Original Article

High Plasma Norepinephrine Levels Associated with β2-Adrenoceptor Polymorphisms Predict Future Renal Damage in Nonobese Normotensive Individuals

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Renal injury is common in obesity and hypertension. In the present study, we examined relationships between renal function alterations, plasma norepinephrine (NE), and β2-adrenoceptor polymorphisms in a longitudinal design over 5 years. In 219 nonobese, normotensive men with entry-normal renal function, we measured serum blood urea nitrogen (BUN), creatinine, creatinine clearance, plasma NE, homeostasis model assessment of insulin resistance (HOMA-IR), body mass index (BMI), total body fat mass, and blood pressure (BP) annually for 5 years. β2 (Arg16Gly, Gln27Glu)-adrenoceptor polymorphisms were determined. The subjects were stable in body weight and BP (<10%) for 5 years. High plasma NE was defined as ≥mean+1 SD at entry. Thirty-seven subjects had entry-high plasma NE and 182 were entry-normal. Entry-high plasma NE subjects had significantly greater total body fat mass and plasma NE and significantly lower creatinine clearance at entry and throughout the study. Increases in BMI, fat mass, BP, plasma NE, BUN, and creatinine, as well as the reduction in creatinine clearance in the 5 years, were significantly greater in entry-high NE subjects. These subjects had significantly higher frequencies of the Gly16 allele of β2-adrenoceptor polymorphisms. Throughout the study, subjects carrying the Gly16 allele had higher plasma NE, HOMA-IR, and fat mass, and significantly greater reductions in creatinine clearance. Plasma NE at entry was a determinant variable for changes in BUN, creatinine, and creatinine clearance over the 5-year period in multiple regression analysis. In conclusion, high plasma NE at entry, associated with the Gly16 allele of the β2-adrenoceptor polymorphisms, predict renal function deterioration (seen in elevations of BUN and creatinine and reduction of creatinine clearance) over a 5-year period accompanying further heightened sympathetic nerve activity and deterioration of insulin resistance. (*Hypertens Res* 2007; 30: 503–511)

***Key Words*: plasma norepinephrine, renal function, blood pressure elevation, weight gain, β2-adrenoceptor polymorphisms**

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Introduction

Renal injury predicts the development of cardiovascular disease (*1*). Hypertension and obesity are currently among the World Health Organization's top 10 global health risks, and are strongly associated with renal injury, chronic renal disease, and end-stage renal disease (*2*, *3*). The incidence of hypertension, one of the primary etiological factors for chronic renal failure, is significantly higher with obesity. Obesity also leads to increases in the incidence of renal disease (*3*), metabolic diseases such as diabetes (*4*), and cardiovascular diseases (*5*). Both diabetes and hypertension, which occur often with obesity, together account for approximately 70% of end-stage renal diseases in the United States and Japan (*6*). Thus, one could speculate that there are strong relationships between hypertension, obesity, and renal injury regardless of its severity. However, most previous studies of these relationships have examined proteinuria/micro-albuminuria as an index of renal injury.

Heightened sympathetic nerve activity is observed in both hypertension and obesity (*7–11*). Further, it has been reported that heightened sympathetic nerve activity predicts incident cardiovascular events (*12*). Many investigations have found that human obesity and hypertension have strong genetic as well as environmental determinants (*13–16*). Several observations show associations of β2-adrenoceptor polymorphisms with hypertension and obesity (*16–18*), but those findings have not been confirmed. Additionally, few investigations have simultaneously taken into account sympathetic nerve activity and renal function as related to adrenoceptor polymorphisms in the same study population followed longitudinally for several years.

Thus, to investigate the relationship between alterations in renal function and certain polymorphisms of the β2-adrenoceptor system that are reportedly associated, with obesity and hypertension (*16*), we created the present study. In this study, we examined renal functions (blood urea nitrogen [BUN], creatinine, and creatinine clearance) accompanying sympathetic nerve activity (plasma norepinephrine [NE]) and the β2-adrenoceptor polymorphisms in nonobese, normotensive subjects with originally normal renal functions in a 5-year longitudinal study, though in previous studies we examined a series of studies (*i.e.*, relationships between the β2-adrenoceptor polymorphisms *vs*. insulin resistance, weight gain, or blood pressure [BP] elevation), in the same cohort (*16*, *19*). To our knowledge, the present study is the first observation regarding the relationships between the β2-adrenoceptor polymorphisms and renal function.

