	.005	−.643∼−.115	−0.127	.009	.003	−.873∼−.186	−0.077	.152		
Total cholesterol	0.042	.189	.015	.001∼−.007	0.022	.611			0.132	.005	.028	.001∼.009
HbA_{1c}	−0.134	<.001	.031	−.114∼−.005	−0.082	.063			−0.158	.001		
Age	0.103	.001			0.136	.002			0.103	.027		
WHR	0.045	.184			0.055	.232			0.095	.061		
Known diabetes duration	0.139	<.001			0.126	.005			0.165	.001		
Age of DM onset	−0.027	.420			0.011	.815			−0.048	.353		
Systolic BP	0.159	<.001			0.171	<.001			0.202	<.001		
Triglycerides	0.133	<.001			0.152	<.001	.004	.001∼.004	0.099	.033		
LDL-cholesterol	−0.213	<.001			−0.029	.523			0.147	.003		
Fasting glucose	−0.019	.558			0.023	.593			−0.054	.253		

*By Pearson's ccorrelation test.

†By multiple linear stepwise correlation test.

sion.[3-7,20] Almost all of these factors were associated with increased SUA levels. Interestingly, these same factors have been shown to either reduce NO production and/or increase NO destruction.[8] Many were characterized in the present study; however, results measured by multiple linear regression analysis indicate that the ecNOS intron 4 genotype acts as an independent contributor to SUA levels (Table 3). Even though the influence of the ecNOS genotype SUA variability measured only 2% ($P = .003$), we maintain the opinion that physiologic or pathophysiological associations are still implied.

The recognition of SUA as a risk factor in cardiovascular disease has been debated for nearly 50 years. Virtually all studies have shown that higher uric acid levels are associated with increased cardiovascular risk,[1,2] and an elevated SUA level has been found to be a strong predictor of cerebrovascular disease.[21,22] Furthermore, the association between SUA levels and cardiovascular diseases in patients with type 2 diabetes has also been noted.[2,23] The observed association between uric acid levels and cardiovascular disease may represent an epiphenomenona, reflecting the complex interaction between uric acid and other risk factors. Recently, several plausible explanations for the mechanism whereby uric acid directly affects atherosclerosis and/or on the clinical course of cardiovascular disease have been presented.[1] Maxwell and Bruinsma[15] reported that SUA level was second only to age in correlation with flow-mediated brachial artery vasodilation (FMV), accounting for 7% of the variability in FMV; SUA levels and FMV were related to the degree of cardiovascular disease. They concluded that the association of elevated SUA with cardiovascular disease may be a consequence of impaired vascular NO activity. Additionally, a close circadian relationship between SUA and serum NO was reported in healthy males.[14] Taken together with the role that vascular NO activity plays in the progression of cardiovascular diseases,[24] the current results support the hypothesis that SUA is regulated by NO activity and may explain the mechanism for SUA levels as factors in cardiovascular diseases.

SUA in patients with type 2 diabetes has been reported to be lower, normal, or higher than in control subjects.[25-27] In general, decreased SUA was observed in these patients and elevated SUA could be associated with impaired renal function, severe obesity, elevated blood pressure, dyslipidemia, male gender, and poor glycemic control.[28] The mean SUA level of female diabetic subjects (5.7 $\pm$ 1.7 mg/dL) was lower when compared to the overall control group (6.6 $\pm$ 1.8 mg/dL), control female subjects (6.4 $\pm$ 1.6 mg/dL), and male diabetic patients (6.5 $\pm$ 1.7 mg/dL). In fact, female diabetic patients showed the lowest SUA levels, demonstrating a significant correlation between SUA and ecNOS genotypes as observed in these subjects only. This implies that in subjects with a tendency for low SUA, NO may have had a regulatory effect on the generation of SUA. In those with a tendency towards elevated SUA, such as male subjects, affects of NO on uric acid production may have been countered and therefore statistical significance was lost.

In conclusion, this report reveals that SUA levels in female diabetic patients are associated with the ecNOS intron 4 polymorphism, and supports the theory that NO has a regulatory effect on uric acid production.

ACKNOWLEDGMENT

We are grateful to Yi-Su Chung for her excellent technical assistance and to the staff of the clinical and nutrition diabetes section for their assistance in various measurements and other organizational aspects of this study.

