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Possible interactions of the endothelial constitutive nitric oxide synthase genotype with alcohol drinking and walking time for high serum uric acid levels among Japanese

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Abstract

A variable number of tandem repeat polymorphism located in intron 4 of the gene for endothelial constitutive nitric oxide synthase (*ecNOS*) is reported to be significantly associated with the nitric oxide level, which influences serum uric acid (SUA). To cast light on any association between the polymorphism and hyperuricemia, as well as gene-environment interactions, a cross-sectional study was conducted for 703 health checkup examinees (213 men and 490 women). The age-adjusted odds ratio (aOR) of hyperuricemia (≥7 mg/dL) for *ecNOS 4*/*4*, *4*/*5*, or *5*/*6* genotypes (*non-5*/*5* group) as compared with the *5*/*5* genotype was 2.41 (95% confidence interval [CI], 1.09-5.30) in men. The aORs for drinking alcohol relative to never drinking were found to be 8.93 (95% CI, 1.02-78.16) among men with *non-5*/*5* genotypes and 1.76 (95% CI, 0.59-5.26) for their *5*/*5* counterparts. Moreover, the aORs for heavy drinking (≥50 mL/d) were 23.16 (95% CI, 2.14-250.35) and 2.48 (95% CI, 0.75-8.15), respectively. The interaction between the genotype and current drinking was 3.10 (95% CI, 0.45-21.41). The aORs for more than 30 minutes of daily walking relative to 30 minutes or less of daily walking were found to be 1.54 (95% CI, 0.40-5.95) among men with *non-5*/*5* genotypes and 0.31 (95% CI, 0.12-0.81) for their *5*/*5* counterparts. The interaction between the genotype and more than 30 minutes of daily walking was 4.92 (95% CI, 0.95-25.64). This study indicated that the *ecNOS* variable number of tandem repeat polymorphism influences the SUA level in men. Although the interactions were not significant, alcohol intake may be more influential among men with *non-5*/*5* genotypes and walking may be more effective among men with the *5*/*5* genotype. These findings would be informative for men with high SUA levels.

1. Introduction

It is well known that high serum uric acid (SUA) levels cause gout. In addition, both epidemiological and experimental evidence suggest that SUA is a powerful independent risk factor to stratify risk for cardiovascular disease [1-3]. Furthermore, hyperuricemia (≥7 mg/dL) is often accompanied with the "metabolic syndrome" [4]. Although urate is an antioxidant, its association with cancer risk is unclear [5].

Elevated SUA is commonly detected in subjects with abnormal purine metabolism, reflecting overproduction of uric acid (UA) and/or insufficient UA excretion from the kidney [6]. Inhibitors of xanthine oxidase (XO), which catalyzes the oxidation of hypoxanthine to xanthine and of xanthine to UA, thus block synthesis to provide a therapeutic approach for the treatment of hyperuricemia [7]. Nitric oxide (NO) is also known to affect XO activity (Fig. 1) [8-10]. Nitric oxide synthase (NOS) has 3 isozymes: neuronal constitutive NOS encoded by *NOS-1*; inducible NOS encoded by *NOS-2*; and endothelial constitutive NOS (ecNOS) encoded by *NOS-3*. The ecNOS form is present in airway and vascular endothelia, maintaining basal vascular NO production [11]. Although many environmental factors and disease states may alter ecNOS activity, genetic factors may also play a role and a 27-base pair (bp) variable number of tandem repeat (VNTR) polymorphism in intron 4, T-786C in the promoter region, and Glu298Asp in exon 7 of the *ecNOS* gene have been foci of interest as potential

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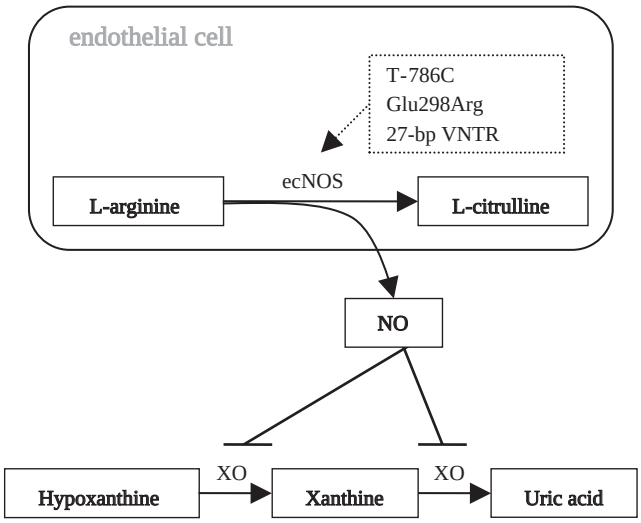

Fig. 1. Biosynthesis of UA.

sources of interindividual variation in ecNOS regulation and NO activity. Previous studies have shown a tight linkage between VNTR and T-786C polymorphisms in Asians [12-15]. Recently, it was reported that ecNOS activity has a significant association with VNTR polymorphism but not with Glu298Asp polymorphism [13,16,17]. Furthermore, all previous investigations with 100 subjects or more showed no association between Glu298Asp polymorphism and exhaled or plasma NO levels [18-20]. In contrast, all except one study in the United Kingdom [20] showed significant associations between VNTR polymorphism and plasma NO levels [21-23].