Methods

Subjects

Subjects were recruited from 1,121 men employed at a company in Osaka, Japan, as part of their annual medical evaluation. At study entry, subjects were excluded if they were >50 years of age, obese (body mass index [BMI] ≥25 kg/m^2), or had any of the following: diabetes mellitus (fasting glucose level >100 mg/dL), hypertension (≥140/90 mmHg), renal dysfunction (proteinuria, microscopic hematuria, BUN ≥20.0 mg/dL, creatinine ≥1.3 mg/dL, creatinine clearance <80 mL/min), or hyperuricemia (serum uric acid ≥6.5 mg/dL). Subjects were also excluded if they were taking medications for hypertension, hyperlipidemia, hyperuricemia, or other illnesses. The goal of the present study was to clarify the relationships among the genetic variance in β2-adrenoceptor polymorphisms, sympathetic nerve activity (plasma NE levels), and renal function (proteinuria, BUN, creatinine, and creatinine clearance). Thus, to minimize the influence of changes in body weight or BP levels on renal function and plasma NE levels, only subjects who had been free from significant changes (<10%) in body weight and BP levels over a 5-year period were enrolled in this study (*9–11*, *16*, *19*). After the exclusions, the present study analyzed 219 young, nonobese (BMI <25 kg/m^2), normotensive (<140/90 mmHg) men with normal renal function (no proteinuria, no microscopic hematuria, BUN<20.0 mg/dL, creatinine <1.3 mg/dL, creatinine clearance ≥80 mL/min) who were taking no medications. Informed consent was obtained from each subject, as approved by the Ethics Committee of Osaka University Graduate School of Medicine, Japan.

Measurements

After an overnight fast of >12 h, BMI, total body fat mass, ratio of waist circumference to hip circumference (waist-to-hip ratio), BP, heart rate, and venous sampling for BUN, creatinine, serum uric acid, plasma NE, insulin, leptin, and glucose were sampled after a 30-min rest period in the supine position in a quiet room. Blood samples for the extraction of genomic DNA from leukocytes were also taken. Measurements were made at entry and every year for 5 years. BP and heart rate were measured at each review at least three times in the supine position by an automated sphygmomanometer (TM-2713, A&D Co., Tokyo, Japan) using an adjustable cuff based on arm circumference, which was standardized against a mercury sphygmomanometer, and were averaged. Those who had wide variability in BP and heart rate were asked to return for repeated measurements at least three separate visits to exclude chance variation. The percentage body fat mass was determined with impedance measurements (BF-102, Tanita Co., Tokyo, Japan), and total body fat mass (kg) was calculated according to the following formula: [percentage body fat mass (%)/100] × body weight (kg). Creatinine clearance was calculated according to the following formula established by Cockroft and Gault (*20*): [(140 − age) × body weight] (kg)/[72 × serum creatinine (mg/dL)]. Homeostasis model assessment of insulin resistance (HOMA-IR) was defined as the product of fasting plasma insulin (μU/mL) and

Table 1. Comparisons of Characteristic in Subjects with Normal Plasma Norepinephrine at Entry (<Mean+1 SD) *vs*. High Plasma Norepinephrine at Entry (≥Mean+1 SD)

