

# Associations Between Plasma Betatrophin Levels and Coronary and Peripheral Artery Disease

Hanako Niki<sup>1</sup>, Yoshimi Kishimoto<sup>2</sup>, Susumu Ibe<sup>1</sup>, Emi Saita<sup>2</sup>, Kenji Sasaki<sup>1</sup>, Kotaro Miura<sup>1</sup>, Yukinori Ikegami<sup>1</sup>, Reiko Ohmori<sup>3</sup>, Kazuo Kondo<sup>2,4</sup> and Yukihiko Momiyama<sup>1</sup>

<sup>1</sup>Department of Cardiology, National Hospital Organization Tokyo Medical Center, Tokyo, Japan

<sup>2</sup>Endowed Research Department “Food for Health”, Ochanomizu University, Tokyo, Japan

<sup>3</sup>Faculty of Regional Design, Utsunomiya University, Tochigi, Japan

<sup>4</sup>Institute of Life Innovation Studies, Toyo University, Gunma, Japan

**Aim:** Betatrophin, a recently identified circulating adipokine, affects lipid and glucose metabolism. However, association between plasma betatrophin levels and atherosclerotic diseases, such as coronary artery disease (CAD) and peripheral artery disease (PAD), has not been elucidated.

**Methods:** We investigated plasma betatrophin levels in 457 patients undergoing elective coronary angiography who also had ankle-brachial index (ABI) test for PAD screening.

**Results:** Of the 457 study patients, CAD was present in 241 patients (53%) (1-vessel [1-VD],  $n=99$ ; 2-vessel [2-VD],  $n=71$ ; 3-vessel disease [3-VD],  $n=71$ ). Compared to 216 patients without CAD, 241 with CAD had higher betatrophin levels (median 1120 vs. 909 pg/mL,  $p<0.001$ ). A stepwise increase in betatrophin levels was found depending on the number of  $>50\%$  stenotic coronary vessels: 909 in CAD(-), 962 in 1-VD, 1097 in 2-VD, and 1393 pg/ml in 3-VD ( $p<0.001$ ). Betatrophin levels correlated with the number of  $>25\%$  stenotic segments ( $r=0.24$ ,  $p<0.001$ ). PAD (ABI $<0.9$ ) was found in 41 patients (9%). Plasma betatrophin levels were also significantly higher in 41 patients with PAD than in 416 without PAD (1354 vs. 981 pg/mL,  $p<0.001$ ). In the multivariate analysis, betatrophin levels were not a factor for CAD, but they were a significant factor for 3-VD and PAD independent of atherosclerotic risk factors. The odds ratios for 3-VD and PAD were 1.06 (95%CI=1.01-1.11) and 1.07 (95%CI=1.01-1.13) for a 100-pg/mL increase in betatrophin levels, respectively ( $p<0.05$ ).

**Conclusion:** Plasma betatrophin levels were associated with the presence and severity of CAD and PAD, suggesting betatrophin has a role in atherosclerosis.

**Key words:** Betatrophin, Coronary artery disease, Peripheral artery disease

## Introduction

Betatrophin, also called angiopoietin-like protein 8 (ANGPTL8), is a recently identified circulating adipokine, mainly produced in the liver and adipose tissues, which affects both glucose and lipid metabolism<sup>1</sup>. In animal models, betatrophin promoted pancreatic beta cell proliferation<sup>2</sup> and regeneration<sup>3</sup>, thereby improving glucose tolerance. However, another study indicated betatrophin affected neither

beta cell expansion nor glucose metabolism<sup>4</sup>. Most studies showed that betatrophin levels in diabetic patients are higher than in non-diabetic patients<sup>5-8</sup>, but some studies reported decreased levels in diabetic patients<sup>9</sup> or no difference between diabetic and non-diabetic patients<sup>10, 11</sup>. In lipid metabolism, betatrophin inhibited lipoprotein lipase (LPL) activity, thereby causing increased triglyceride (TG) levels<sup>12, 13</sup>. Although some studies reported blood betatrophin levels positively correlated with TG levels<sup>7, 14</sup>, others

Address for correspondence: Yukihiko Momiyama, Department of Cardiology, National Hospital Organization Tokyo Medical Center, 2-5-1 Higashigaoka, Meguro-ku, Tokyo 152-8902, Japan E-mail: ymomiyamajp@gmail.com

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showed no correlation between betatrophin and TG levels<sup>5, 6, 11</sup>). Thus, the associations between blood betatrophin levels and impaired glucose metabolism or atherogenic lipid profiles remain controversial.

