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Association of a polymorphism of the transforming growth factor-β1 gene with blood pressure in Japanese individuals

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Abstract Transforming growth factor-β1 (TGF-β1) is an important regulator of blood pressure (BP) and vascular remodeling, and thus may contribute to the pathogenesis of hypertension. A T→C transition at nucleotide 869 of the *TGF-β1* gene results in a Leu→Pro substitution at amino acid 10 of the signal peptide. We have now examined the possible association of the 869T→C polymorphism of the *TGF-β1* gene with BP and the prevalence of hypertension in 2241 community-dwelling Japanese individuals (1126 men and 1115 women). TGF-β1 genotype was determined by an allele-specific polymerase chain reaction method. For women, both systolic and diastolic BP was significantly higher in individuals with the *CC* genotype than in those with the *TT* or *TC* genotype. No significant association between *TGF-β1* genotype and BP was detected in men. The frequency of the *CC* genotype was significantly higher in women with hypertension than in those with normal BP. These results suggest that the *TGF-β1* gene at chromosome 19q13.1 may be a candidate susceptibility locus for hypertension in Japanese women.

Key words Transforming growth factor-β1 · Gene polymorphism · Blood pressure · Hypertension · Population-based study

Introduction

The regulation of blood pressure (BP) involves both the integration of a variety of biological systems that control the structure and tone of the vasculature and the volume and composition of body fluid, as well as the adaptation of these systems to constantly changing physiological needs (Lalouel and Rohrwasser 2001). Hypertension is a complex multifactorial and polygenic disorder that is thought to result from an interaction between an individual's genetic background and various environmental factors (Lifton et al. 2001). Recent advances in genetic epidemiology have revealed that certain genetic variants, including polymorphisms in the genes encoding angiotensinogen (Jeunemaitre et al. 1992), the β3 subunit of G proteins (Siffert et al. 1998), and the β2-adrenergic receptor (Bray et al. 2000), increase the risk of hypertension.

Transforming growth factor-β (TGF-β) is the prototype of a large family of cytokines (Heldin et al. 1997). Three isoforms of TGF-β (TGF-β1, -β2, and -β3) have been identified in mammals, and these isoforms exhibit similar biological properties. TGF-β directly stimulates the synthesis of extracellular matrix proteins and inhibits matrix degradation (Roberts et al. 1992a). It may influence BP by stimulating both the production of endothelin-1 by vascular endothelial cells (Kurihara et al. 1989) and the release of renin from juxtaglomerular cells of the kidney (Antonipillai et al. 1993). Li et al. (1999) demonstrated a positive correlation between the circulating concentration of TGF-β1 and BP in humans. In addition, the upregulation of TGF-β1 expression was shown to be associated with cardiovascular and renal alterations in individuals with hypertension (August et al. 2000).

Several single-nucleotide polymorphisms (SNPs) have been detected in the TGF-β1 gene, including a 869T→C (Leu10Pro) transition at codon 10 and a 914G→C (Arg25Pro) transversion at codon 25 in the region encoding the signal peptide of this protein (Derynck et al. 1987; Cambien et al. 1996; Grainger et al. 1999). Cambien et al. (1996) showed that the 914G→C polymorphism was associated with hypertension, with the *C* allele reflecting a lower systolic pressure and a lower frequency of a history of hypertension. Suthanthiran et al. (2000) showed that the *C* allele of the 869T→C polymorphism was more frequent in African–Americans than in Caucasian Americans and was

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associated with both a greater abundance of TGF-β1 mRNA in peripheral blood mononuclear cells and higher serum concentrations of the protein. Recently, Rivera et al. (2001) showed that the 869T→C polymorphism was associated with systolic BP measured at rest as well as at moderate and maximal exercise intensities in Caucasians.

Clarification of the role of TGF-β1 in the development of hypertension should be facilitated by characterization of the relations of genetic variants that may affect the production, secretion, or activity of this cytokine to BP in various ethnic groups. We have now studied the relation of the 869T→C polymorphism of the *TGF-β1* gene to both BP and the prevalence of hypertension in community-dwelling Japanese men and women.