Variables	Subjects with normal plasma norepinephrine levels			Subjects with high plasma norepinephrine levels		
	At entry	After 5 years	Changes for 5 years	At entry	After 5 years	Changes for 5 years
Subjects (*n*)	182	182	182	37	37	37
Age (years)	39±3	44±3§	5	37±3	42±3§	5
BMI (kg/m^2)	21.3±1.4	21.4±2.9	0.1±1.0	21.5±2.4	22.4±2.9	0.9±1.2*
Total body fat mass (kg)	12.9±4.7	13.7±4.4	0.9±3.2	14.3±6.2*	17.1±4.8*,‡	2.8±5.1†
Waist-to-hip ratio	0.84±0.07	0.84±0.09	−0.01±0.06	0.87±0.10	0.93±0.09*	0.05±0.09
Systolic BP (mmHg)	123±7	124±8	1±12	126±9	133±9†	8±7*
Diastolic BP (mmHg)	71±5	73±6	3±4	76±13	79±6*	3±10
Mean BP (mmHg)	88±6	90±6	1±8	93±7	97±7*	5±5*
Norepinephrine (pmol/mL)	1.28±0.27	1.68±0.45	0.40±0.52	1.89±0.25†	2.85±0.46†,§	0.99±0.41†
HOMA-IR	1.53±0.76	2.17±0.93	0.64±0.75	1.71±0.60	2.58±0.75‡	0.87±1.02
Leptin (ng/mL)	3.2±2.0	3.2±2.1	−0.1±1.9	3.4±1.8	3.5±1.0	0.2±1.0
BUN (mg/dL)	10.5±2.9	11.2±3.7	0.6±1.0	10.8±2.3	12.3±2.6*,‡	1.5±0.9*
Creatinine (mg/dL)	0.93±0.12	0.95±0.16	0.02±0.08	0.95±0.09	1.06±0.14*,‡	0.11±0.16*
Creatinine clearance (mL/min)	98.0±19.0	94.9±21.8	−3.1±21.4	85.1±9.9†	72.3±18.9†,§	−12.8±14.8†
Uric acid (mg/dL)	4.3±1.3	4.8±1.5	0.4±1.8	5.0±1.0	5.8±1.5*,‡	0.8±1.5*

BMI, body mass index; BP, blood pressure; HOMA-IR, homeostasis model assessment of insulin resistance; BUN, blood urea nitrogen. *$p<0.05$, †$p<0.01$ *vs*. subjects with normal plasma norepinephrine levels; ‡$p<0.05$, §$p<0.01$ *vs*. values at entry. Data are shown mean±SD. $N=219$.

glucose (mg/dL) divided by 405.

Laboratory Determinations

Plasma NE was measured by high-performance liquid chromatography with a fluorometric method as described previously in detail (*10*). Plasma immunoreactive insulin was measured by a standard radioimmunoassay method as described in detail (insulin RIABEAD II, Dinabott Co., Tokyo, Japan) (*10*). Plasma leptin was measured by radioimmunoassay as described (human leptin RIA kit, Linco Research, St. Charles, USA) (*10*). Serum BUN, creatinine, uric acid, and glucose were measured by an Autoanalyzer (Hitachi-7050, Hitachi Medical Co., Tokyo, Japan).

Genotyping

Genotyping was performed by the TaqMan assay as previously described (*16*, *19*, *21*, *22*). Two polymorphisms in the β2-adrenergic receptors (arginine/glycine substitution, Arg16Gly; glutamine/glutamate substitution, Gln27Glu) of the β2-adrenoceptor genes (*16*, *17*) were evaluated. The probes and primers used in the TaqMan assay were as follows. For Arg16Gly, the probes were CGCATGGCTTCC ATTGGGTGC and CGCATGGCTTCTATTGGGTGC, and the primers were GGAACGGCAGCGCCTTCT and CAG GACGATGAGAGACATGACGAT; for Gln27Glu, the probes were CTCGTCCCTTTCCTGCGTGACGT and CTCGTCCCTTTGCTGCGTGACGT (the primers used in this assay were the same as those used for Arg16Gly).

Statistical Analysis

Values shown are means±SD. Changes in measured parameters within each group and differences among groups were examined by two-way analysis of variance. When these differences were significant, the Dunnett test was used to determine whether the differences of the mean measured variables at year 5 were significantly different from baseline within the groups and among the groups. The Mantel-Haenszel χ^2 test was used to compare differences in the genotype frequencies between the groups with and without high plasma NE at the entry period. Multiple linear regression analyses were used to examine relationships among variables using changes in creatinine clearance, serum BUN, or creatinine for 5 years as the dependent variables to evaluate the relationships with plasma NE, insulin sensitivity (HOMA-IR), body weight, total body fat mass, BP levels, and heart rates at entry. The Hardy-Weinberg equilibrium was estimated with the χ^2 test.

