CLINICAL STUDY

Thrombospondin-2 as a Potential Risk Factor in a General Population

Nagisa Morikawa,[1] MD, Hisashi Adachi,[1,2] MD, Mika Enomoto,[1] MD, Ako Fukami,[1] MD, Eita Kumagai,[1] MD, Sachiko Nakamura,[1] MD, Yume Nohara,[1] MD, Erika Nakao,[1] MD, Shoko Kono,[1] MD, Tomoko Tsuru,[1] MD, Akiko Sakaue,[1] MD, Hitoshi Hamamura,[1] MD and Yoshihiro Fukumoto,[1] MD

Summary

Serum thrombospondin-2 (TSP-2) is a glycoprotein expressed in the extracellular matrix (ECM), which increases during tissue remodeling. It has been shown in recent studies that TSP-2 is a useful predictor of cardiovascular death in patients with heart failure (HF). However, the clinical importance of serum TSP-2 levels in a general population is still unknown. Therefore, we aimed to clarify the association between TSP-2 and clinical risk factors. A periodic epidemiological survey was performed in a community dwelling in the town of Uku, Nagasaki, Japan. A total of 445 residents received a health checkup examination including blood tests such as fasting serum levels of TSP-2. Uni- and multivariate analyses were performed to examine the relationship between TSP-2 and clinical risk factors. All statistical analyses were performed using SAS v9.4 program. The mean ± standard deviation of age was 67.0 ± 9.4 years old. Although serum TSP-2 levels (mean: 20.9 ± 8.5 ng/mL) showed no significant sex difference, they were significantly correlated with the levels of plasma glucose ($P < 0.001$), insulin ($P < 0.01$), homeostasis model assessment of insulin resistance (HOMA-IR) ($P < 0.001$), estimated glomerular filtration rate (eGFR) ($P < 0.01$, inversely), high-sensitivity C-reactive protein (hs-CRP) ($P < 0.001$), history of atrial fibrillation ($P < 0.001$), history of cardiovascular diseases ($P < 0.001$), and N-terminal prohormone of brain natriuretic peptide (NT-proBNP) ($P < 0.001$). Moreover, in the multiple stepwise linear regression analysis, the levels of TSP-2 were independently and significantly associated with the history of atrial fibrillation ($P < 0.0001$), HOMA-IR ($P < 0.001$), high-sensitivity CRP ($P = 0.011$), and NT-proBNP ($P = 0.043$). These results indicated the significant relationship between TSP-2 and clinical risk factors in a general population, suggesting its role as a predictor of heart disease morbidity and mortality.

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Key words: TSP-2, Epidemiology

Thrombospondins are a family of glycoproteins expressed in the extracellular matrix (ECM), which have no direct structural role in the ECM, but they act as regulators of cell-cell and cell-matrix associations and interact with other ECM molecules, affecting their function.[1)] Among five types of thrombospondins, the role of thrombospondin-2 (TSP-2) in cardiac remodeling has been recently focused on in cardiovascular fields.[1,2)]

A previous *in vivo* experiment showed that the absence of TSP-2 in older mice displayed a severe dilated cardiomyopathy with impaired systolic function, increased cardiac dilatation, and fibrosis.[3)] Further, TSP-2 knockout mice have also been reported to exhibit increased mortality during viral myocarditis or doxorubicin-induced cardiomyopathy,[4,5)] indicating that TSP-2 plays a protective role in the cardiac function.

Further, it has been suggested in recent clinical studies that TSP-2 is a useful predictor of heart failure (HF) in patients with both reduced and preserved ejection fraction (EF) and that it is associated with cardiovascular death and all-cause deaths.[6,7)] In addition, much attention has been recently paid to the role of TSP-2 in various fields, such as coronary artery diseases,[8)] abdominal aortic aneurysms,[9)] systemic sclerosis,[10)] preeclampsia,[11)] and ischemic stroke.[12)]

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However, the clinical importance of serum TSP-2 in a general population is still unknown. Therefore, herein, we performed a cross-sectional study in order to examine the relationship between TSP-2 and clinical risk factors in

From the [1]Division of Cardiovascular Medicine, Department of Internal Medicine, Kurume University School of Medicine Kurume, Japan and [2]Department of Community Medicine, Kurume University School of Medicine, Kurume, Japan.