The present study was therefore conducted to examine whether *ecNOS* VNTR polymorphism is related to hyperuricemia in a Japanese population. In addition, interactions of the *ecNOS* VNTR genotype with lifestyle and physical conditions were investigated. This study was approved by the Nagoya University Graduate School of Medicine Ethics Committee (approval no. 48, issued on June 16, 2003).

2. Materials and methods

2.1. Subjects

Eight hundred twelve subjects (285 men and 527 women) aged 39 years or older were recruited from 864 residents in a rural area of Hokkaido, Japan, who attended a health checkup program in August 2003. They worked in fishing, dairy farming, or commerce, with roughly equal numbers from each of these fields. After excluding 109 examinees who reported that they were taking medication for gout or who had levels of blood urea nitrogen greater than 20 mg/dL, creatinine greater than 1.1 mg/dL, aspartate aminotransferase greater than 100 IU/L, alanine aminotransferase greater than 100 IU/L, or HbA_{1c} of 8.0% or higher at the health checkup, the remaining 213 men and 490 women were considered eligible for the present analysis.

2.2. Lifestyle questionnaire and biomarker measurements

The participants were requested to respond to a questionnaire on health and daily lifestyle at the time of the health examination. The questionnaire included items on drinking habits (current drinker, ex-drinker, never drinker), alcohol consumption (amount per day), smoking (current smoker, ex-smoker, never smoker), physical exercise (rarely, 1-2 h/wk, 3-4 h/wk, or $\geq$5 h/wk), walking time (rarely, 30 min/d, 30-60 min/d, $\geq$1 h/d), consumption of 4 kinds of meats (ie, beef, pork, chicken, and liver) and 3 kinds of fish dishes (ie, undried fish, dried fish, and shellfish) (rarely, 1-2 times/mo, 1-2 times/wk, 3-4 times/wk, or almost daily), and drug use for hypertension, as well as history of gout, as described in our previous article [24].

Written informed consent on providing lifestyle information and residual blood for genotyping was obtained. All patients underwent complete physical examinations and routine biochemical analyses of blood and urine after overnight fasting. Biochemical analysis of the sampled sera was performed using an auto-analyzer (JCA-RX20, Nihon Denshi Co Ltd, Tokyo, Japan), and the SUA level was measured by the uricase-POD method. Body height and weight were measured at the health checkup and the body mass index (BMI) was calculated as body weight (kilograms) divided by height (meters) squared.

2.3. Genotyping procedure

DNA was extracted from residual blood using a BioRobot EZ1 (QIAGEN Group, Tokyo, Japan) for genotyping of the *ecNOS* VNTR polymorphism by a polymerase chain reaction (PCR) method. Each 25-μL reaction tube contained 50 to 80 ng of DNA, 0.12 mmol/L of dNTP, 12.5 pmol of each primer, 0.5 U of AmpliTaq Gold (Perkin-Elmer, Foster

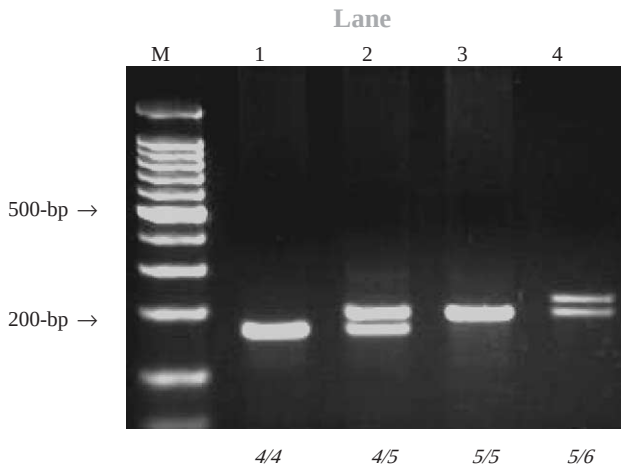

Fig. 2. Representative PCR results for the *ecNOS* 27-bp VNTR polymorphism in intron 4. DNA fragments stained with ethidium bromide are shown. Lane M, a 100-bp ladder; lane 1, a *4/4* homozygote with a fragment of 161 bp; lane 2, a *4/5* heterozygote with fragments of both 161 and 188 bp; lane 3, a *5/5* homozygote with a fragment of 188 bp; lane 4, a *5/6* homozygote with fragments of both 188 and 215 bp.

Table 1
Characteristics of study participants according to SUA levels (mg/dL)