Table 2. Comparisons of the Frequencies of Genotype of the β2-Adrenoceptor Polymorphisms between Subjects with and without High Plasma Norepinephrine (Plasma Norepinephrine Levels ≥ Mean + 1 SD)

	Genotype frequency				Allele frequency		Normal- *vs*. high-plasma norepinephrine χ^2 test	
							For genotype	For allele
Arg16Gly, β2-adrenoceptor	Arg/Arg	Arg/Gly	Gly/Gly	missing	Arg16 allele	Gly16 allele	χ^2=31.86, p<0.001	χ^2=58.32, p<0.001
Normal SNA (*n* (%))	62 (34.1)	93 (51.1)	27 (14.8)	0 (0.0)	217 (59.6)	147 (40.4)		
High SNA (*n* (%))	5 (13.5)	11 (29.7)	21 (56.8)	0 (0.0)	21 (28.4)	53 (71.6)		
Gln27Glu, β2-adrenoceptor	Gln/Gln	Gln/Glu	Glu/Glu	missing	Gln27 allele	Glu27 allele	χ^2=—, p=—	χ^2=2.82, p=0.093
Normal SNA (*n* (%))	156 (86.0)	19 (10.0)	0 (0.0)	7 (4.0)	331 (90.0)	19 (5.2)		
High SNA (*n* (%))	35 (94.6)	0 (0.0)	0 (0.0)	2 (5.4)	70 (94.6)	0 (0.0)		

SNA, sympathetic nervous system measured by plasma norepinephrine.

Table 3. Comparisons of Characteristics between Subjects with and without the Gly16 Allele of Arg16Gly, β2-Adrenoceptor Gene

Variables	Subjects without Gly16 allele			Subjects with Gly16 allele		
	At entry	After 5 years	Changes for 5 years	At entry	After 5 years	Changes for 5 years
Subjects (*n*)	67	67	67	152	152	152
Age (years)	39±3	44±3§	5	39±3	44±3§	5
BMI (kg/m^2)	21.7±1.4	21.7±1.7	0.1±1.2	21.2±1.7	21.5±3.0	0.3±2.3
Total body fat mass (kg)	12.7±5.3	13.4±6.7	0.5±4.0	13.4±5.3*	14.7±5.1*,‡	1.4±4.1*
Waist-to-hip ratio	0.83±0.06	0.85±0.08	0.01±0.05	0.87±0.06*	0.91±0.08*,‡	0.04±0.06*
Systolic BP (mmHg)	126±10	126±9	1±6	120±9	124±8	4±7
Diastolic BP (mmHg)	74±6	73±6	−1±7	71±6	75±6	4±8*
Mean BP (mmHg)	91±6	91±7	−1±5	87±6	91±6	4±9
Norepinephrine (pmol/mL)	1.10±0.41	1.69±0.49‡	0.59±0.40	1.50±0.40*	1.98±0.51*,‡	0.47±0.45
HOMA-IR	1.34±0.63	1.95±0.74‡	0.61±1.62	1.67±0.51*	2.44±0.65*,‡	0.77±0.95
Leptin (ng/mL)	3.4±2.6	3.7±2.0	0.3±1.6	3.2±2.9	3.1±3.3	−0.1±4.4
BUN (mg/dL)	10.4±2.6	11.0±2.8	0.6±1.1	10.6±2.9	11.6±3.7	1.1±2.0
Creatinine (mg/dL)	0.92±0.14	0.97±0.14	0.05±0.12	0.94±0.11	0.97±0.16	0.04±0.09
Creatinine clearance (mL/min)	98.2±22.8	95.8±21.6	−2.4±22.5	91.1±21.9	80.3±23.1†	−10.8±20.7†
Uric acid (mg/dL)	4.0±0.7	4.4±1.2	0.4±1.4	4.6±0.5*	5.2±1.3*,‡	0.6±1.5

BMI, body mass index; BP, blood pressure; HOMA-IR, homeostasis model assessment of insulin resistance; BUN, blood urea nitrogen. *p<0.05, †p<0.01 *vs*. values in subjects without Gly16 allele, ‡p<0.05, §p<0.01 *vs*. values at entry. Data are mean±SD. N=219.

Results

Characteristics of Entry-Normal (<Mean+1 SD) and Entry-High (≥Mean+1 SD) Plasma Norepinephrine Study Groups

The subjects were subdivided into two groups—one with high plasma NE and the other with normal plasma NE—using the cut-off limit of mean+1 SD (1.34+0.33 pmol/mL) at entry. There were 37 subjects who had entry-high plasma NE and 182 subjects who had entry-normal plasma NE (Table 1). None of the participants had proteinuria or microscopic hematuria throughout the study.