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Address for correspondence: Hisashi Adachi, MD, Department of Community Medicine, Kurume University School of Medicine, 67 Asahi-machi, Kurume, 830-0011, Japan. E-mail: hadac@med.kurume-u.ac.jp

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a Japanese general population.

Methods

Study population: A periodical epidemiological survey was performed in 2013 and 2014 in a small fishing community in southwestern Japan (a town called Uku).[13-15] This town is an island in the prefecture of Nagasaki, with a total population of about 3,700 people. A total of 446 subjects (183 males and 263 females), aged 40-91, received a population-based health examination. In this study, we excluded five subjects who had missing TSP-2 data or had renal dysfunction (serum creatinine levels greater than 2 mg/dL). In the remaining 441 subjects, a cross-sectional analysis was performed to examine the relationship between serum TSP-2 levels and clinical risk factors.

Data collection: At the beginning of a health checkup, we confirmed the diseases that the subjects were currently suffering from and prescribed medications via questionnaires or interviews. Then, they were checked regarding smoking habits and alcohol intake. Smoking habits were determined as follows: nonsmokers/past smokers = 0, current smokers = 1. Alcohol intake was determined as follows: no intake/past intake/casual intake (three days or less per week) = 0, intake for four days or more per week = 1. We investigated the presence or absence of histories of cardiovascular diseases (angina pectoris, myocardial infarction, and arrhythmia, as well as other diseases such as valvular diseases and abnormal ECG like CRBBB) and cerebrovascular diseases (cerebral hemorrhage, ischemic stroke, and subarachnoid hemorrhage).

Height and weight were measured, and the body mass index (BMI) was calculated as weight (kg) divided by the square of the height (m^2) as an index of the presence or absence of obesity. Waist circumference was measured at the level of the umbilicus in the standing position. Blood pressure (BP) was measured twice in the sitting (first) and supine (second) positions. The second BP with the fifth-phase diastolic pressure was used for analysis. Vigorous physical activity and smoking were avoided for at least 30 minutes before BP measurements.

Blood was drawn from the antecubital vein in the morning after a 12-hour fast for the determination of lipid profile (total cholesterol, triglycerides, and high-density lipoprotein cholesterol [HDL-C]), fasting plasma glucose (FPG), fasting immune-reactive insulin, glycosylated hemoglobin A1c [HbA1c (NGSP)], blood urea nitrogen, creatinine, and uric acid. Fasting blood samples were centrifuged within an hour after collection. The estimated glomerular filtration rate (eGFR) was calculated using the Modification of Diet in Renal Disease study equation modified with a Japanese coefficient.[16] Insulin resistance (IR) was calculated using FPG level × fasting insulin level/405,[17] that is, homeostasis model assessment of IR (HOMA-IR). Serum TSP-2 was measured using the enzyme-linked immunosorbent assay technique. The intra- and interassay coefficients of variation of TSP-2 at a commercially available laboratory (SRL Inc., Fukuoka, Japan) were 3.4% and 5.4%, respectively. High-sensitivity C-reactive protein (hs-CRP) was measured by the latex method as an inflammatory marker.[18] N-terminal prohormone of brain natriuretic peptide (NT-proBNP) was measured using an enzyme immunoassay as a marker of cardiac dysfunction. Albuminuria was determined as the ratio of urinary albumin to creatinine (UACR) from first morning void urine. Microalbuminuria was defined as UACR ≥ 30 mg/g Cr. All the blood and urine tests were conducted in a commercially available laboratory (Kyodo Igaku Laboratories, Fukuoka, Japan).