Characteristics	Men				Women			
	<5 (n = 54)	5-6.9 (n = 125)	≥7 (n = 34)	P[a]	<5 (n = 330)	5-6.9 (n = 151)	≥7 (n = 9)	P[a]
Age range (y)	42-87	40-83	39-83	–	39-86	39-80	55-69	–
Age mean ± SD (y)	63.3 ± 10.8	61.9 ± 10.2	62.4 ± 11.3	.72	59.2 ± 9.9	62.4 ± 9.7	61.3 ± 5.0	.06
Ex-drinker (%)	5.6	8.0	8.8	.81	2.4	2.0	0.0	.86
Current drinker (%)	63.0	53.6	73.5	.09	13.6	15.2	0.0	.43
≥50 mL ethanol/d (%)	25.9	24.0	47.1	.03	1.21	1.99	0.0	.75
Ex-smoker (%)	44.4	46.4	58.8	.17	10	11.3	0.0	.19
Current smoker (%)	33.3	32.8	29.4	.37	7.6	12	0.0	.54
Exercise (≥1 h/wk, %)	33.3	36.8	29.4	.70	37.3	37.1	44.4	.91
Walking time (>30 min/d, %)	74.1	60.8	50.0	.06	66.4	61.6	77.8	.43
Daily consumer of meat (%)	3.7	4.8	8.8	.55	7.0	4.6	0.0	.45
Daily consumer of fish (%)	50.0	49.6	61.8	.44	46.1	58.9	55.6	.03
Obesity (BMI, ≥25; %)	14.8	37.6	44.1	.004	31.5	42.4	88.9	<.001
BMI (mean ± SD)	22.4 ± 2.3	24.1 ± 3.0	24.9 ± 2.5	<.001	23.5 ± 3.2	24.5 ± 3.4	29.3 ± 3.6	<.001
Drug use for hypertension (%)	29.6	29.6	32.4	.95	20.3	36.4	77.8	<.001
Systolic blood pressure (mean ± SD, mmHg)	136.8 ± 18.6	139.0 ± 20.4	142.1 ± 19.3	.48	133.4 ± 19.6	137.9 ± 18.5	144.0 ± 12.5	.02
Diastolic blood pressure (mean ± SD, mmHg)	86.8 ± 10.8	88.9 ± 11.3	89.3 ± 9.9	.45	83.5 ± 10.3	85.7 ± 10.8	89.1 ± 7.9	.04
HbA_{1c} (mean ± SD, %)	5.3 ± 0.7	5.2 ± 0.5	5.3 ± 0.6	.52	5.1 ± 0.5	5.2 ± 0.4	5.4 ± 0.7	.01
Total cholesterol (>240 mg/dL, %)	14.8	12.1	11.8	.87	19.6	26.0	55.6	.09
Triglyceride (≥150 mg/dL, %)	11.1	20.8	23.5	.23	7.6	13.9	11.1	.02
Urinary protein (+, %)	0.0	3.2	0.0	.24	1.5	3.3	0.0	.39
Blood urea nitrogen (mean ± SD, mg/dL)	14.6 ± 3.0	14.6 ± 2.8	14.6 ± 2.2	.99	13.4 ± 3.0	14.2 ± 2.8	15.7 ± 2.7	.003
Creatinine (mean ± SD, mg/dL)	0.75 ± 0.1	0.79 ± 0.12	0.82 ± 0.13	.03	0.59 ± 0.10	0.65 ± 0.11	0.62 ± 0.10	<.001

[a] *P* values for continuous variables were computed by analysis of variance; *P* values for all categorical variables computed by a 2×3 χ^2 test.

City, Calif), and 2.5 μL of 10× PCR buffer including 15 mmol/L of $MgCl_2$. The PCR conditions were denaturation at 95°C for 10 minutes, 30 cycles of denaturing at 95°C for 1 minute, annealing at 63°C for 1 minute, 72°C for 1 minute, and a final extension at 72°C for 5 minutes. The primers were 5′ -ATG GTA GTG CCT TGG CTG GA-3′ and 5′ -CAG GGA AGC TTC GCT CAG-3′ and the amplified DNA fragments were 161 bp for the *4*-repeat allele, 188 bp for the *5*-repeat allele, and 215 bp for the *6*-repeat allele, as illustrated in Fig. 2.

2.4. Statistical analysis

Current ethanol drinkers of 50 mL/d or more were defined as heavy drinkers. The frequencies of meat and fish consumption were calculated by adding 4 meat items and 3 fish items, respectively. Then, the subjects with 7 times/wk or more of consumption were defined as daily consumers.

All statistical analyses were performed using STATA SE/8.0 software (STATA, College Station, Tex). Accordance with the Hardy-Weinberg equilibrium, which indicates an absence of discrepancy between genotype and allele frequencies, was checked with a χ^2 test. Comparisons among drinking habit, physical activity, daily walking time, daily meat consumption, daily fish consumption, and drug use for hypertension among groups defined by SUA levels (<5, 5-6.9, and ≥7 mg/dL) were made with Pearson's χ^2 tests, and differences in the means of age and clinical characteristics among the SUA groups (<5, 5-6.9, and ≥7

Table 2
Frequencies of *ecNOS* genotypes and SUA levels by sex

	Men				Women			
	4/4 (n = 3)	*4/5* (n = 45)	*5/5* (n = 163)	*5/6* (n = 2)	*4/4* (n = 9)	*4/5* (n = 103)	*5/5* (n = 376)	*5/6* (n = 2)
Genotype frequency (%)	1.4	21.1	76.5	0.9	1.8	21.0	76.7	0.4
SUA level								
Mean ± SD	6.6 ± 1.2	6.1 ± 1.2	5.7 ± 1.3	5.3 ± 0.1	4.7 ± 1.3	4.5 ± 1.0	4.5 ± 1.1	6.1 ± 1.8
<5 mg/dL [% (n)]	0 (0)	15.6 (7)	28.8 (47)	0 (0)	66.7 (6)	67.0 (69)	67.6 (254)	50.0 (1)
5-6.9 mg/dL [% (n)]	66.7 (2)	57.8 (26)	58.3 (95)	1.6 (2)	33.3 (3)	32.4 (33)	30.6 (115)	0.0 (0)
≥7 mg/dL [% (n)]	33.3 (1)	26.7 (12)	12.9 (21)	0 (0)	0.0 (0)	1.0 (1)	1.9 (7)	50.0 (1)