Recently, Huang *et al.*<sup>15</sup>) reported serum betatrophin levels were high in 22 diabetic patients with coronary artery disease (CAD) or stroke. Maurer *et al.*<sup>14</sup>) also showed that serum betatrophin levels correlated with carotid arterial wall thickness ( $r=0.26$ ) in 535 subjects, of whom 32 (6%) had DM. However, blood betatrophin levels in patients with atherosclerotic diseases, such as CAD and peripheral artery disease (PAD), have not been clarified yet. To elucidate whether or not plasma betatrophin levels are associated with the presence and severity of CAD and PAD, independent of glucose and lipid metabolism, our cross-sectional study was performed in 457 patients undergoing elective coronary angiography who also underwent an ankle-brachial index (ABI) test to screen for PAD<sup>16</sup>).

## Methods

### Study Patients

We investigated plasma betatrophin levels in 457 consecutive patients undergoing elective coronary angiography for suspected CAD at Tokyo Medical Center who also had an ABI test to screen for PAD from July 2008 to September 2016. The ABI on each leg was measured in a supine position after five mins of rest using VaSera VS-1000 instrument (Fukuda Denshi, Tokyo, Japan). The lower of the two ABIs was used for further analysis, and PAD was defined as an ABI of  $<0.9$ <sup>16</sup>). Patients with a history of percutaneous coronary intervention or cardiac surgery were excluded from this study. Any patients with acute coronary syndrome, such as acute myocardial infarction and unstable angina, were also excluded. Hypertension was defined as blood pressure of  $\geq 140/90$  mmHg or on drugs, and 272 (60%) patients were taking anti-hypertensive drugs. Hypercholesterolemia was defined as an LDL-cholesterol (LDL-C) level of  $> 140$  mg/dL or on drugs, and 159 (35%) patients were taking LDL-C lowering drugs, such as a statin or ezetimibe. Hypertriglyceridemia was defined as an TG level of  $>150$  mg/dL or on drugs, and 33 (7%) were taking TG lowering drugs, such as fibrates, nicotinate, or EPA. DM (a fasting plasma glucose [FPG] level of  $\geq 126$  mg/dL or on treatment) was present in 117 (26%) patients, and 171 (37%) were smokers ( $\geq 10$  pack-years). Our study was approved by the institutional ethics committee of our hospital (R07-054/R15-056). After written informed consent was obtained, overnight-fasting blood samples were taken

on the morning of the day when coronary angiography was performed.

### Measurement of Plasma Betatrophin and C-reactive Protein Levels

Blood samples were collected in EDTA-containing tubes. Plasma was stored at  $-80^{\circ}\text{C}$ . Plasma betatrophin levels were measured by an enzyme-linked immunosorbent assay using a commercially available kit (WUHAN EIAab Science; Catalog number E11644h, China) at Ochanomizu University according to the manufacturer's instructions. The intra-assay and inter-assay coefficients of variation were  $<8\%$  and  $<10\%$ , respectively. Plasma high-sensitivity C-reactive protein (hsCRP) levels were also measured by a BNII nephelometer (Dade Behring, Tokyo, Japan).

### Coronary Angiography

Angiograms were recorded on a cineangiogram system (Philips Electronics Japan, Tokyo, Japan). CAD was defined as at least one coronary artery having  $>50\%$  luminal diameter stenosis on angiograms. The CAD severity was represented as the number of  $>50\%$  stenotic vessels and the numbers of  $>50\%$  and  $>25\%$  stenotic segments. Coronary artery segments were defined as 29 segments according to the Coronary Artery Surgery Study classification. All angiograms were evaluated by a single cardiologist (Y.M.), blinded to the clinical and laboratory data.

### Statistical Analysis

Any differences between two groups were evaluated by an unpaired t-test for parametric variables, by Mann-Whitney U test for nonparametric variables, and by chi-squared test for categorical variables. Any differences among  $\geq 3$  groups were evaluated by an analysis of variance with Scheffe's test for parametric variables, by Kruskal-Wallis test with Steel-Dwass test for nonparametric variables, and by chi-squared test for categorical variables. The correlations between plasma betatrophin levels and lipid and glucose parameters as well as the severity of CAD or PAD were evaluated by Spearman's rank correlation test and multiple linear regression analysis. A multiple logistic regression analysis was used to determine the independent associations between betatrophin levels and the presence of CAD or PAD. In model 1, only 7 and 4 variables were entered in the analysis of CAD or 3-VD and of PAD, because the numbers of patients with 3-VD and PAD were 71 and 41, respectively<sup>17</sup>). In addition to betatrophin levels, we selected these variables because they are associated with CAD or PAD among atherosclerotic risk factors<sup>18, 19</sup>). However, all