Echocardiography: The carotid intima-media thickness (c-IMT) of the common carotid artery was determined using duplex ultrasonography (SonoSite TITAN, Aloka, Tokyo, Japan) with a 10 MHz transducer in the supine position. A single well-trained sonographer recorded longitudinal B-mode images at the diastolic phase of the cardiac cycle. The images were magnified and printed with a high-resolution line recorder (LSR-100A; Toshiba, Tochigi, Japan). The c-IMT defined by Pignoli, *et al*.[19,20] was measured as the distance from the leading edge of the first echogenic line to the leading edge of the second echogenic line. The first line represented the lumen-intimal interface; the collagen-containing upper layer of the tunica adventitia formed the second line. At each longitudinal projection, the site of the greatest thickness, including plaque, was sought along the arterial walls nearest to the skin and farthest from the skin from the common carotid artery to the internal carotid artery. Three determinations of c-IMT were carried out at the site of the greatest thickness and two other points, 1 cm upstream and 1 cm downstream from this site. These three determinations were averaged. The greatest value among the six averaged IMTs (three from the left and three from the right) was used as the representative value for everyone.

All individuals underwent standard M-mode and two-dimensional echocardiography (SonoSite 180 Plus Ultrasound System). The left ventricular (LV) dimension was measured according to the recommendations of the American Society of Echocardiography.[21] The LV mass was calculated according to the formula of Devereux and Relchek:[22] LV mass (g) = 1.04 [(LVEDD + IVSd + PWd)^3 − (LVEDD)^3] − 13.6, where LVEDD is the end-diastolic LV internal diameter, IVSd is the end-diastolic interventricular septum thickness, and PWd is the end-diastolic LV posterior wall thickness. The LV mass index was calculated by dividing the LV mass by the body surface area. LVH was defined as LV mass index ≥ 125 cm^2 (males) and LV mass index ≥ 110 cm^2 (females) using the 2007 Guidelines for the Management of Arterial Hypertension of the European Society of Hypertension and the European Society of Cardiology (ESC).[23] The intra- and interobserver variabilities for the measurement of the LV mass index were less than 5%.

We defined hypertension as the use of antihypertensive drugs and/or systolic BP ≥ 140 mmHg or diastolic BP ≥ 90 mmHg. Dyslipidemia was defined as the use of lipid-lowering drugs and/or plasma total cholesterol ≥ 240 mg/dL and/or triglycerides ≥ 150 mg/dL and/or HDL < 40 mg/dL. Diabetes mellitus was diagnosed using antidiabetic drugs and/or FPG ≥ 110 mg/dL or HbA1c ≥ 6.5%.

This study was approved by the mayor and the welfare department of Uku town, as well as by the ethics

committee of Kurume University. All participants provided informed consent.

Statistical analysis: Because of the skewed distributions, natural logarithmic (ln) transformations were performed for triglycerides, insulin, HOMA-IR, NT-proBNP, and hs-CRP. Log-transformed values were reconverted to antilogarithm forms in the tables. The medications for hypertension, dyslipidemia, and diabetes mellitus were coded as dummy variables. Gender, smoking habits, and alcohol intake were also coded as dummy variables.

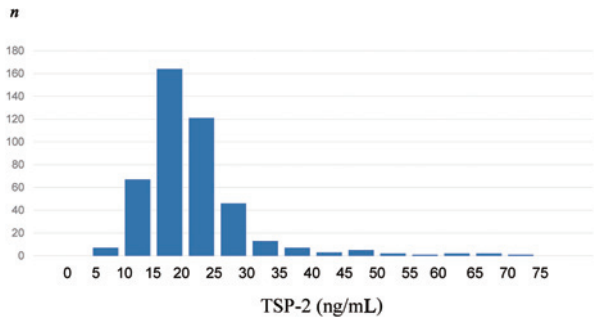

Figure 1. Distribution of serum TSP-2 levels.