Subjects with entry-high plasma NE had greater total body fat mass and plasma NE levels at entry compared to those with entry-normal plasma NE, although BMI, waist-to-hip ratios, BP, HOMA-IR, BUN, and creatinine levels were similar. Creatinine clearance was significantly lower in subjects with entry-high plasma NE than in those with entry-normal plasma NE at entry and throughout the study. Further, increases in BMI, total body fat mass, BP, plasma NE, BUN, creatinine, and uric acid levels, as well as the reduction in creatinine clearance, over the 5-year period were significantly greater in the subjects with entry-high plasma NE levels (Table 1).

Table 4. Characteristic of the 4 Study Groups According to Entry Plasma Norepinephrine Levels and the Gly16 Allele of the β2-Adrenoceptor Polymorphisms

Variables	Subjects with normal plasma norepinephrine levels				Subjects with high plasma norepinephrine levels			
	Without Gly16 allele		With Gly16 allele		Without Gly16 allele		With Gly16 allele	
	At entry	After 5 years	At entry	After 5 years	At entry	After 5 years	At entry	After 5 years
Subjects (*n*)	62	62	120	120	5	5	32	32
Age (years)	40±4	45±4$^{\#}$	38±3	43±3$^{\#}$	35±6	40±6$^{\#}$	39±3	44±3$^{\#}$
BMI (kg/m^2)	20.9±1.3	20.8±2.7	21.5±1.4	21.7±2.8	20.9±2.2	21.8±2.8	21.6±2.0	22.5±2.3
Total body fat mass (kg)	11.9±3.8	12.5±3.9	13.4±4.0	14.3±4.5	12.3±2.8	15.1±4.3	14.9±3.8	17.4±4.5*,§
Waist-to-hip ratio	0.82±0.06	0.80±0.08	0.85±0.09	0.87±0.10	0.83±0.08	0.87±0.05	0.88±0.07	0.94±0.10*,§
Systolic BP (mmHg)	121±6	121±9	124±9	126±7	125±8	127±7	127±8	134±9*,§
Diastolic BP (mmHg)	71±5	71±6	71±6	74±7	76±11	79±9	77±9*	80±9*
Mean BP (mmHg)	88±6	88±6	89±6	91±7	92±5	95±6	94±7*	98±7*
Norepinephrine (pmol/mL)	1.22±0.41	1.54±0.37	1.31±0.35	1.71±0.45§	1.51±0.20	2.40±0.40§	1.95±0.62*	2.92±0.71†,§
HOMA-IR	1.24±0.42	1.42±0.94	1.73±0.65‡	2.55±0.56‡,§	1.39±0.54	1.68±0.75	1.76±0.72	2.57±0.73§
Leptin (ng/mL)	3.0±1.9	3.0±2.0	3.3±2.1	3.4±1.8	3.0±1.5	3.2±1.0	3.4±1.4	3.6±1.5
BUN (mg/dL)	10.1±2.7	10.2±3.3	10.5±2.8	12.1±3.4‡	9.6±2.1	10.4±2.5	11.0±2.3	12.7±2.9
Creatinine (mg/dL)	0.91±0.09	0.93±0.12	0.94±0.12	0.96±0.15	0.90±0.10	1.00±0.13	0.96±0.10	1.07±0.11*,§
Creatinine clearance (mL/min)	98.3±17.9	96.4±22.3	97.9±22.2	91.8±22.2	93.8±10.8	90.1±21.8	84.4±10.0†,‡	70.9±18.8$^{\dagger,\ddagger,\#}$
Uric acid (mg/dL)	4.0±1.3	4.2±1.2	4.5±1.4	5.2±1.5‡	4.4±1.0	5.1±1.4	5.0±1.3	5.9±1.6§

BMI, body mass index; BP, blood pressure; HOMA-IR, homeostasis model assessment of insulin resistance; BUN, blood urea nitrogen. *$p<0.05$, $^{\dagger}p<0.01$ *vs*. subjects with entry-normal plasma norepinephrine levels; $^{\ddagger}p<0.05$ *vs*. subjects without the Gly16 allele; $^{\S}p<0.05$, $^{\#}p<0.01$ *vs*. value at the entry period.

Comparisons of the Frequencies of the β2-Adrenoceptor Polymorphisms According to Plasma NE Levels

Subjects with high plasma NE levels had significantly higher frequencies of the homozygous Gly16 genotype and the Gly16 allele of the Arg16Gly, the β2-adrenoceptor polymorphism. However, the frequencies of the Glu27 allele of the Gln27Glu were similar between subjects with and without high plasma NE levels (Table 2).