**Table 1.** Clinical characteristics and plasma betatrophin levels of patients with and without CAD

	CAD(-) (n=216)	P value CAD(-) vs. CAD	CAD (n=241)	1-VD (n=99)	2-VD (n=71)	3-VD (n=71)	P value Among 4 groups
Age (years)	66 ± 12	<0.001	70 ± 10	68 ± 10	69 ± 10	72 ± 7	<0.001
Gender (male)	137 (63%)	0.003	184 (76%)	76 (77%)	50 (70%)	58 (82%)	0.010
BMI (kg/m <sup>2</sup> )	24.6 ± 12.3	0.282	23.7 ± 3.6	24.0 ± 4.0	23.8 ± 3.2	23.1 ± 3.2	0.655
Hypertension	133 (62%)	<0.001	191 (79%)	77 (78%)	55 (77%)	59 (83%)	<0.001
SBP (mmHg)	130 ± 20	0.285	133 ± 21	131 ± 20	138 ± 21	129 ± 24	0.049
Diabetes mellitus	30 (14%)	<0.001	87 (36%)	30 (30%)	27 (38%)	30 (42%)	<0.001
FPG (mg/dL)	100 ± 25	0.003	108 ± 31	104 ± 24	109 ± 32	111 ± 37	0.010
HbA1c (%)	5.9 ± 0.6	<0.001	6.3 ± 1.0	6.2 ± 0.9	6.4 ± 1.0	6.5 ± 1.1	<0.001
Smoking	66 (31%)	0.005	105 (44%)	45 (45%)	30 (42%)	30 (42%)	0.037
Hypercholesterolemia	81 (38%)	<0.001	135 (56%)	51 (52%)	43 (61%)	41 (58%)	0.001
LDL-C (mg/dL)	111 ± 29	0.354	114 ± 32	112 ± 34	116 ± 32	115 ± 29	0.629
Medication(+)	53 (28%)	<0.001	106 (44%)	41 (41%)	32 (45%)	33 (46%)	<0.001
Statin	52 (24%)	<0.001	105 (44%)	40 (40%)	32 (45%)	33 (46%)	<0.001
Ezetimibe	1 (0%)	0.628	2 (1%)	1 (1%)	1 (1%)	0 (0%)	0.706
Hypertriglyceridemia	63 (29%)	0.034	93 (39%)	35 (35%)	31 (44%)	27 (38%)	0.123
TG (mg/dL)	122 ± 64	0.013	137 ± 67	136 ± 65	147 ± 76	128 ± 60	0.028
Medication(+)	11 (5%)	0.096	22 (9%)	8 (8%)	7 (7%)	7 (7%)	0.385
Fibrates	1 (0%)	0.063	8 (3%)	3 (3%)	3 (4%)	2 (3%)	0.154
Nicotinates	6 (3%)	0.415	4 (2%)	3 (3%)	0 (0%)	1 (1%)	0.481
EPA	4 (2%)	0.155	10 (4%)	2 (2%)	4 (6%)	4 (6%)	0.204
HDL-C (mg/dL)	58 ± 15	<0.001	51 ± 13	54 ± 14	50 ± 11	49 ± 12	<0.001
hsCRP (mg/L)	0.49 [0.25, 1.27]	0.004	0.69 [0.35, 1.61]	0.62 [0.30, 1.33]	0.64 [0.40, 1.42]	0.88 [0.42, 2.00]	0.008
Betatrophin levels (pg/mL)	909 [645, 1333]	<0.001	1120 [761, 1517]	962 [641, 1384]	1097 [830, 1421]	1393 [957, 1621]	<0.001
PAD (ABI < 0.9)	6 (3%)	<0.001	35 (15%)	9 (9%)	7 (10%)	19 (27%)	<0.001

Data represent the mean ±SD or the number (%) of patients, with the exception of hsCRP and betatrophin levels which are presented as the median value and interquartile range.

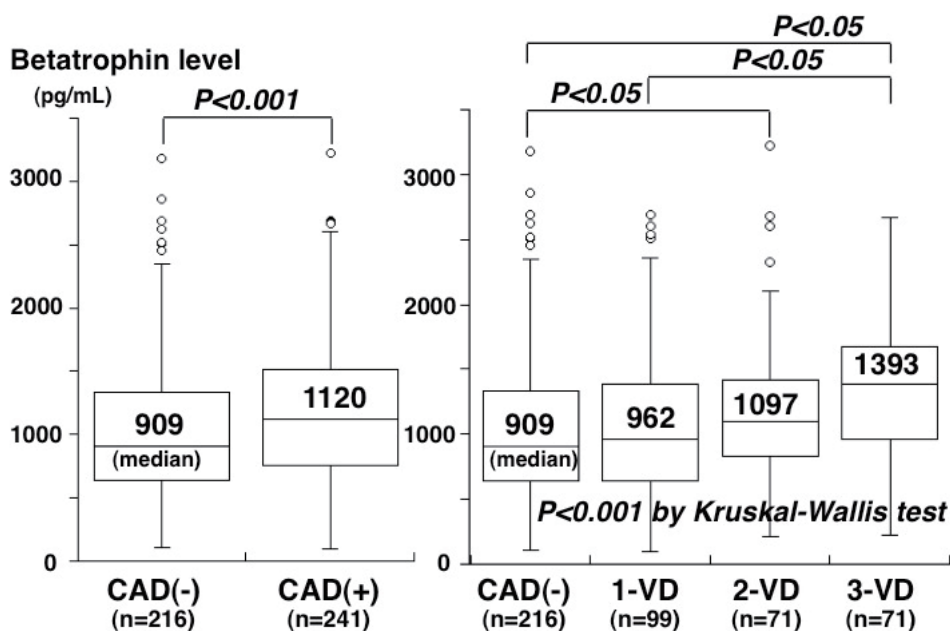
BMI=body mass index; SBP=systolic blood pressure; FPG=fasting plasma glucose; LDL-C=low-density lipoprotein cholesterol; HDL-C=high-density lipoprotein cholesterol; TG=triglyceride.