Variables that were not normally distributed and/or showed homogeneity of variances were analyzed using the Mann-Whitney test for independent samples. Normally distributed variables were analyzed using the independent *t*-test. First, the mean ± standard deviations (SDs) and frequencies were presented by gender. Second, the association between serum TSP-2 levels and clinical risk factors was tested using single and multiple regression analysis. Then, we performed multiple stepwise regression analysis using significant factors shown in univariate analysis. Finally, in order to investigate the impact of IR and NT-proBNP on TSP-2, we created a hierarchical model stratified by two groups of HOMA-IR (< 1.73 versus ≥ 1.73) and NT-proBNP (< 72 versus ≥ 72). The cut-off level in HOMA-IR was used as shown by Ura, *et al.*,[24] and the median level of NT-proBNP (72 pg/mL) was used. *P*-values < 0.05 were considered statistically significant. All statistical analyses were carried out using SAS version 9.4

Table I. Clinical Characteristics of Subjects

Characteristics	Total (*n* = 445)	Males (*n* = 183)	Females (*n* = 262)
Age (years)	67.0 ± 9.4	67.8 ± 8.6	66.4 ± 9.9
BMI (kg/m^2)	23.5 ± 3.5	24.0 ± 3.2	23.2 ± 3.6
Waist circumference (cm)	84.1 ± 9.8	87.7 ± 9.2	81.6 ± 9.4
Systolic BP (mmHg)	139.6 ± 20.6	139.0 ± 19.1	140.0 ± 21.6
Diastolic BP (mmHg)	78.3 ± 11.1	80.3 ± 11.3	77.0 ± 10.7
Heart rate (bpm)	62.8 ± 9.5	61.3 ± 9.4	63.9 ± 9.5
Creatinine (mg/dL)	0.8 ± 0.3	0.9 ± 0.2	0.7 ± 0.2
Uric acid (mg/dL)	5.2 ± 1.3	5.9 ± 1.3	4.8 ± 1.1
eGFR (mL/minute/1.73 m^2)	70.4 ± 16.5	70.7 ± 16.0	70.2 ± 16.8
Urinary albumin (mg/g Cr)*	6.4 (1.4-877)	6.4 (1.4-122)	6.5 (1.7-877)
Triglycerides (mg/dL)*	88.8 (30-537)	90.0 (30-537)	81.5 (35-311)
HDL-C (mg/dL)	62.0 ± 15.8	57.0 ± 15.7	65.5 ± 14.9
LDL-C (mg/dL)	118.3 ± 30.5	113.7 ± 29.1	121.4 ± 31.1
FPG (mg/dL)	97.5 ± 15.9	104.9 ± 19.0	92.4 ± 10.8
Insulin (μU/mL)*	4.3 (0.8-27.3)	4.5 (0.9-27.3)	4.1 (0.8-19)
HOMA-IR*	1.0 (0.2-9.4)	1.2 (0.2-9.4)	0.9 (0.2-6.5)
HbA1c (%)	5.6 ± 0.5	5.7 ± 0.6	5.5 ± 0.4
EF (%)	67.2 ± 7.0	66.4 ± 6.6	67.8 ± 7.2
LVMI (g/m^2)	113.6 ± 35.5	128.4 ± 36.1	103.3 ± 31.2
c-IMT (mm)	0.8 ± 0.1	0.8 ± 0.1	0.7 ± 0.1
hs-CRP (mg/L)*	0.047 (0.003-5.3)	0.067 (0.003-5.3)	0.037 (0.004-3.5)
NT-proBNP (pg/mL)*	73.0 (5-1980)	66.7 (5-1720)	81.5 (9-1980)
TSP-2 (ng/mL)	20.9 ± 8.5	21.6 ± 9.2	20.5 ± 8.0
Smoking habits (*n*: yes) (%)	43 (9.7%)	38 (20.8%)	5 (1.9%)
Alcohol intake (*n*: yes) (%)	124 (27.9%)	100 (54.6%)	24 (9.2%)
Hypertension (*n*: yes) (%)	312 (70.1%)	139 (76.0%)	173 (66.0%)
Diabetes mellitus (*n*: yes) (%)	53 (11.9%)	34 (18.6%)	19 (7.3%)
Dyslipidemia (*n*: yes) (%)	235 (53.1%)	87 (48.1%)	148 (56.5%)
History of atrial fibrillation (*n*: yes) (%)	14 (3.1%)	10 (5.5%)	4 (1.5%)
History of cardiovascular disease (*n*: yes) (%)	72 (16.2%)	39 (21.3%)	33 (12.6%)
History of cerebrovascular disease (*n*: yes) (%)	21 (4.7%)	11 (6.0%)	10 (3.8%)
History of cancer (*n*: yes) (%)	37 (8.3%)	21 (11.5%)	16 (6.1%)