Comparisons of Characteristics between Subjects with and without the Gly16 Allele of the Arg16Gly β2-Adrenoceptor Polymorphism

The finding that subjects with high plasma NE had higher frequencies of the Gly16 allele of the Arg16Gly, but not of the Glu27 allele, indicates that the Gly16 allele is closely linked to high plasma NE levels. Thus, we compared the parameters between the subjects with and without the Gly16 allele.

Subjects carrying the Gly16 allele had greater total body fat mass, waist-to-hip ratio, plasma NE, HOMA-IR, and uric acid at entry and after 5 years compared to those without the Gly16 allele, but BUN and creatinine levels throughout the study were similar. Creatinine clearance at entry was similar between those with and without the Gly16 allele, but subjects with the Gly16 allele had significantly lower creatinine clearance after 5 years and significantly greater reductions in creatinine clearance over the 5-year period compared to those without the Gly16 allele. Subjects with the Gly16 allele also had significantly greater increases in total body fat mass and waist-to-hip ratio over the 5-year period (Table 3).

Comparisons of the Characteristics of the Study Groups According to Entry Plasma Norepinephrine Levels and the Gly16 Allele of β2-Adrenoceptor Polymorphisms

When we compared the four study groups according to the entry plasma NE levels and the Gly16 allele of the β2-adrenoceptor polymorphisms, total body fat mass, waist-to-hip ratio, plasma NE, HOMA-IR, BUN, creatinine, and uric acid levels at entry were greatest in the group that had both entry-high plasma NE and the Gly16 allele. BP elevations over the 5-year period and increases in total body fat mass, waist-to-hip ratio, plasma NE, HOMA-IR, BUN, creatinine, and uric acid were also greatest in the group that had both entry-high plasma NE and the Gly16 allele (Table 4).

Further, subjects with both entry-high plasma NE and the Gly16 allele had the lowest creatinine clearance rate throughout the study period, as well as the greatest reduction in creatinine clearance over the 5-year period. Only in subjects with entry-high plasma NE, creatinine clearance was significantly lower in subjects carrying the Gly16 allele than in those with-

Table 5. Multiple Linear Regression Analyses

Independent variables	Dependent variables		
	*Δ*Creatinine clearance	*Δ*BUN	*Δ*Creatinine
A: At entry			
Plasma NE	$p=0.011$	$p=0.008$	$p=0.018$
Plasma leptin	n.s.	n.s.	n.s.
HOMA-IR	n.s.	n.s.	n.s.
Mean BP	n.s.	n.s.	n.s.
Heart rate	n.s.	n.s.	n.s.
BMI	n.s.	n.s.	n.s.
Total body fat mass	n.s.	n.s.	n.s.
Creatinine clearance	$p<0.001$	$p=0.035$	$p=0.027$
Serum BUN	n.s.	n.s.	$p=0.025$
Serum creatinine	n.s.	n.s.	$p=0.041$
	$F=8.842$	$F=2.750$	$F=2.18$
	$p<0.001$	$p=0.033$	$p=0.032$
	$r^2=0.630$	$r^2=0.271$	$r^2=0.325$
B: Change over a 5-year period			
*Δ*Plasma NE	$p=0.028$	$p=0.013$	$p=0.038$
*Δ*Plasma leptin	$p=0.048$	$p=0.024$	n.s.
*Δ*HOMA-IR	n.s.	$p=0.014$	$p=0.029$
*Δ*Mean BP	$p=0.025$	$p=0.030$	n.s.
*Δ*Heart rate	$p=0.037$	n.s.	n.s.
*Δ*BMI	n.s.	n.s.	n.s.
*Δ*Total body fat mass	$p=0.011$	$p=0.018$	$p=0.049$
*Δ*Creatinine clearance	—	$p=0.022$	$p=0.011$
*Δ*Serum BUN	n.s.	—	$p=0.018$
*Δ*Serum creatinine	$p=0.012$	$p=0.018$	—
	$F=3.838$	$F=1.866$	$F=4.830$
	$p=0.011$	$p=0.049$	$p=0.002$
	$r^2=0.348$	$r^2=0.172$	$r^2=0.475$

NE, norepinephrine; HOMA-IR, homeostasis of model assessment of insulin-resistance; BP, blood pressure; BMI, body mass index; BUN, blood urea nitrogen; *Δ*, change over a 5-year period.

out the Gly16 allele. However, in subjects with entry-normal plasma NE, creatinine clearance was similar between those with and without the Gly16 allele (Table 4).