atherosclerotic risk factors and betatrophin levels were entered into model 2. In model 2 for PAD, CAD was also entered to see the association between betatrophin levels and PAD independent of CAD. A *p* value of < 0.05 was considered to be statistically significant. The results are presented as the mean ±SD or the median value.

### Results

Among the 457 study patients, CAD was present in 241 patients (53%) (1-vessel disease [1-VD], *n*=99; 2-vessel disease [2-VD], *n*=71; 3-vessel disease [3-VD], *n*=71). Compared to 216 patients without CAD, 241 patients with CAD were older, generally male, and had a higher prevalence of hypertension, hypercholesterolemia, hypertriglyceridemia, DM, and smoking (Table 1). CAD patients also had lower HDL-cholesterol and higher TG and hsCRP levels

and were more often taking LDL-C lowering drugs, but not TG lowering drugs, than those without CAD. Notably, plasma betatrophin levels were significantly higher in patients with CAD than in those without CAD (median 1120 vs. 909 pg/mL, *p*<0.001) (Fig. 1). A stepwise increase in betatrophin levels was found depending on the number of >50% stenotic coronary vessels: 909 in CAD(-), 962 in 1-VD, 1097 in 2-VD, and 1393 pg/ml in 3-VD (*p*<0.001) (Fig. 1). Especially, betatrophin levels in 3-VD were significantly higher than those in CAD(-) and 1-VD (*p*<0.05). Betatrophin levels also significantly correlated with the numbers of >50% and >25% stenotic coronary segments (*r*s=0.22 and *r*s=0.24, respectively, *p*<0.001 by Spearman's rank correlation test). However, in multiple linear regression analysis which included age, BMI, SBP, HbA1c and LDL-C, TG, and HDL-C levels as variables, betatrophin levels significantly correlated with the number of >50% ste-



**Fig. 1.** Associations between plasma betatrophin levels and the presence of CAD or the number of stenotic coronary vessels

The plasma betatrophin levels were significantly higher in CAD than in CAD(-) (left). Furthermore, betatrophin levels in four groups of CAD(-), 1-VD, 2-VD, and 3-VD were 909, 962, 1097, and 1393 pg/mL, respectively, and were highest in 3-VD ( $p < 0.001$  by Kruskal-Wallis test) (right). Betatrophin levels in 3-VD were significantly higher than those in CAD(-) and 1-VD ( $p < 0.05$ ). The central line represents the median, and the box represents the 25th to 75th percentiles. The whiskers represent the lowest and highest value in the 25th percentile minus 1.5 IQR and 75th percentile plus 1.5 IQR, respectively.

notic vessels ( $\beta = 0.02$ ,  $p < 0.05$ ) but did not with the numbers of  $> 50\%$  and  $> 25\%$  stenotic segments.

Among the 457 study patients, PAD (ABI  $< 0.9$ ) was found in 41 (9%) patients (6 of 216 [3%] patients without CAD and 35 of 241 [15%] with CAD). Compared to 416 patients without PAD, 41 patients with PAD were older and had higher prevalence of hypertension, hypercholesterolemia, and smoking (Table 2). Patients with PAD also had lower HDL-cholesterol and higher hsCRP levels and were more often taking LDL-C lowering drugs but not TG lowering drugs than those without PAD. Notably, patients with PAD had a higher prevalence of CAD (85% vs. 50%), especially 3-VD (46% vs. 13%), than those without PAD ( $p < 0.001$ ). Plasma betatrophin levels were significantly higher in patients with PAD than in those without PAD (median 1354 vs. 981 pg/mL,  $p < 0.001$ ) (Fig. 2). Betatrophin levels also significantly correlated with ABI ( $r_s = -0.17$ ,  $p < 0.001$  by Spearman's rank correlation test). In multiple linear regression analysis including age, BMI, SBP, HbA1c, LDL-C, TG and HDL-C levels, betatrophin levels significantly correlated with ABI ( $\beta = -0.01$ ,  $p < 0.001$ ).