Table 3
Age-adjusted odds ratios and 95% CIs for *ecNOS* genotypes regarding high SUA by sex

	aOR (95% CI) of ≥5 mg/dL		aOR (95% CI) of ≥7 mg/dL	
	Men	Women	Men	Women
5/5	1 (reference)	1 (reference)	1 (reference)	1 (reference)
non-5/5	2.63 (1.09-6.32)	0.97 (0.62-1.53)	2.41 (1.09-5.30)	0.90 (0.18-4.45)

mg/dL) were tested with analysis of variance. Age-adjusted odds ratios (aORs) and 95% confidence intervals (CIs) were estimated using an unconditional logistic regression model, with 2-sided P values less than .05 considered statistically significant. Gene-environment interactions were also estimated with the logistic model [25].

3. Results

Subject characteristics according to SUA levels (<5, 5-6.9, and ≥7 mg/dL) are summarized in Table 1. In men, the percentages in each SUA group were 25.4%, 58.7%, and 16.0%. In women, the corresponding values were 67.3%, 30.8%, and 1.8%. The male hyperuricemia group (≥7 mg/dL) had a larger proportion of heavy drinkers (47.1%) compared with other groups (24.6%; $P = .008$) and a higher mean BMI (24.9 vs 23.6; $P = .02$). In women, hyperuricemia was associated with a BMI of 25 or higher (88.9% vs 34.9%; $P = .001$), high mean BMI (29.3 vs 23.8; $P < .001$), use of hypertension drugs (77.8% vs 25.4%; $P < .001$), total cholesterol level higher than 240 mg/dL (55.6% vs 21.6%; $P = .02$), and a high mean blood urea nitrogen level (15.7 vs 13.6; $P = .04$).

The *ecNOS* genotype frequencies by sex are presented in Table 2. The distributions were in Hardy-Weinberg equilibrium for men ($P = .85$), women ($P = .60$), and the sexes combined ($P = .73$). As shown in Table 3, the aORs for high SUA levels (≥5 mg/dL or ≥7 mg/dL) in the *non-5/5* group relative to the *5/5* group were 2.63 (95% CI, 1.09-6.32) and 2.41 (95% CI, 1.09-5.30) in men. There were no significant associations in women.Table 4 shows the aORs for hyperuricemia (≥7 mg/dL) in men with reference to combinations of lifestyle and genotype, for genotype according to lifestyle factors, and for lifestyle factors according to genotype. The aORs for current drinking vs nondrinking were 8.93 (95% CI, 1.02-78.16) for the *non-5/5* genotypes and 1.76 (95% CI, 0.59-5.26) for the *5/5* genotype. The difference in aORs was much larger for heavy drinking (≥50 mL/d). The gene-environment interactions between the *ecNOS non-5/5* genotypes and alcohol drinking were 3.10 (95% CI, 0.45-21.41) for current drinkers in the logistic model including age, genotype, 2 dummy variables for ex-drinkers and current drinkers, and an interaction term for genotype × current drinkers, and 2.81 (95% CI, 0.52-15.28) for heavy drinkers

Table 4
Age-adjusted odds ratios and 95% CIs for *ecNOS* genotypes and environmental factors relative to 3 references in men