Subjects and methods

Study population

The National Institute for Longevity Sciences–Longitudinal Study of Aging (NILS-LSA), a population-based prospective cohort study of aging and age-related diseases, was begun in 1997 (Shimokata et al. 2000). We have examined 2241 participants (1126 men and 1115 women) of the NILS-LSA, all of whom were community-dwelling individuals aged 40 to 79 years and randomly recruited from Obu City and regions close to NILS in Aichi Prefecture, Japan. A total of 1477 participants (746 men and 731 women) had normal BP (systolic BP of <140 mmHg and diastolic BP of <90 mmHg), and 754 individuals (377 men and 377 women) had hypertension (systolic BP of ≥140 mmHg or diastolic BP of ≥90 mmHg, or both) or had taken antihypertensive drugs; the remaining 10 subjects (3 men and 7 women) had borderline hypertension (hypertension and normal BP at the first and second measurements, respectively) and did not take antihypertensive medication. Individuals with congenital malformations of the heart or vessels, valvular heart disease, or renal or endocrinologic diseases that cause secondary hypertension were excluded from the study. BP was measured with subjects in the seated position according to the guidelines of the American Heart Association (Perloff et al. 1993). The study protocol was approved by the Committee on the Ethics of Human Research of National Chubu Hospital and the National Institute for Longevity Sciences, and written informed consent was obtained from each subject.

Genotyping of the *TGF-β1* gene polymorphism

Venous blood (7 ml) was collected from each subject into tubes containing 50 mmol/l ethylenediaminetetraacetate (disodium salt), and genomic DNA was isolated with an automated system (model NA-1000; Kurabo, Osaka, Japan). TGF-β1 genotype was determined by an allele-specific polymerase chain reaction (PCR)–based method, as previously described (Yamada et al. 1998, 2000; Yokota et al. 2000), with two sense primers (S1, 5′-CTCCGGGCTGCGGCTGCTGCT-3′; S2, 5′-CTCCGGGCTGCGGCTGCTGCC-3′) and one antisense primer (AS, 5′-GTTGTGGGTTTCCACCATTAG-3′). Amplification reactions were performed in a total volume of 25 μL containing 0.25 μg of genomic DNA, 10 pmol of each primer, 0.2 mmol/l each of deoxycytidine triphosphate, deoxythymidine triphosphate, deoxyguanosine triphosphate, and deoxyadenosine triphosphate, 0.5 U of *Taq* DNA polymerase (Amplitaq Gold; Perkin Elmer, Foster City, CA, USA), 50 mmol/ KCl, 1.5 mmol/l $MgCl_2$, 1.5% dimethyl sulfoxide, 0.01% gelatin, and 10 mmol/l Tris-HCl (pH 8.3). The thermocycling procedure consisted of an initial denaturation at 94°C for 5 min; 35 cycles of denaturation (94°C for 30 s), annealing (60°C for 30 s), and extension (72°C for 30 s); and a final extension at 72°C for 5 min. The PCR products were analyzed by 1% agarose gel electrophoresis and visualized by ethidium bromide staining. The expected size of the specific amplification product was 346 bp.

Statistical analysis

Data are presented as means ± SD. BP and other quantitative data were compared among TGF-β1 genotypes by one-way analysis of variance and the Tukey-Kramer post hoc test. BP values were analyzed with adjustment for age, body mass index (BMI), smoking status, alcohol consumption, and salt intake by the least squares method in a general linear model. BP values were also subjected to analysis of covariance and the Tukey-Kramer test in a general linear model. Qualitative data were compared by the chi-square test. Allele frequencies were estimated by the gene counting method, and the significance of deviation from Hardy-Weinberg equilibrium was analyzed by the chi-square test. A P value of <0.05 was considered statistically significant.