DM was present in 117 (26%) of 457 study patients. Compared to 340 patients without DM, 117

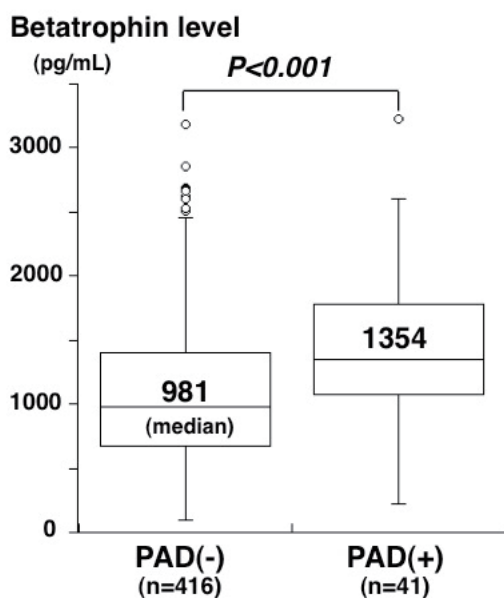
with DM more often had CAD (74% vs. 45%), especially 3-VD (26% vs. 12%) ( $p < 0.001$ ). Betatrophin levels were significantly higher in patients with DM than in those without DM (median 1305 vs. 1045 pg/mL,  $p < 0.001$ ). Betatrophin levels significantly correlated with age ( $r_s = 0.42$ ) and HbA1c ( $r_s = 0.18$ ) and hsCRP ( $r_s = 0.22$ ) levels ( $p < 0.001$ ), but they did not correlate with BMI and FPG or TG levels. Even after excluding 159 patients with LDL-C lowering drugs and 33 patients with TG lowering drugs, betatrophin levels correlated with age ( $r_s = 0.42$ ), HbA1c ( $r_s = 0.19$ ) and hsCRP ( $r_s = 0.21$ ) ( $p < 0.001$ ), but not with BMI and FPG or TG levels.

Table 3 shows the odds ratios of atherosclerotic risk factors and betatrophin levels for CAD and 3-VD in univariate and multivariate analysis. To elucidate the independent associations between betatrophin levels and CAD or 3-VD, variables (age, gender, BMI, hypertension, smoking, DM, LDL-C lowering and TG lowering drugs, and LDL-C, HDL-C, TG, and betatrophin levels) were entered into a multiple logistic regression model. In both models 1 and 2, plasma betatrophin levels were not a significant factor for CAD but were a significant factor for 3-VD independent of atherosclerotic risk factors. The odds ratio for

**Table 2.** Clinical characteristics and plasma betatrophin levels of patients with and without PAD

	PAD(-) (n=416)	PAD (n=41)	P value
Age (years)	67 ± 11	73 ± 9	0.001
Gender (male)	290 (70%)	31 (76%)	0.431
BMI (kg/m <sup>2</sup> )	24.2 ± 9.2	23.4 ± 3.6	0.576
Hypertension	286 (69%)	38 (93%)	0.001
Systolic blood pressure (mmHg)	131 ± 20	133 ± 30	0.755
Diabetes mellitus	102 (25%)	15 (37%)	0.091
FPG (mg/dL)	104 ± 28	105 ± 32	0.737
HBA1c (%)	6.1 ± 0.8	6.4 ± 1.0	0.115
Smoking	148 (36%)	23 (56%)	0.010
Hypercholesterolemia	188 (45%)	28 (68%)	0.005
LDL-cholesterol (mg/dL)	112 ± 30	115 ± 31	0.542
Medication(+)	138 (33%)	21 (51%)	0.021
Statin	136 (33%)	21 (51%)	0.017
Ezetimibe	3 (1%)	0 (0%)	0.585
Hypertriglyceridemia	136 (33%)	20 (49%)	0.057
TG (mg/dL)	129 ± 67	137 ± 63	0.458
Medication(+)	29 (7%)	4 (10%)	0.733
Fibrates	8 (2%)	1 (2%)	0.821
Nicotimates	9 (2%)	1 (2%)	0.908
EPA	12 (3%)	2 (5%)	0.480
HDL-cholesterol (mg/dL)	55 ± 14	48 ± 10	0.004
hsCRP (mg/L)	0.56 [0.28, 1.32]	1.29 [0.45, 2.82]	0.002
Betatrophin levels (ng/mL)	981 [670, 1402]	1354 [1072, 1783]	<0.001
CAD	206 (50%)	35 (85%)	<0.001
1-VD	90 (22%)	9 (22%)	0.975
2-VD	64 (15%)	7 (17%)	0.950
3-VD	52 (13%)	19 (46%)	<0.001

Data represent the mean ± SD or the number (%) of patients, with the exception of hsCRP and betatrophin levels which is presented as the median value and interquartile range.