Multiple Regression Analysis Using Changes in Creatinine Clearance, BUN, or Creatinine over the 5-Year Period as a Dependent Variable

Multiple linear regression analyses using changes in creatinine clearance, serum BUN, or serum creatinine over the 5-year period as dependent variables demonstrated that plasma NE level at entry was a significant determinant variable for changes in creatinine clearance, BUN, or creatinine (Table 5, A). Change in plasma NE and change in total body fat mass over the 5-year period were also significant determinant variables for changes in creatinine clearance, serum BUN, or serum creatinine (Table 5, B).

Discussion

The present study shows that subjects carrying baseline high plasma NE have significantly greater increases in total body fat mass, BP levels, plasma NE, BUN, and creatinine, as well as greater reductions in creatinine clearance, over the 5-year period. Subjects with high plasma NE have significantly higher frequencies of the Gly16 of the Arg15Gly, the β2-adrenoceptor polymorphism. Significant deterioration in renal function (shown in creatinine clearance, serum creatinine, and BUN) is observed especially in subjects who carry both the Gly16 allele and entry-high plasma NE. Multiple regression analyses demonstrate that the plasma NE level at entry is a significant determinant of changes in BUN, creatinine, and creatinine clearance over the 5-year period. These findings demonstrate that high plasma NE levels associated with the Gly16 allele of a β2-adrenoceptor polymorphism are

related to the deterioration in renal function (BUN, creatinine, and creatinine clearance) over the 5-year period in originally normal renal function, nonobese, normotensive Japanese men.

Mortality caused by cardiovascular disease is more than three times higher in subjects with renal dysfunction than in those with normal renal function (*23*). Many investigators have reported that plasma NE and sympathetic nerve overactivity predict survival and incident cardiovascular events (*12*, *24*). Recently, Joles and Koomans have reported that sympathetic nerve stimulation contributes to the progression of renal disease (*25*). The 40-min infusion of NE into the renal artery of dogs produces a reversible ischemic model of acute renal failure (*26*). Another study demonstrates renal protection by β-adrenergic receptor blockade in a nephrectomized rat experiment without any BP changes (*27*). The findings that high plasma NE levels at entry are closely linked to future renal function (BUN, creatinine, and creatinine clearance) could also indicate that sympathetic nerve overactivity (high plasma NE) might be a cause of renal injury. These investigations demonstrate that direct effects of sympathetic nerve overactivity may lead to proteinuria and renal injury (*25*), and that renal dysfunctions associated with sympathetic overactivity is related to much higher mortality compared to that in individuals with normal renal function.

Previous epidemiological and clinical studies have used micro-albuminuria as a marker of renal injury (*1*). However, it should be noted that we used BUN, creatinine levels, and creatinine clearance, which are more direct markers of renal function relative to micro-albuminuria, and that those are deteriorated over the 5-year period in association with further heightened sympathetic nerve activity. Prolonged sympathetic nerve overactivity (high plasma NE) can induce changes in intrarenal blood vessels. Catecholamines induce proliferation of smooth muscle cells and adventitial fibroblasts in the vascular wall (*28*). The findings that reductions in creatinine clearance were significantly greater in subjects carrying entry-high plasma NE, the Gly16 allele, or both indicated that subjects carrying high sympathetic nerve activity (entry-high plasma NE), the β2-adrenoceptor polymorphisms, or both are related to deterioration of renal function. Further, the finding that, in subjects with high plasma NE, the deterioration in renal function is significantly greater in subjects carrying the Gly16 allele, suggests that the Gly16 allele might have additional effects on future renal injury, especially in subjects who carry heightened sympathetic nerve activity. The novel findings in the present study are that the Gly16 allele of the β2-adrenoceptor associated with entry-high plasma NE might be related to future renal injury. To our knowledge, this is one of the first investigations into the relationships between the β2-adrenoceptor polymorphisms and renal injury.

Hypertension and obesity are also risk factors for progressive renal function loss in patients with known renal disease and may damage the kidneys in otherwise healthy subjects (*29*). Typically, significant hypertension initially affects the renal vasculature, resulting in hyaline thickening of small arteries and arterioles. At an earlier stage, hypertension and atherosclerosis may be intimately linked through their effects on endothelial function. A dysfunctional endothelium allows adhesion of lipid-filled macrophages and consequent chemotaxis and aggregation of inflammatory cells. In large vessels, hypertension favors atherosclerosis progression primarily by accelerating the conversion of fatty streaks to atheroma (*30*).