Results

We examined the effect of TGF-β1 genotype on BP in a total of 1758 subjects (876 men and 882 women), consisting of 1477 individuals with normal BP and 281 untreated individuals (130 men and 151 women) with borderline or mild hypertension (Tables 1 to 3). The characteristics of these subjects are shown in Table 1. The distribution of TGF-β1 genotype was in Hardy-Weinberg equilibrium both in men and in women. For women, the fasting blood sugar concentration was significantly greater in individuals with the *CC* genotype than in those with the *TC* genotype. BMI and serum asparate aminotransferase activity were lower and the serum concentration of creatinine was higher in women with the *TC* genotype than in those with the *TT* genotype. For men, the serum concentrations of total cholesterol and triglycerides were lower in individuals with the *CC* genotype than in those with the *TT* or *TC* genotype. BMI was greater in men with the *TC* genotype than in those with the *TT* genotype.

For women, both systolic and diastolic BP values were significantly greater in individuals with the *CC* genotype

Table 1. Characteristics of 1758 subjects according to TGF-β1 genotype

Characteristic	Men (n = 876)			Women (n = 882)		
	TT	*TC*	*CC*	*TT*	*TC*	*CC*
No. of subjects	238 (27.2%)	441 (50.3%)	197 (22.5%)	266 (30.2%)	465 (52.7%)	151 (17.1%)
Age (years)	55.7 ± 10.8	57.4 ± 12.6	57.6 ± 11.2	57.3 ± 11.4	56.4 ± 10.8	55.3 ± 12.3
BMI (kg/m^2)	22.4 ± 3.1	23.0 ± 2.1*	22.7 ± 2.8	23.0 ± 3.3	22.4 ± 2.2*	22.9 ± 3.7
Smoking (pack-year)	23.6 ± 20.0	25.3 ± 18.9	24.3 ± 21.0	1.8 ± 4.9	1.3 ± 6.5	1.8 ± 6.2
Alcohol consumption (g/day)	29.1 ± 37.0	26.0 ± 37.8	23.3 ± 37.8	6.0 ± 17.9	4.7 ± 17.3	5.8 ± 19.7
Salt intake (g/day)	13.3 ± 4.6	13.1 ± 4.2	12.8 ± 4.2	11.3 ± 3.3	11.2 ± 4.3	11.5 ± 3.7
Blood examination						
Hemoglobin (g/dL)	15.6 ± 1.5	15.6 ± 2.1	15.4 ± 1.4	13.6 ± 1.6	13.6 ± 2.2	13.7 ± 1.2
Asparate aminotransferase (IU/L)	26.5 ± 15.4	27.6 ± 16.8	26.7 ± 16.8	25.5 ± 13.0	23.2 ± 13.0†	23.8 ± 12.3
Alanine aminotransferase (IU/L)	27.9 ± 20.0	27.7 ± 21.0	26.3 ± 21.0	22.4 ± 16.3	19.6 ± 17.3	20.7 ± 17.2
γ-Glutamyl transferase (IU/L)	56.2 ± 70.8	61.5 ± 71.4	57.2 ± 72.8	29.8 ± 24.5	26.7 ± 25.9	29.7 ± 24.6
Total cholesterol (mg/dL)	215 ± 31	212 ± 21	206 ± 28‡	228 ± 33	225 ± 43	227 ± 37
HDL-cholesterol (mg/dL)	57.4 ± 13.9	58.2 ± 12.6	56.2 ± 14.0	66.0 ± 14.7	66.9 ± 15.1	64.5 ± 14.8
Triglycerides (mg/dL)	127 ± 77	139 ± 84	120 ± 84§	108 ± 49	105 ± 65	113 ± 62
Fasting blood sugar (mg/dL)	104 ± 15	103 ± 21	103 ± 14	99 ± 16	98 ± 22	102 ± 12§
Hemoglobin A_{1c} (%)	5.29 ± 0.62	5.36 ± 0.63	5.29 ± 0.70	5.13 ± 0.49	5.13 ± 0.43	5.23 ± 0.49
Blood urea nitrogen (mg/dL)	16.3 ± 13.9	16.3 ± 14.7	14.6 ± 25.2	13.7 ± 11.4	15.5 ± 13.0	14.4 ± 11.1
Creatinine (mg/dL)	1.00 ± 0.31	1.03 ± 0.21	1.00 ± 0.28	0.76 ± 1.63	0.78 ± 2.16*	0.77 ± 0.12
Sodium (mEq/L)	143 ± 5	143 ± 6	144 ± 9	143 ± 6	142 ± 6	143 ± 5
Potassium (mEq/L)	4.14 ± 1.23	4.20 ± 1.26	4.28 ± 2.24	4.07 ± 1.14	4.22 ± 1.30	4.10 ± 0.98
Calcium (mg/dL)	9.27 ± 0.31	9.27 ± 0.21	9.26 ± 0.28	9.25 ± 0.33	9.27 ± 0.22	9.26 ± 0.37
Magnesium (mg/dL)	2.20 ± 0.15	2.20 ± 0.21	2.21 ± 0.14	2.17 ± 0.16	2.19 ± 0.22	2.17 ± 0.12