**Fig. 2.** Association between plasma betatrophin levels and the presence of PAD

The plasma betatrophin levels were significantly higher in PAD than in PAD(-). The central line represents the median, and the box represents the 25th to 75th percentiles. The whiskers represent the lowest and highest value in the 25th percentile minus 1.5 interquartile range (IQR) and 75th percentile plus 1.5 IQR, respectively.

**Table 3.** Factors associated with CAD (Univariate and multivariate regression analysis of 457 study patients)

	Univariate		Multivariate			
	Odds ratio (95%CI)	P value	Model 1		Model 2	
			Odds ratio (95%CI)	P value	Odds ratio (95%CI)	P value
<b>CAD</b>						
Age (per 10 yrs increase)	1.45 (1.21-1.73)	< 0.001	1.40 (1.16-1.70)	0.001	1.51 (1.23-1.86)	< 0.001
Male gender	1.86 (1.24-2.79)	0.003			1.78 (1.07-2.94)	0.025
BMI (per 1 kg/m <sup>2</sup> increase)	0.99 (0.96-1.02)	0.346			----	NS
Hypertension	2.38 (1.57-3.61)	< 0.001	1.75 (1.11-2.77)	0.016	----	NS
Diabetes mellitus	3.50 (2.20-5.59)	< 0.001	2.50 (1.52-4.11)	< 0.001	2.12 (1.27-3.55)	0.004
Smoking	1.76 (1.19-2.58)	0.004	----	NS	----	NS
LDL-C (per 10 mg/dL increase)	1.03 (0.97-1.09)	0.384	----	NS	1.10 (1.02-1.18)	0.013
LDL-C lowering drug	2.42 (1.62-3.61)	< 0.001			2.50 (1.57-3.99)	< 0.001
TG (per 10 mg/dL increase)	1.04 (1.01-1.07)	0.013			----	NS
TG lowering drug	1.87 (0.89-3.96)	0.101			----	NS
HDL-C (per 10 mg/dL increase)	0.68 (0.59-0.79)	< 0.001	0.72 (0.62-0.84)	< 0.001	0.72 (0.61-0.85)	< 0.001
Betatrophin (per 100 mg/dL increase)	1.07 (1.03-1.10)	< 0.001	----	NS	----	NS
<b>3-vessel disease</b>						
Age (per 10 yrs increase)	1.79 (1.33-2.39)	< 0.001	1.75 (1.26-2.42)	0.001	1.80 (1.29-2.51)	0.001
Male gender	2.09 (1.10-3.95)	0.024			2.59 (1.23-5.43)	0.012
BMI (per 1 kg/m <sup>2</sup> increase)	0.95 (0.89-1.02)	0.151			----	NS
Hypertension	2.25 (1.16-4.33)	0.016	----	NS	----	NS
Diabetes mellitus	2.51 (1.48-4.26)	0.001	----	NS	----	NS
Smoking	1.27 (0.76-2.13)	0.360	----	NS	----	NS
LDL-C (per 10 mg/dL increase)	1.03 (0.95-1.12)	0.500	1.10 (1.01-1.21)	0.041	1.14 (1.03-1.26)	0.009
LDL-C lowering drug	1.79 (1.07-2.99)	0.026			2.02 (1.13-3.61)	0.017
TG (per 10 mg/dL increase)	1.00 (0.96-1.04)	0.879			----	NS
TG lowering drug	1.51 (0.63-3.64)	0.353			----	NS
HDL-C (per 10 mg/dL increase)	0.69 (0.56-0.85)	< 0.001	0.65 (0.52-0.82)	< 0.001	0.71 (0.56-0.91)	0.006
Betatrophin (per 100 mg/dL increase)	1.09 (1.05-1.14)	< 0.001	1.06 (1.01-1.11)	0.020	1.06 (1.01-1.12)	0.014

3-VD was 1.06 (95%CI=1.01-1.11) for a 100-pg/mL increase in betatrophin levels ( $p < 0.025$ ) (Table 3). Table 4 shows the odds ratios of atherosclerotic risk factors and betatrophin levels for PAD in univariate and multivariate analyses. To elucidate the independent association between betatrophin levels and PAD, variables (age, gender, BMI, hypertension, smoking, DM, LDL-C lowering and TG lowering drugs, LDL-C, HDL-C, TG and betatrophin levels, and CAD) were entered into a multiple logistic regression model. In both models 1 and 2, betatrophin levels were a significant factor for PAD independent of atherosclerotic risk factors. The odds ratio for PAD was 1.07 (95%CI=1.01-1.13) for a 100-pg/mL increase in betatrophin levels ( $p < 0.05$ ) (Table 4).