Data are represented as means ± SD or percentage, unless otherwise indicated. BMI indicates body mass index; BP, blood pressure; eGFR, estimated glomerular filtration rate; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; FPG, fasting plasma glucose; HOMA-IR, FPG × insulin/405; HbA1c, glycosylated hemoglobin A1c; EF, ejection fraction; LVMI, left ventricular mass index; c-IMT, carotid intima-media thickness; hs-CRP, high-sensitivity C-reactive protein; NT-proBNP, N-terminal prohormone of brain natriuretic peptide; and TSP-2, thrombospondin-2. *Log-transformed values were used for the calculation and reconverted into antilogarithm forms.

Table II. Univariable Analysis for Correlates of Serum TSP-2 Levels

Characteristics	β	SE	*P*
Age	0.02	0.04	0.6
Sex	−1.1	0.8	0.2
BMI	0.07	0.1	0.6
Waist circumference	0.051	0.041	0.22
Systolic BP	−0.002	0.02	0.9
Diastolic BP	0.05	0.04	0.2
Heart rate	0.12	0.042	0.006
Creatinine	7.5	1.6	< 0.0001
Uric acid	0.5	0.3	0.1
eGFR	−0.07	0.02	< 0.01
Urinary albumin*	1.0	0.5	< 0.01
Triglycerides*	2.8	0.9	< 0.01
HDL-C	−0.05	0.03	< 0.05
LDL-C	−0.03	0.01	< 0.05
FPG	0.1	0.03	< 0.001
Insulin*	2.2	0.7	< 0.01
HOMA-IR*	2.2	0.6	< 0.001
HbA1c	1.9	0.8	< 0.05
EF	−0.09	0.06	0.1
LVMI	−0.01	0.01	0.3
c-IMT	7.8	3.2	< 0.05
hs-CRP*	1.2	0.3	< 0.001
NT-proBNP*	1.6	0.4	< 0.001
Smoking habits	0.9	1.4	0.5
Alcohol intake	1.9	0.9	< 0.05
Hypertension	0.2	0.9	0.8
Diabetes mellitus	3.3	1.2	< 0.01
Dyslipidemia	1.0	0.8	0.2
History of atrial fibrillation	11.7	2.2	< 0.0001
History of cardiovascular disease	4.3	1.1	< 0.0001
History of cerebrovascular disease	5.5	1.9	< 0.01
History of cancer	0.4	1.5	0.8

β indicates standardized regression coefficients; SE, standard error; sex, male = 0, female = 1; BMI, body mass index; BP, blood pressure; eGFR, estimated glomerular filtration rate; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; FPG, fasting plasma glucose; HOMA-IR, FPG × insulin/405; HbA1c, glycosylated hemoglobin A1c; EF, ejection fraction; LVMI, left ventricular mass index; c-IMT, carotid intima-media thickness; hs-CRP, high-sensitivity C-reactive protein; and NT-proBNP, N-terminal prohormone of brain natriuretic peptide. *Log-transformed values were used for the calculation and reconverted into antilogarithm forms.

(SAS Inc., Cary, NC, USA).