	aOR (95% CI)		aOR (95% CI)		aOR (95% CI)		Interaction term
	5/5	*non-5/5*	5/5	*non-5/5*	5/5	*non-5/5*	aOR (95% CI)
Alcohol drinking							
Never drinker	1 (reference)	0.66 (0.07-6.11)	1 (reference)	0.63 (0.06-6.17)	1 (reference)	1 (reference)	
Ex-drinker	1.88 (0.33-11.13)	5.26 (0.40-69.83)	1 (reference)	3.52 (0.18-70.28)	1.78 (0.30-10.57)	9.31 (0.38-288.25)	
Current drinker	1.68 (0.57-5.01)	5.57 (1.71-18.13)	1 (reference)	2.93 (1.12-7.64)	1.76 (0.59-5.26)	8.93 (1.02-78.16)	3.10 (0.45-21.41)
<50 mL/d	1.09 (0.29-4.05)	2.85 (0.67-12.09)	1 (reference)	2.59 (0.60-11.20)	1.16 (0.31-4.32)	4.37 (0.42-45.03)	
≥50 mL/d	2.33 (0.71-7.60)	12.26 (2.90-51.76)	1 (reference)	3.78 (0.95-15.02)	2.48 (0.75-8.15)	23.16 (2.14-250.35)	2.81 (0.52-15.28)
Smoking							
Never smoker	1 (reference)	0.72 (0.07-7.70)	1 (reference)	0.72 (0.07-7.69)	1 (reference)	1 (reference)	
Ex-smoker	1.56 (0.41-6.00)	4.43 (1.02-19.32)	1 (reference)	2.79 (0.95-8.15)	1.57 (0.41-6.04)	6.34 (0.67-58.59)	
Current smoker	1.01 (0.23-4.40)	3.76 (0.70-20.25)	1 (reference)	3.79 (0.88-16.32)	1.03 (0.23-4.55)	5.01 (0.47-53.56)	1.71 (0.30-9.70)
Exercise							
<1 h/wk	1 (reference)	2.76 (1.08-7.07)	1 (reference)	2.67 (1.04-6.87)	1 (reference)	1 (reference)	
≥1 h/wk	0.90 (0.34-2.40)	1.52 (0.38-6.07)	1 (reference)	1.70 (0.38-7.56)	0.89 (0.33-2.36)	0.55 (0.13-2.36)	0.61 (0.11-3.54)
Walking time							
≤30 min/d	1 (reference)	1.00 (0.28-3.54)	1 (reference)	0.95 (0.27-3.38)	1 (reference)	1 (reference)	
>30 min/d	0.31 (0.12-0.81)	1.53 (0.57-4.14)	1 (reference)	5.30 (1.79-15.75)	0.31 (0.12-0.81)	1.54 (0.40-5.95)	4.92 (0.95-25.64)
Consumption of meat							
Non-daily	1 (reference)	2.41 (1.07-5.48)	1 (reference)	2.37 (1.05-5.33)	1 (reference)	1 (reference)	
Daily	2.09 (0.40-10.93)	7.62 (0.45-130.29)	1 (reference)	3.50 (0.14-84.69)	2.04 (0.39-10.71)	3.39 (0.19-61.21)	1.50 (0.06-40.41)
Consumption of fish							
Non-daily	1 (reference)	2.74 (0.79-9.52)	1 (reference)	2.68 (0.78-9.23)	1 (reference)	1 (reference)	
Daily	1.72 (0.67-4.42)	3.69 (1.23-11.11)	1 (reference)	2.13 (0.77-5.88)	1.72 (0.67-4.40)	1.33 (0.36-4.87)	0.78 (0.16-3.88)
BMI							
<25	1 (reference)	1.94 (0.66-5.71)	1 (reference)	1.92 (0.67-5.94)	1 (reference)	1 (reference)	
≥25	1.50 (0.58-3.90)	4.50 (1.50-13.48)	1 (reference)	2.98 (0.89-9.92)	1.50 (0.58-3.89)	2.27 (0.61-8.41)	1.54 (0.31-7.73)
Drugs for hypertension							
Non-user	1 (reference)	2.19 (0.80-6.00)	1 (reference)	2.19 (0.80-5.97)	1 (reference)	1 (reference)	
User	0.92 (0.30-2.80)	2.58 (0.82-8.11)	1 (reference)	2.78 (0.74-10.43)	0.87 (0.28-2.69)	1.33 (0.32-5.48)	1.28 (0.24-6.73)

in models including age, genotype, 3 dummy variables for ex-drinkers, current drinkers with ethanol levels less than 50 mg/d, and current drinkers with ethanol levels greater than 50 mg/d, and an interaction term for genotype × current drinkers with ethanol levels greater than 50 mg/d.

When male subjects were analyzed according to walking time, the aORs for *non-5/5* compared with *5/5* were much higher for men with more than 30 minutes of daily walking (aOR = 5.30; 95% CI, 1.79-15.75) than for men with 30 minutes or less of daily walking (aOR = 0.95; 95% CI, 0.27-3.38). The aORs for more than 30 minutes of daily walking in men were 0.31 (95% CI, 0.12-0.81) for the *5/5* genotype and 1.54 (95% CI, 0.40-5.95) for *non-5/5* genotypes. The aOR for the gene-environment interaction between the genotype and walking time was 4.92 (95% CI, 0.95-25.64).

Significant aORs were found for ex-smokers with *non-5/5* genotypes relative to never smokers with the *5/5* genotype, for *non-5/5* genotypes among those exercising less than 1 h/wk and among those not consuming meat daily, for daily meat consumers with *non-5/5* genotypes relative to non–daily meat consumers with the *5/5* genotype, and for *non-5/5* genotypes with a BMI of 25 or higher relative to the *5/5* genotype with a BMI of less than 25 (Table 4).

4. Discussion

Although a genetically determined reduction in SUA levels was recently reported [26], elevation is more commonly associated with genetic traits and lifestyle. At present, age, sex, menopause, food consumption, alcohol intake, obesity, a sedentary lifestyle, dyslipidemia, insulin resistance, blood pressure, renal function, and drug use for hypertension can be listed as factors related to SUA levels [27-32]. Among these factors, elevated SUA was associated with heavy drinking and high BMI in men and with obesity, drug use for hypertension, hyperlipidemia, and reduced renal function in women in the present study. In addition, associations with the *ecNOS* VNTR genotype were evident in male checkup examinees within the reference ranges of blood tests. Because of the low number of women with hyperuricemia (SUA, ≥7 mg/dL), we could not make a precise evaluation of risk. When women with a SUA level of 6 mg/dL or higher (n = 54) were regarded as having hyperuricemia, the aOR of *non-5/5* genotypes was 0.87 (95% CI, 0.44-1.73), such that any link appears limited.

The VNTR polymorphism genotype distribution found here is similar to that reported in a larger Japanese study (N = 832): 1.3% for the *4/4* genotype, 18.4% for the *4/5* genotype, and 80.3% for the *5/5* genotype [33]. Two individuals with a *6*-repeat allele were found in the present sample. This has been previously reported in Japan [34] as well as in Germany [35]. In 197 of our study participants, VNTR polymorphism was completely linked with T-786C: the *4*-repeat allele to the *-786C* allele and the *5*- or *6*-repeat allele to the *-786T* allele, as found in other studies on Asians [12-15]. Exclusion or inclusion of the *5/6* genotype into the *5/5* group did not change the results substantially.