Data are means ± SD
BMI, Body mass index
* P = 0.03 versus *TT*, $^\dagger P$ = 0.04 versus *TT*, $^\ddagger P$ = 0.01 versus *TT*, $^\S P$ = 0.02 versus *TC*

Table 2. Systolic and diastolic BP in 1758 subjects according to TGF-β1 genotype

	Men (n = 876)			Women (n = 882)		
	TT (n = 238)	*TC* (n = 441)	*CC* (n = 197)	*TT* (n = 266)	*TC* (n = 465)	*CC* (n = 151)
Systolic BP (mmHg)	120.0 ± 16.9	121.0 ± 16.8	122.0 ± 18.2	119.7 ± 19.6	118.9 ± 19.4	124.7 ± 18.5*
Diastolic BP (mmHg)	74.9 ± 10.8	75.5 ± 10.5	75.7 ± 11.2	72.3 ± 11.4	72.0 ± 10.8	75.6 ± 11.1†
Adjusted for age (years), BMI (kg/m^2), smoking (pack-year), alcohol consumption (g/day), and salt intake (g/day)						
Systolic BP (mmHg)	121.1 ± 16.9	120.1 ± 16.8	122.0 ± 16.8	118.1 ± 17.9	119.0 ± 17.3	123.9 ± 18.5‡
Diastolic BP (mmHg)	75.1 ± 10.8	75.1 ± 10.5	75.9 ± 9.8	72.0 ± 11.4	72.2 ± 10.8	75.4 ± 11.1§

Data are means ± SD
BP, Blood pressure; BMI, body mass index
* P = 0.03 versus *TT*, P = 0.003 versus *TC*; $^\dagger P$ = 0.02 versus *TT*, P = 0.001 versus *TC*; $^\ddagger P$ = 0.006 versus *TT*, P = 0.015 versus *TC*; $^\S P$ = 0.007 versus *TT*, P = 0.006 versus *TC*

Table 3. General linear model for analysis of factors that affect systolic and diastolic BP in 1758 subjects

	Men (n = 876)				Women (n = 882)			
	Systolic BP		Diastolic BP		Systolic BP		Diastolic BP	
	SRC	*P*	SRC	*P*	SRC	*P*	SRC	*P*
TGF-β1 genotype (*TT* = *TC* = 0, *CC* = 1)	0.038	0.273	0.034	0.318	0.103	0.002	0.113	<0.001
Age (years)	0.123	<0.001	0.035	0.331	0.246	<0.001	0.156	<0.001
BMI (kg/m^2)	0.252	<0.001	0.273	<0.001	0.305	<0.001	0.300	<0.001
Smoking (pack-year)	−0.057	0.110	−0.051	0.150	−0.011	0.757	−0.012	0.728
Alcohol consumption (g/day)	0.159	<0.001	0.191	<0.001	0.014	0.681	0.020	0.562
Salt intake (g/day)	−0.064	0.074	−0.030	0.390	−0.026	0.437	−0.033	0.341