Results

Serum TSP-2 levels exhibited a normal distribution (Figure 1). The characteristics of the male and female subjects are shown in Table I. The mean ± SD in serum TSP-2 levels was 20.9 ± 8.5 ng/mL, and there was no significant sex difference. The mean ± SD of waist circumference was 87.7 ± 9.2 cm in males and 81.6 ± 9.4 cm in females. The mean HOMA-IR was 1.2 in males and 0.9 in females. The prevalence of hypertension was 76% in males and 66% in females. There were statistically significant differences between males and females in terms of the BMI, waist circumference, diastolic BP, heart rate, triglycerides, HDL-C, FPG, insulin, HOMA-IR, HbA1c, creatinine, uric acid, and hs-CRP. The prevalence of smoking, alcohol intake, and histories of cardiovascular diseases, hypertension, and diabetes mellitus were significantly higher in males than in females.

Table II shows a univariate regression analysis with TSP-2. Serum TSP-2 was associated with heart rate ($P < 0.01$), triglycerides ($P < 0.01$), HDL-c ($P < 0.05$, inversely), FPG ($P < 0.001$), insulin ($P < 0.01$), HOMA-IR ($P < 0.001$), HbA1c ($P < 0.05$), creatinine ($P < 0.0001$), eGFR ($P < 0.01$, inversely), microalbuminuria ($P < 0.01$), c-IMT ($P < 0.05$, Figure 2), hs-CRP ($P < 0.001$), NT-proBNP ($P < 0.001$), the prevalence of alcohol intake ($P < 0.05$), DM ($P < 0.01$), history of atrial fibrillation ($P < 0.001$), and history of cardiovascular ($P < 0.0001$) and cerebrovascular ($P < 0.01$) diseases, but not with echocardiographic parameters such as EF and LV mass index. Moreover, in a multivariate regression analysis adjusted for age and sex, significant associations with TSP-2 were found in the same factors, except for HDL and alcohol intake (Table III).

In order to clarify the significance and independence of TSP-2, a multiple stepwise regression analysis was performed, which showed that the history of atrial fibrillation ($P < 0.0001$), HOMA-IR ($P < 0.001$), hs-CRP ($P = 0.011$), and NT-proBNP ($P = 0.043$) were independently correlated with serum TSP-2 levels ($r^2 = 0.111$) (Table

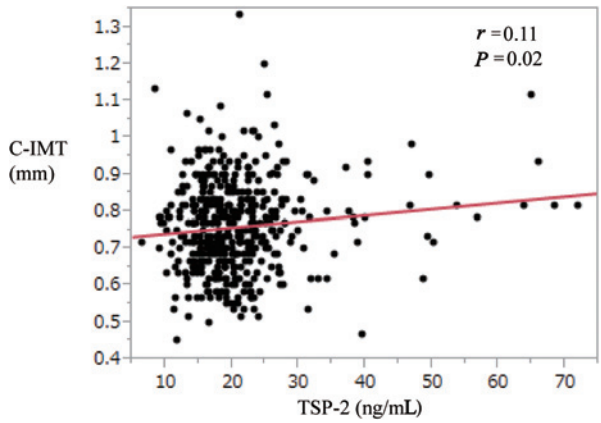

Figure 2. Association between c-IMT and serum TSP-2.

IV).

When the participants were stratified by TSP-2 tertiles, we further investigated the association between serum TSP-2 levels and HOMA-IR using multiple logistic regression analysis (Group 1 versus Group 3). In Table V, a significant odds ratio (2.00, 95% confidence interval [CI]: 1.03-3.90) in the final model (Model 3) was obtained.

When the subjects were divided into low- and high-NT-proBNP groups using the median of NT-proBNP (72 pg/mL), the hierarchical model indicated that high TSP-2 was associated with high NT-proBNP and HOMA-IR levels (Figure 3).