One previous study provided evidence that SUA levels may be genetically predetermined by VNTR *ecNOS* gene polymorphism in diabetic female subjects; subjects with the *ecNOS 5/5* genotype had significantly higher SUA levels ($P < .01$) than those with other genotypes, whereas in healthy subjects or diabetic men, the study documented the insignificant opposite association [36]. Our findings were consistent with the latter results and fit the biologic mechanism to regulate the SUA under the assumption that the *4*-repeat allele is a lower expression allele, as shown in Fig. 1.

Associations of the *4*-repeat allele or the *-786C* allele have been reported with low ecNOS protein level, ecNOS activity, and *ecNOS* gene promoter activity [13,16,17]. In addition, levels of exhaled NO or NO metabolite levels were significantly lower in subjects with the *4/4* and *4/5* genotypes than in their *5/5* counterparts [23,37,38] although inverse associations were also reported [21,23]. One previous study pointed to a close circadian inverse relationship between NO and SUA [39], and another study showed a significant association between impairment of vascular NO activity and elevated SUA [40]. This may be caused by the ability of NO to modulate UA production through its influence on XO activity. These findings indicate that subjects with *non-5/5* genotypes have lower enzyme activity, resulting in lower NO levels, reduced suppression of XO, and increased synthesis of UA (see Fig. 1). The present study supported the hypothesis with the finding that SUA levels were high in healthy subjects with the *non-5/5* genotypes. However, because the NO and XO levels were not measured in this study's subjects, another mechanism for the observed associations could not be denied.

Interactions of genetic factors with lifestyle or physical conditions appear important for the control of SUA. To the best of our knowledge, this is the first report about possible gene-environment interactions for hyperuricemia. Although the interaction term was not significant, our men with *non-5/5* genotypes had a higher aOR (8.93) for drinking than men with the *5/5* genotype did (aOR = 1.76), suggesting that their SUA levels may be effectively lowered by reducing alcohol intake. Because the aOR for *non-5/5* genotypes among male never drinkers was less than unity, no association between SUA and the *ecNOS* genotype among women may partly be explained by the large proportion of never drinkers. The amount of alcohol intake was also smaller among women than among men, which made it difficult to evaluate the effects of drinking among women.

Alcohol is known to increase SUA through several mechanisms: increased production of UA by increasing purine nucleotide degradation in processes of ethanol metabolism [41]; reduction in urinary UA output by

increasing blood lactate produced by the oxidation of ethanol [42]; and increased production of UA by loading of purine from alcohol itself [43]. However, it was reported that ethanol induced a rapid increase of ecNOS protein and mRNA expression levels and activity [44-48]. Ethanol modulates tyrosine kinase activity [49], and tyrosine kinase regulates expression of ecNOS via posttranscriptional mechanisms [50,51]. In addition, ethanol dose dependently increased basal ecNOS activity via a mechanism involving a pertussis toxin-sensitive G protein in the absence of any effect on cell viability or NOS protein expression [46]. Therefore, it was supposed that ethanol is a possibility with the function to suppress production of UA. The influence of ethanol on ecNOS activity and UA production may differ among those with different *ecNOS* genotypes.

Moreover, our men with a *5/5* genotype had a lower aOR (0.31) for walking more than 30 min/d than men with *non-5/5* genotypes did (aOR = 1.54). It is well known that SUA is temporarily elevated by strenuous muscular exercise [52]. Exercise decreases UA excretion [53] and accelerates purine degradation (adenine nucleotide degradation) in muscles, leading to an increase in the production of hypoxanthine, the end product of purine degradation [54-56]. A study showed that the accelerated purine degradation was observed at the status exceeding anaerobic threshold but not at that below the threshold [57]. Although the question on frequency of sports did not show the association with SUA among men with the *5/5* genotype, more than 30 minutes of daily walking decreased risk among them. This finding may reflect that regular aerobic exercise stimulates ecNOS activity and increases NO release with a high-expression homozygous genotype [58]. Because exercise is known to elevate UA levels, clinicians may hesitate to recommend exercise to patients with hyperuricemia. However, mild exercise such as walking would be recommended at least to men with the *5/5* genotype.

Significantly elevated aORs were found for subgroups defined by the genotype, meat consumption, fish consumption, and BMI. The point estimations for the interactions were not marked. Accordingly, the effect modification by *ecNOS* genotype did not seem plausible.

In conclusion, the present study showed a significant association between hyperuricemia and the *ecNOS* 27-bp VNTR polymorphism in male health checkup examinees. Because the prevalence of hyperuricemia in men is higher than that in women, our results are important in terms of preventive strategies for men. Taking into consideration the observed possible interactions of drinking and walking with the *ecNOS* genotype for hyperuricemia, reducing alcohol intake may be more protective among men with the *non-5/5* genotypes and more than 30 minutes of daily walking may be more protective among men with the *5/5* genotype. Although confirmation is required, our results could be applied to optimize preventive programs based on individual host factors against hyperuricemia, an important risk factor for cardiovascular disease.