## Discussion

In the present study, plasma betatrophin levels were significantly higher in patients with CAD, espe-

cially in 3-VD, than in those without CAD, and they correlated with CAD severity, defined as the numbers of stenotic vessels and segments. Moreover, betatrophin levels were also higher in patients with PAD than in those without PAD, and they correlated with PAD severity, defined as ABI. Although betatrophin levels correlated with HbA1c, but not with TG levels, betatrophin levels were found to be a significant factor for 3-VD as well as PAD independent of atherosclerotic risk factors.

Betatrophin was suggested to inhibit LPL activity and increase TG levels<sup>12, 13</sup>. However, the association between blood betatrophin levels and TG levels remains controversial, with some studies indicating positive correlation<sup>7, 14</sup> and others indicating no correlation<sup>5, 6, 11</sup>. In our study, 159 (35%) and 33 (7%) of the 457 study patients were taking LDL-C lowering drugs (statin or ezetimibe) and TG lowering drugs (fibrates, nicotines or EPA), respectively. However, we also found no correlation between plasma betatro-

**Table 4.** Factors associated with PAD (Univariate and multivariate regression analysis of 457 study patients)

	Univariate		Multivariate			
	Odds ratio (95%CI)	P value	Model 1		Model 2	
			Odds ratio (95%CI)	P value	Odds ratio (95%CI)	P value
PAD						
Age (per 10 yrs increase)	1.96 (1.33-2.91)	0.001	1.71 (1.11-2.66)	0.016	----	NS
Male gender	1.35 (0.64-2.83)	0.432			----	NS
BMI (per 1 kg/m <sup>2</sup> increase)	0.97 (0.90-1.06)	0.527			----	NS
Hypertension	5.76 (1.75-18.9)	0.004	4.36 (1.30-14.6)	0.017	3.96 (1.17-13.4)	0.027
Diabetes mellitus	1.78 (0.91-3.48)	0.095			----	NS
Smoking	2.31 (1.21-4.43)	0.011	2.51 (1.27-4.98)	0.008	----	NS
LDL-C (per 10 mg/dL increase)	1.03 (0.93-1.14)	0.577			----	NS
LDL-C lowering drug	2.12 (1.11-4.03)	0.023			----	NS
TG (per 10 mg/dL increase)	1.02 (0.97-1.07)	0.428			----	NS
TG lowering drug	1.44 (0.48-4.33)	0.513			----	NS
HDL-C (per 10 mg/dL increase)	0.69 (0.53-0.90)	0.007			----	NS
Betatrophin (per 100 mg/dL increase)	1.11 (1.05-1.16)	<0.001	1.07 (1.01-1.13)	0.028	1.09 (1.03-1.15)	0.002
CAD	5.95 (2.45-14.4)	<0.001			4.56 (1.84-11.3)	0.001

phin and TG levels. Even after excluding patients taking LDL-C or TG lowering drugs, no correlation was found between betatrophin and TG levels. Yue *et al.*<sup>8)</sup> performed a meta-analysis of 11 studies and showed higher betatrophin levels in diabetic patients than in non-diabetic patients. Furthermore, some studies reported correlations between betatrophin and FPG<sup>5,6)</sup> or HbA1c<sup>6, 14)</sup> levels. Our study also showed that plasma betatrophin levels were higher in patients with DM than in those without DM and they correlated weakly, but significantly, with HbA1c levels. Plasma betatrophin levels were a significant factor associated with CAD, especially 3-VD, and PAD independent of lipid levels and DM.

The ANGPTL protein family contains seven typical members, which are characterized by a coiled-coil domain at N-terminus, a fibrinogen-like domain at C-terminus, and a signal peptide for protein secretion<sup>1)</sup>. Betatrophin (ANGPTL8) is an atypical member of ANGPTL because it lacks a fibrinogen-like domain but shares common ancestors with ANGPTL3 and ANGPTL4. Both ANGPTL3 and ANGPTL4 are shown to inhibit LPL *in vitro*, suggesting a role in lipid metabolism<sup>20)</sup>. In animal models, ANGPTL4 overexpression reduced atherosclerosis with no change in lipid levels<sup>21)</sup>, suggesting a protective effect of ANGPTL4 on atherosclerosis. However, Smart-Halajko *et al.*<sup>22)</sup> reported no association between plasma ANGPTL4 levels and CAD risk or TG levels in 666 subjects. Despite similar biochemical effects *in vitro*, reduced ANGPTL3 expression reduced atherosclerosis and TG levels<sup>23)</sup>. In humans, lower levels of

plasma ANGPTL3 were associated with a lower risk of MI and lower TG levels<sup>24)</sup>. Furthermore, plasma ANGPTL3 levels were correlated with carotid arterial wall thickness<sup>25)</sup>. Therefore, ANGPTL3 may have a promotive effect on atherosclerosis progression.