Discussion

To the best of our knowledge, this is the first report

Table III. Multivariable Analysis Adjusted for Age and Sex for Correlates of Serum TSP-2 Levels

Characteristics	β	SE	*P*
BMI	0.06	0.1	0.6
Waist circumference	0.039	0.043	0.37
Systolic BP	–0.005	0.02	0.8
Diastolic BP	0.04	0.04	0.2
Heart rate	0.13	0.044	0.0035
Creatinine	8.4	1.8	< 0.0001
Uric acid	0.4	0.4	0.3
eGFR	–0.08	0.03	< 0.01
Urinary albumin*	1.0	0.5	< 0.05
Triglycerides*	2.7	0.9	< 0.01
HDL-C	–0.05	0.03	0.07
LDL-C	–0.03	0.01	< 0.05
FPG	0.1	0.03	< 0.001
Insulin*	2.1	0.7	< 0.01
HOMA-IR*	2.2	0.6	< 0.001
HbA1c	1.8	0.8	< 0.05
EF	–0.08	0.06	0.2
LVMI	–0.02	0.01	0.1
c-IMT	7.7	3.7	< 0.05
hs-CRP*	1.2	0.3	< 0.001
NT-proBNP*	2.1	0.5	< 0.001
Smoking habits	0.6	1.5	0.7
Alcohol intake	1.8	1.0	0.09
Hypertension	0.06	0.9	1.0
Diabetes mellitus	3.1	1.3	< 0.05
Dyslipidemia	1.2	0.8	0.2
History of atrial fibrillation	11.6	2.3	< 0.0001
History of cardiovascular disease	4.2	1.1	< 0.001
History of cerebrovascular disease	5.3	1.9	< 0.01
History of cancer	0.2	1.5	0.9

β indicates standardized regression coefficients; SE, standard error; BMI, body mass index; BP, blood pressure; eGFR, estimated glomerular filtration rate; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; FPG, fasting plasma glucose; HOMA-IR, FPG × insulin/405; HbA1c, glycosylated hemoglobin A1c; EF, ejection fraction; LVMI, left ventricular mass index; c-IMT, carotid intima-media thickness; hs-CRP, high-sensitivity C-reactive protein; and NT-proBNP, N-terminal prohormone of brain natriuretic peptide. *Log-transformed values were used for the calculation and reconverted into antilogarithm forms.

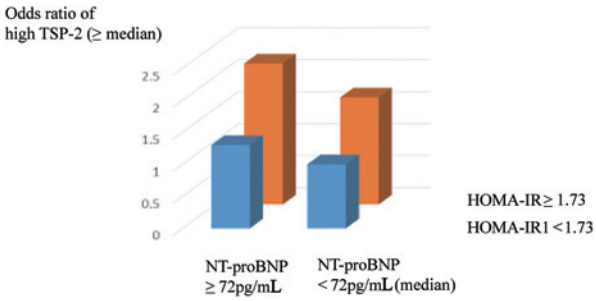

Figure 3. Mean TSP-2 levels stratified by four groups of NT-proBNP and HOMA-IR levels divided into low and high levels of NT-proBNP and low and high levels of HOMA-IR as the hierarchical model.

Table IV. Multiple Stepwise Regression Analysis for Correlates of Serum TSP-2 Levels

	β	SE	*P*
History of atrial fibrillation	9.0	2.4	< 0.0001
HOMA-IR*	2.2	0.6	< 0.001
hs-CRP*	0.8	0.3	0.011
NT-proBNP*	0.9	0.5	0.043

β indicates standardized regression coefficients; SE, standard error; HOMA-IR, fasting plasma glucose × insulin/405; hs-CRP, high-sensitivity C-reactive protein; and NT-proBNP, N-terminal prohormone of brain natriuretic peptide. *Log-transformed values were used for the calculation and reconverted into antilogarithm forms.