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References

[1] Alderman M, Aiyer KJ. Uric acid: role in cardiovascular disease and effects of losartan. Curr Med Res Opin 2004;20:369-79.
[2] Niskanen LK, Laaksonen DE, Nyyssonen K, et al. Uric acid level as a risk factor for cardiovascular and all-cause mortality in middle-aged men: a prospective cohort study. Arch Intern Med 2004;164:1546-51.
[3] Tomita M, Mizuno S, Yamanaka H, et al. Does hyperuricemia affect mortality?: a prospective cohort study of Japanese male workers. J Epidemiol 2000;10:403-9.
[4] Zimmet P, Alberti KG, Shaw J. Global and societal implications of the diabetes epidemic. Nature 2001;414:782-7.
[5] Abiaka C, Al-Awadi F, Gulshan S, et al. Plasma concentrations of alpha-tocopherol and urate in patients with different types of cancer. J Clin Pharm Ther 2001;26:265-70.
[6] Wortmann RL. Gout and hyperuricemia. Curr Opin Rheumatol 2002; 14:281-6.
[7] Emmerson BT. The management of gout. N Engl J Med 1996;334: 445-51.
[8] Fukahori M, Ichimori K, Ishida H, et al. Nitric oxide reversibly suppresses xanthine oxidase activity. Free Radic Res 1994;21:203-12.
[9] Cote CG, Yu FS, Zulueta JJ, et al. Regulation of intracellular xanthine oxidase by endothelial-derived nitric oxide. Am J Physiol 1996;271: 869-74.
[10] Houston M, Chumley P, Radi R, et al. Xanthine oxidase reaction with nitric oxide and peroxynitrite. Arch Biochem Biophys 1998;355:1-8.
[11] Moncada S, Higgs A. The L-arginine–nitric oxide pathway. N Engl J Med 1993;329:2002-12.
[12] Tanus-Santos JE, Desai M, Flockhart DA. Effects of ethnicity on the distribution of clinically relevant endothelial nitric oxide variants. Pharmacogenetics 2001;11:719-25.
[13] Song J, Yoon Y, Park KU, et al. Genotype-specific influence on nitric oxide synthase gene expression, protein concentrations, and enzyme activity in cultured human endothelial cells. Clin Chem 2003;49: 847-52.
[14] Yoshimura M, Yasue H, Nakayama M, et al. Genetic risk factors for coronary artery spasm: significance of endothelial nitric oxide synthase gene T-786→C and missense Glu298Asp variants. J Investig Med 2000;48:367-74.
[15] Asakimori Y, Yorioka N, Taniguchi Y, et al. T^{-786}→C polymorphism of the endothelial nitric oxide synthase gene influences the progression of renal disease. Nephron 2002;91:747-51.
[16] Wang XL, Sim AS, Wang MX, et al. Genotype dependent and cigarette specific effects on endothelial nitric oxide synthase gene expression and enzyme activity. FEBS Lett 2000;471:45-50.
[17] Nakayama M, Yasue H, Yoshimura M, et al. T^{-786}→C mutation in the 5′-flanking region of the endothelial nitric oxide synthase gene is associated with coronary spasm. Circulation 1999;99:2864-70.
[18] Grasemann H, van's Gravesande KS, Buscher R, et al. Effects of sex and of gene variants in constitutive nitric oxide synthases on exhaled nitric oxide. Am J Respir Crit Care Med 2003;167:1113-6.
[19] Moon J, Yoon S, Kim E, et al. Lack of evidence for contribution of Glu298Asp (G894T) polymorphism of endothelial nitric oxide