Betatrophin (ANGPTL8) and ANGPTL3 are suggested to be most closely related among the ANGPTL family because of their similar gene structure<sup>1)</sup>. Betatrophin and ANGPTL3 co-operate in the regulation of TG levels<sup>26, 27)</sup>. Moreover, betatrophin was reported to promote ANGPTL3's ability to bind and then inhibit LPL<sup>28, 29)</sup>. These findings suggest that betatrophin, as well as ANGPTL3, may have atherogenic effect. One study reported that serum betatrophin levels were higher in 22 diabetic patients with CAD or stroke than in 101 diabetic patients without such disease<sup>15)</sup>. Another study reported that serum betatrophin levels correlated positively with carotid arterial wall thickness<sup>14)</sup>. Our study showed that plasma betatrophin levels were significantly higher in patients with CAD, especially in 3-VD, than in those without CAD, and that they correlated with CAD severity, defined as the numbers of stenotic vessels and segments. Although betatrophin levels correlated with HbA1c levels, they were a significant factor associated with CAD, especially 3-VD, independent of DM and lipid levels. Our findings thus suggest that betatrophin may play a role in CAD development.

Atherosclerosis is a progressive disease that affects multiple vascular beds, and CAD patients are often complicated by PAD<sup>16)</sup>. In our study, PAD was found in 6 of 216 patients without CAD (3%) and 35 of

241 with CAD (15%). The PAD prevalence was similar to that reported by Lee *et al.*<sup>16)</sup> (5 of 119 without CAD [4%] and 385 of 2424 with CAD [16%] among 2,543 patients undergoing coronary angiography). No previous studies have investigated blood betatrophin levels in patients with PAD. We reported high levels of plasma betatrophin in patients with PAD. Betatrophin levels correlated with PAD severity, defined as ABI, and were a significant factor for PAD independent of atherosclerotic risk factors, suggesting a role of betatrophin in atherosclerosis progression. However, the mechanism of how betatrophin affects atherosclerosis and the direct atherogenic effect of betatrophin has not been clarified. Further studies are needed to elucidate the mechanism of the atherogenic effect of betatrophin and determine whether or not betatrophin has a direct atherogenic effect in addition to its effects on glucose and lipid metabolism.

### Study Limitations

Our study has several limitations. First, we used the betatrophin kit manufactured by EIAab Science, which recognizes the N-terminus of betatrophin. However, some studies used the kit by Phoenix, which recognizes the C-terminus. The N-terminal kit measures the full-length protein, while the C-terminal kit measures total betatrophin species, including the full-length protein and C-terminal fragments<sup>30)</sup>. Using different kits may have caused different results. However, increased levels were reported in spite of the use of different kits, and the N-terminal kit is more commonly used than the C-terminal kit<sup>30)</sup>. Moreover, we did not measure LPL activity and insulin levels. One of the study limitations is lack of data on the role of betatrophin in atherosclerosis. Second, coronary angiography was used to evaluate coronary atherosclerosis. Angiography cannot visualize plaques and only shows lumen characteristics. However, IVUS, which can visualize coronary plaques, was not always performed in our patients. Third, PAD was found in 6 of 216 patients without CAD (3%) and 35 of 241 with CAD (15%). This prevalence of PAD was similar to that reported by Lee *et al.*<sup>16)</sup> However, the small number of patients with PAD was one of major limitations. Moreover, in our study, an ABI test was used to screen for PAD, and PAD was defined as ABI of  $<0.9$ <sup>16)</sup>. Angiography or computed tomography were not always performed to confirm the PAD diagnosis. This is one of study limitations. Fourth, our study was cross-sectional in nature and could not establish causality since it only showed some associations and proposed some hypotheses. Finally, our study was performed on Japanese patients undergoing coronary angiography, who are generally considered to be a highly select population

at high risk for CAD. Our results, therefore, may not be applicable to the general or other ethnic populations.

### Conclusion

Plasma betatrophin levels were high in CAD patients, especially in 3-VD, and they correlated with the severity of CAD. Furthermore, betatrophin levels were also high in patients with PAD. Although betatrophin levels correlated with HbA1c but not TG levels, betatrophin levels were found to be a significant factor associated with 3-VD and PAD independent of atherosclerotic risk factors. Our findings, thus, suggest that betatrophin may promote atherosclerotic disease development, such as CAD and PAD.

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### Conflict of interest

Our study has no conflict of interest to disclose.

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