Several investigators have reported that cardiac sympathetic nerve activity is not substantially elevated in obese humans, whereas sympathetic nerve activity is increased in the kidneys (*3*, *31*). Their observations show that the kidneys play an important role in heightened sympathetic nerve activity in obesity. Obesity can also produce renal injury by early up-regulation of numerous pro-inflammatory (*e.g.*, leptin, interleukins, adiponectin, tumor-necrosis factor-α) and growth-promoting (*e.g.*, angiotensin II, TGF-β, leptin) factors, leading to mesangial matrix production and thickening of the glomerular and tubular basement membrane lesions that may precede glomerulosclerosis (*29*). Obesity can lead to hypertension by increasing renal tubular sodium reabsorption, impairing pressure natriuresis, and inducing volume expansion, as well as by physical compression of the kidneys. With prolonged obesity, there is increasing urinary protein excretion and a gradual loss of nephron function that worsens with time and exacerbates hypertension (*3*). Further, obesity is considered the phenotypic hallmark of metabolic syndrome (insulin resistance). Patients with metabolic syndrome have a high prevalence of micro-albuminuria, which is considered an early marker of renal endothelial dysfunction and chronic kidney disease as well as generalized endothelial dysfunction. With the worsening of metabolic syndrome and the development of type II diabetes in some obese patients, kidney damage progresses much more rapidly and seriously. Thus, aggressive obesity-lowering therapies are needed to ameliorate renal disease progression (*3*, *32*). Importantly, both the pathogenesis of hypertension and obesity are associated with sympathetic nerve overactivity, as previously documented.

It should be noted that subjects with entry-high plasma NE in part associated with the Gly16 allele of the Arg16Gly polymorphism in the present study had greater increases in total body fat mass and BP elevations accompanied by greater increases in plasma NE, even though we tried to minimize (<10% in changes in BMI or BP levels over 5 years) the influence of weight gain (obesity) or BP elevation (hypertension) on renal function. Further, those subjects who had greater increases in adiposity and BP levels had more deterioration in renal function. We previously reported the associations of the Gly16 allele of the Arg16Gly, a β2-adrenoceptor polymorphism, with weight gain and BP elevations (*16*). These observations suggest that BP elevation and weight gain determined by the Gly16 allele of Arg16Gly (even though the degree of those elevations was very small [<10%]), might contribute to renal function accompanied by sympathetic

nerve overactivity (high plasma NE). Thus, one could speculate on the possibility that weight gain and BP elevation associated with the Gly16 allele of the Arg16Gly might lead to high plasma NE levels at entry, greater increases in plasma NE over 5 years, and resultant renal injury. Subjects carrying entry-high plasma NE had greater increases in plasma leptin levels over the 5-year period associated with greater increases in fat mass, suggesting that greater increases in plasma leptin (adiposity) might lead to further heightened sympathetic activity (*33*).

There is an association between the Gly16 allele of Arg16Gly and insulin resistance (*19*). The close relationships between plasma NE (sympathetic nerve activity) and insulin (insulin resistance) were also observed in this cohort of nonobese, normotensive subjects with a cross-sectional design (*34*). Further, we have reported that heightened sympathetic nerve activity (high plasma NE) precedes hyperinsulinemia in nonobese, normotensive individuals (*10*, *11*). Taken together with previous observations, in nonobese, normotensive individuals, heightened sympathetic nerve activity associated with the Gly16 allele might play a primary role in renal injury, and hyperinsulinemia linked to the Gly16 allele as well as high plasma NE might be an ancillary mechanism.

In conclusion, the findings demonstrate that baseline plasma NE in part associated with the Gly16 allele of Arg16Gly, a β2-adrenoceptor polymorphism, could predict future renal injury (BUN and creatinine elevation, and reduction in creatinine clearance) accompanied by increases in abdominal obesity (waist-to-hip ratio and total body fat mass), BP elevation, and insulin resistance over 5 years in nonobese, normotensive male individuals. Thus, we would propose that plasma NE levels, a β2-adrenoceptor polymorphism (Arg16Gly), and relative abdominal obesity might be useful for predicting future renal injury.

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