REFERENCES

1. Rich MW: Uric acid: Is it a risk factor for cardiovascular disease? Am J Cardiol 85:1018-1021, 2000

2. Alderman MH: Uric acid and cardiovascular risk. Curr Opin Pharmacol 2:126-130, 2002

3. Burack RC, Keller JB, Higgins MW: Cardiovascular risk factors and obesity: Are baseline levels of blood pressure, glucose, cholesterol and uric acid elevated prior to weight gain? J Chronic Dis 38:865-872, 1985

4. Benedek TG: Correlations of serum uric acid and lipid concentrations in normal, gouty, and atherosclerotic men. Ann Intern Med 66:851-861, 1967

5. Alderman MH: Uric acid in hypertension and cardiovascular disease. Can J Cardiol 15:20F22F, 1999 (suppl F)

6. Bengtsson C, Tibblin E: Serum uric acid levels in women: An epidemiological survey with special reference to women with high serum uric acid values. Acta Med Scand 196:93-102, 1974

7. Rathmann W, Funkhouser E, Dyer AR, et al: Relations of hyperuricemia with the various components of the insulin resistance syndrome in young black and white adults: The CARDIA Study. Am Epidemiol 8:250-261, 1998

8. Maxwell AJ, Tao PS, Cooke JP: Modulation of the nitric oxide synthase pathway in atherosclerosis. Exp Physiol 83:573-584, 1998

9. Houston M, Chumley P, Radi R, et al: Xanthine oxidase reaction with nitric oxide and peroxynitrite. Arch Biochem Biophys 355:1-8, 1998

10. Lee C, Liu X, Zweier JL: Regulation of xanthine oxidase by nitric oxide and peroxynitrite. J Biol Chem 275:9369-9376, 2000

11. Cote CG, Yu FS, Zulueta JJ, et al: Regulation of intracellular xanthine oxidase by endothelial-derived nitric oxide. Am J Physiol 271:L869-L874, 1996

12. Miyamoto Y, Akaike T, Yoshida M, et al: Potentiation of nitric oxide-mediated vasorelaxation by xanthine oxidase inhibitors. Proc Soc Exp Biol Med 211:366-373, 1996

13. Mazzali M, Hughes J, Kim YG, et al: Elevated uric acid increased blood pressure in the rat by a novel crystal-independent mechanism. Hypertension 38:1101-1106, 2001

14. Kanabrocki EL, Third JL, Ryan MD, et al: Circadian relationship of serum uric acid and nitric oxide. JAMA 283:2240-2241, 2000

15. Maxwell AJ, Bruinsma KA: Uric acid is closely linked to vascular nitric oxide activity: Evidence for mechanism of association with cardiovascular disease. J Am Coll Cardiol 38:1850-1858, 2001

16. Wang XL, Mahaney MC, Sim AS, et al: Genetic contribution of the endothelial constitutive nitric oxide synthase gene to plasma nitric oxide levels. Arterioscler Thromb Vasc Biol 17:147-153, 1997

17. Alberti KG, Zimmet PZ: Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: Diagnosis and classification of diabetes mellitus, provisional report of a WHO consultation. Diabet Med 15:539-553, 1998

18. Lee YJ, Tsai JC: Angiotensin converting enzyme gene insertion/deletion polymorphism associated with 1998 World Health Organiza-

tion definition of metabolic syndrome in Chinese with type 2 diabetic patients. Diabetes Care 25:1002-1008, 2002

19. Wang XL, Sim AS, Badenhop RF, et al: A smoking-dependent risk of coronary artery disease associated with a polymorphism of the endothelial nitric oxide synthase gene. Nature Med 2:41-45, 1996

20. Nakashima M, Uematsu T, Kosuge K, et al: Pilot study of uricosuric effect of DuP-753, a new angiotensin II receptor antagonist, in healthy subjects. Eur J Clin Pharmacol 42:333-335, 1992

21. Seghieri G, Moruzzo D, Fascetti S, et al: Increase in serum uric acid is selectively associated with stroke in type 2 diabetes. Diabetes Care 25:1095, 2002

22. Lehto S, Niskanem L, Ronnemaa T, et al: Serum uric acid is a strong predictor of stroke in patients with non-insulin-dependent diabetes mellitus. Stroke 29:635-639, 1998

23. Rathmann W, Hauner H, Dannehl K, et al: Association of elevated serum uric acid with coronary heart disease in diabetes mellitus. Diabet Metab 19:159-166, 1993

24. Kinlay S, Libby P, Ganz P: Endothelial function and coronary artery disease. Curr Opin Lipidol 12:383-389, 2001

25. Bo S, Cavallo-Perin P, Gentile L, et al: Hypouricemia and hyperuricemia in type 2 diabetes: Two different phenotypes. Eur J Clin Invest 31:318-321, 2001

26. Wun YT, Chan CS, Lui CS: Hyperuricaemia in type 2 diabetes mellitus. Diabetes Nutr Metab 12:286-291, 1999

27. Ishihara M, Shinoda T, Yamada T: Co-occurrence of hypercalciuria and hypouricaemia in type 2 diabetic patients. Diabet Med 6:406-411, 1989

28. Ishihara M, Shinoda T, Aizawa T, et al: Hypouricemia in NIDDM patients. Diabetes Care 11:796-797, 1988