synthase gene to plasma nitric oxide levels. Thromb Res 2002;107: 129-34.
[20] Jeerooburkhan N, Jones LC, Bujac S, et al. Genetic and environmental determinants of plasma nitrogen oxides and risk of ischemic heart disease. Hypertension 2001;38:1054-61.
[21] Yoon S, Moon J, Shin C, et al. Smoking status-dependent association of the 27-bp repeat polymorphism in intron 4 of endothelial nitric oxide synthase gene with plasma nitric oxide concentrations. Clin Chim Acta 2002;324:113-20.
[22] Tsukada T, Yokoyama K, Arai T. Evidence of association of the *ecNOS* gene polymorphism with plasma NO metabolite levels in humans. Biochem Biophys Res Commun 1998;245:190-3.
[23] 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 1997;17:3147-53.
[24] Ito Y, Suzuki S, Yagyu K, et al. Relationship between serum carotenoid levels and cancer death rates in the residents, living in a rural area of Hokkaido, Japan. J Epidemiol 1997;7:1-8.
[25] Khoury MJ, Flanders WD. Non-traditional epidemiologic approaches in the analysis of gene-environment interaction: case-control studies with no controls. Am J Epidemiol 1996;144:207-13.
[26] Komoda F, Sekine T, Inatomi J, et al. The W258X mutation in SLC22A12 is the predominant cause of Japanese renal hypouricemia. Pediatr Nephrol 2004;19:728-33.
[27] Burack RC, Keller JB, Higgins HW. Cardiovascular risk factors and obesity: are baseline levels of blood pressure, glucose, cholesterol and uric acid elevated prior to weight gain? J Chronic Dis 1985;38:865-72.
[28] Benedek TG. Correlations of serum uric acid and lipid concentrations in normal, gouty, and atherosclerotic men. Ann Intern Med 1967; 66:851-61.
[29] Alderman MH. Uric acid in hypertension and cardiovascular disease. Can J Cardiol 1999;15(Suppl F):20-2.
[30] 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 1974;196:93-102.
[31] 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. Ann Epidemiol 1998;8:250-61.
[32] 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 1992;42:333-5.
[33] Kimura T, Yokoyama T, Matsumura Y, et al. NOS3 genotype–dependent correlation between blood pressure and physical activity. Hypertension 2003;41:355-60.
[34] Droma Y, Hanaoka M, Ota M, et al. Positive association of the endothelial nitric oxide synthase gene polymorphisms with high-altitude pulmonary edema. Circulation 2002;106:826-30.
[35] Sigusch HH, Surber R, Lehmann MH, et al. Lack of association between 27-bp repeat polymorphism in intron 4 of the endothelial nitric oxide synthase gene and the risk of coronary artery disease. Scand J Clin Lab Invest 2000;60:229-35.
[36] Lee YJ, Chang DM, Tsai JC. 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. Metabolism 2003;52:1448-53.
[37] Thomas S, Bruce C, Birkhead A, et al. Effect of *ecNOS* polymorphisms and coronary artery disease upon exhaled nitric oxide. J Mol Med 2002;80:181-6.
[38] Tanus-Santos JE, Desai M, Deak LR, et al. Effects of endothelial nitric oxide synthase gene polymorphisms on platelet function, nitric oxide release, and interactions with estradiol. Pharmacogenetics 2002;12: 407-13.
[39] Kanabrocki EL, Third JL, Ryan MD, et al. Circadian relationship of serum uric acid and nitric oxide. JAMA 2000;283:2240-1.
[40] 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 2001;38:1850-8.
[41] Nishimura T, Shimizu T, Mineo I, et al. Influence of daily drinking habits on ethanol-induced hyperuricemia. Metabolism 1994;43: 745-8.
[42] Lieber CS, Jones DP, Losowsky MS, et al. Interrelation of uric acid and ethanol metabolism in man. J Clin Invest 1962;41:1863-70.
[43] Gibson T, Rodgers AV, Simmonds HA, et al. A controlled study of diet in patients with gout. Ann Rheum Dis 1983;42:123-7.
[44] Srivastava VK, Hiney JK, Rettori V, et al. Effects of ethanol on intraovarian nitric oxide production in the prepubertal rat. J Endocrinol 1999;161:69-75.
[45] Venkov CD, Myers PR, Tanner MA, et al. Ethanol increases endothelial nitric oxide production through modulation of nitric oxide synthase expression. Thromb Haemost 1999;81:638-42.
[46] Hendrickson RJ, Cahill PA, Sitzmann JV, et al. Ethanol enhances basal and flow-stimulated nitric oxide synthase activity in vitro by activating an inhibitory guanine nucleotide binding protein. J Pharmacol Exp Ther 1999;289:1293-300.
[47] Kay HH, Grindle KM, Magness RR. Ethanol exposure induces oxidative stress and impairs nitric oxide availability in the human placental villi: a possible mechanism of toxicity. Am J Obstet Gynecol 2000;182:682-8.
[48] Acevedo CG, Carrasco G, Burotto M, et al. Ethanol inhibits L-arginine uptake and enhances NO formation in human placenta. Life Sci 2001;18:2893-903.
[49] Thurston Jr AW, Shukla SD. Ethanol modulates epidermal growth factor–stimulated tyrosine kinase and phosphorylation of PLC-gamma 1. Biochem Biophys Res Commun 1992;30:1062-8.
[50] Greenberg SS, Jie O, Zhao X, et al. The potential mechanism of induction of inducible nitric oxide synthase mRNA in alveolar macrophages by lipopolysaccharide and its suppression by ethanol, in vivo. Alcohol Clin Exp Res 1998;22:260S-5S.
[51] Marsen TA, Egink G, Suckau G, et al. Tyrosine-kinase–dependent regulation of the nitric oxide synthase gene by endothelin-1 in human endothelial cells. Pflugers Arch 1999;438:538-44.
[52] Sutton JR, Toews CJ, Ward GR, et al. Purine metabolism during strenuous muscular exercise in man. Metabolism 1980;29:254-60.
[53] Ka T, Yamamoto T, Moriwaki Y, et al. Effect of exercise and beer on the plasma concentration and urinary excretion of purine bases. J Rheumatol 2003;30:1036-42.
[54] Lowenstein JM. The purine nucleotide cycle revisited [corrected]. Int J Sports Med 1990;11(Suppl 2):S37-S46.
[55] Westing YH, Ekblom B, Sjodin B. The metabolic relation between hypoxanthine and uric acid in man following maximal short-distance running. Acta Physiol Scand 1989;137:341-5.
[56] Sjodin B, Hellsten Westing Y. Changes in plasma concentration of hypoxanthine and uric acid in man with short-distance running at various intensities. Int J Sports Med 1990;11:493-5.
[57] Yamanaka H, Kawagoe Y, Taniguchi A, et al. Accelerated purine nucleotide degradation by anaerobic but not by aerobic ergometer muscle exercise. Metabolism 1992;41:364-9.
[58] Higashi Y, Sasaki S, Kurisu S, et al. Regular aerobic exercise augments endothelium-dependent vascular relaxation in normotensive as well as hypertensive subjects: role of endothelium-derived nitric oxide. Circulation 1999;100:1194-202.