Data were subjected to analysis of covariance and the Tukey-Kramer test in a general linear model
BP, Blood pressure; SRC, standardized regression coefficient; BMI, body mass index

Table 4. Association of TGF-β1 genotype with the prevalence of hypertension in 1477 subjects with normal BP and 754 subjects with hypertension

	Men (n = 1123)		Women (n = 1108)	
	Normal (n = 746)	Hypertension (n = 377)	Normal (n = 731)	Hypertension (n = 377)
TGF-β1 genotype				
TT	210 (28.2%)	94 (24.9%)	226 (30.9%)	114 (30.2%)
TC	372 (49.9%)	203 (53.8%)	392 (53.6%)	184 (48.8%)
TT + *TC*	582 (78.0%)	297 (78.8%)	618 (84.5%)	298 (79.0%)
CC	164 (22.0%)	80 (21.2%)	113 (15.5%)	79 (21.0%)
P (*TT* + *TC* versus *CC*)		0.770		0.017

Normal, systolic BP of <140 mmHg and diastolic BP of <90 mmHg; hypertension, systolic BP of ≥140 mmHg and/or diastolic BP of ≥90 mmHg
BP, Blood pressure

than in those with the *TT* genotype and those with the *TC* genotype (Table 2). Given that aging, obesity, smoking, excessive alcohol consumption, and increased salt intake are conventional risk factors for hypertension, we analyzed BP values after adjustment for age, BMI, smoking status, alcohol consumption, and salt intake by the least squares method. The association of TGF-β1 genotype with BP did not change after adjustment for these factors. For men, however, no difference in systolic or diastolic BP was detected among TGF-β1 genotypes.

Analysis with a general linear model revealed that TGF-β1 genotype, age, and BMI significantly influenced both systolic and diastolic BP in women (Table 3). In men, BMI and alcohol consumption significantly affected both systolic and diastolic BP, with age affecting only systolic BP.

To clarify further the effect of the *C* allele on BP, we compared the distribution of TGF-β1 genotypes between 1477 individuals with normal BP and 754 individuals with hypertension (Table 4). The frequency of the *CC* genotype was significantly higher in subjects with hypertension than in those with normal BP for women. In contrast, the *C* allele was not associated with the prevalence of hypertension in men.

Discussion

TGF-β1 stimulates the expression of endothelin-1 (Kurihara et al. 1989) and inhibits the production of nitric oxide (Roberts et al. 1992b) in vascular endothelial cells, which would be expected to result in an increase in BP. It also stimulates renin release from juxtaglomerular cells (Antonipillai et al. 1993), which likely results in an increased generation of angiotensin II and a consequent increase in BP. In addition, TGF-β1 promotes the deposition of extracellular matrix proteins on vessel walls, thereby influencing their stiffness and compliance (O'Callaghan and Williams 2000). Thus, TGF-β1 appears to play an important role in the regulation of BP and the development of hypertension. We have now examined the association of variants of the *TGF-β1* gene with both BP and the prevalence of hypertension in community-dwelling Japanese. Our results show that the 869T→C polymorphism of the *TGF-β1* gene is associated with BP in women and that the *CC* genotype is more prevalent in women with hypertension than in those with normal BP.

We failed to detect an association of the 869T→C polymorphism of the *TGF-β1* gene with BP or with the prevalence of hypertension in Japanese men. We previously showed that the *T* allele of this polymorphism is a risk factor for predisposition to myocardial infarction in Japanese men but not in women (Yokota et al. 2000). The reason for these gender-dependent differences in the association of TGF-β1 genotype with hypertension or with myocardial infarction remains unclear.

In an association study of hypertension and myocardial infarction with SNPs in the *TGF-β1* gene, Cambien et al. (1996) showed that the 914G→C (Arg25Pro) polymorphism was associated with the prevalence of hypertension among populations in both France and Northern Ireland. The presence of the *C* (Pro25) allele was associated with lower systolic BP in the control group. Li et al. (1999) also showed that homozygosity for the *G* (Arg25) allele of this polymorphism was more frequent in individuals with hypertension than in normotensive controls in the United States. However, we were unable to detect this polymorphism in 102 Japanese subjects (data not shown). In contrast to our results, Cambien et al. (1996) did not detect an association of the 869T→C polymorphism with either BP or the prevalence of hypertension in their European populations. The distribution of the 869T→C polymorphism in European male control subjects [*TT*, 225 (35.8%); *TC*, 294 (47.2%); *CC*, 107 (17.0%)] (Cambien et al. 1996) was significantly different from that in men [*TT*, 238 (27.2%); *TC*, 441 (50.3%); *CC*, 197 (22.5%); $P = 0.0006$, chi-square test] and marginally different from that in women [*TT*, 266 (30.2%); *TC*, 465 (52.7%); *CC*, 151 (17.1%); $P = 0.056$] of our population. Thus, the prevalence of TGF-β1 polymorphisms may differ among races.

Suthanthiran et al. (2000) showed that the distribution of 869T→C genotypes in African–Americans differed significantly from that in Caucasian Americans, in that there was

an excess of the *C* allele in the former when compared with the latter. These researchers also showed that the abundance of TGF-β1 mRNA in peripheral blood mononuclear cells and the serum concentration of TGF-β1 were both greater in individuals with the *CC* or *TC* genotype than in those with the *TT* genotype, consistent with our previous observation that the serum concentration of TGF-β1 increases with the number of *C* alleles (Yamada et al. 1998, 2000; Yokota et al. 2000). Although Suthanthiran et al. (2000) detected an association of the abundance of TGF-β1 mRNA in peripheral blood mononuclear cells and of the serum concentration of TGF-β1 with hypertension, they failed to demonstrate a direct association of the 869T→C polymorphism with the prevalence of hypertension. Rivera et al. (2001) recently showed that the 869T→C polymorphism was associated with systolic BP in Caucasians; the systolic BP of homozygotes for the *T* allele was thus significantly lower than that of homozygotes for the *C* allele, both at rest and at moderate or maximal exercise intensities. In contrast to the observation of Suthanthiran et al. (2000), these researchers observed no difference in allele frequencies between black and white subjects (Rivera et al. 2001). Our results show that the 869T→C polymorphism of the *TGF-β1* gene is associated with BP in Japanese women and that the *CC* genotype is more prevalent among women with hypertension, although the contribution of this polymorphism to hypertension appears to be relatively small.

TGF-β1 is synthesized in a latent form composed of 390 amino acids, with the active protein consisting of two identical disulfide-linked polypeptide chains corresponding to the 112 carboxyl-terminal residues of the precursor (Derynck et al. 1985). The Leu10Pro (869T→C) polymorphism is located in the signal peptide sequence of TGF-β1, which is thought to target newly synthesized protein to the endoplasmic reticulum (Verner and Schatz 1988). We previously showed that the serum concentration of TGF-β1 increases according to the rank order of 869T→C genotypes $TT < TC < CC$ (Yamada et al. 1998, 2000; Yokota et al. 2000). This association suggests that the Leu10Pro substitution may affect the function of the signal peptide, probably influencing intracellular trafficking or export efficiency of the protein. Although the serum concentration of TGF-β1 was not measured in the present study population, our observation that women with the *CC* genotype showed the highest BP is consistent with the previous observation that BP positively correlates with the serum concentration of TGF-β1 (Li et al. 1999).

It is possible that the 869T→C polymorphism of the *TGF-β1* gene is in linkage disequilibrium with some other gene polymorphism that is actually responsible for the development of hypertension. Our results, however, suggest that the *TGF-β1* gene located at chromosome 19q13.1 is a candidate susceptibility locus for hypertension in Japanese women.

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