Table V. Odds Ratio of Insulin Resistance (HOMA-IR ≥ 1.73)

	Group 1 (*n* = 148)	Group 2 (*n* = 148)	Group 3 (*n* = 145)
	Reference	Odds ratio (95%CI)	Odds ratio (95%CI)
Unadjusted	1	1.32 (0.73-2.4)	1.86 (1.04-3.3)
Model 1	1	1.36 (0.74-2.5)	1.88 (1.04-3.39)
Model 2	1	1.73 (0.88-3.4)	2.11 (1.09-4.1)
Model 3	1	1.65 (0.84-3.27)	2.00 (1.03-3.9)

Group 1: TSP-2 6.44-17.2 ng/mL, Group 2: TSP-2 17.3-21.9 ng/mL, Group 3: TSP-2 22.7-71.9 ng/mL. CI indicates confidence interval. Model 1: adjusted for age and sex, Model 2: adjusted for age, sex, BMI and waist, Model 3: adjusted for age, sex, BMI, waist and medication for diabetes mellitus.

examining the serum levels of TSP-2 as a potential risk factor in a general population.

Serum levels of TSP-2: In this study, we observed that the mean ± SD of serum TSP-2 levels was 20.9 ± 8.5 ng/mL, and serum TSP-2 showed no significant sex difference. Regarding the serum levels of TSP-2, previous studies have shown that the mean level was 17.8 ng/mL in patients with HF with reduced EF (EF ≤ 50%) versus 14.8 ng/mL in control (non-HF, preserved EF),[6] 31.2 ng/mL in patients with stroke,[12] and 36.3 mg/dL in patients with systemic sclerosis.[10] The serum TSP-2 levels in a Japanese general population were comparable to those in patients with cardiovascular diseases. The clinical implication in this issue should be clarified in future longitudinal studies.

TSP-2 and IR: In the present study, we focused on the association between serum TSP-2 levels and HOMA-IR. The multivariate linear regression analysis after excluding diabetes also showed that serum TSP-2 levels were significantly associated with HOMA-IR. There have been a few previous studies regarding the association between TSP-2 and IR, some of which have shown that the overexpression of TSP-2 was caused by increased oxidative stress in diabetic mice.[25,26] Another *in vitro* study revealed that TSP-2 levels in vitreous samples from patients with proliferative diabetic retinopathy (PDR) were significantly higher than those in control patients without diabetes,[26] suggesting that the upregulation of TSP-2 might be protective against inflammation and angiogenesis associated with PDR.

TSP-2 and inflammation: In the present study, we demonstrated that there was a significant association between TSP-2 and hs-CRP, a marker of inflammation.[27] TSP-2 may be a natural potent inhibitor of angiogenesis, and the relationship between inflammation and angiogenesis has been widely accepted. Several studies have shown that the overexpression of TSP-2 could inhibit inflammatory responses as well as transforming growth factor-β activation.[28-33] In addition, it has been reported that TSP-2 would be an important regulatory element of T-lymphocytes in inflammatory diseases.[9,34,35] These previous observations were consistent with the significant association between TSP-2 and inflammation.

TSP-2 and history of cardiovascular diseases: Several previous studies have reported an association between TSP-2 and cardiovascular diseases such as viral myocarditis, doxorubicin-induced cardiomyopathy,[4,5] and HF.[6,7] However, Hanatani, *et al*.[6] reported that the levels of TSP-2 with ischemic diseases were comparable to those with nonischemic diseases, which is consistent with the present study. Although TSP-1 has been reported to be correlated to arrhythmias,[36,37] it was reported for the first time in the present study that there is an association between TSP-2 and arrhythmias, especially atrial fibrillation. This issue should be also clarified in the near future.

Limitation: Our study has several limitations. First, the study design was cross-sectional. Thus, nothing conclusive for the association of TSP-2 with morbidity and mortality could be stated. Second, although the medications for hypertension, dyslipidemia, and diabetes were not associated with circulating TSP-2 levels in our analyses, we were not able to exclude the contributions of some therapeutic agents. Finally, TSP-2 levels were measured in this cohort only once in 2013 and 2014. Nevertheless, the significant

and strong association between TSP-2 and potential risk factors is striking and deserves further investigation. We plan to perform longitudinal studies in the future in order to examine the relationship between TSP-2 and cardiovascular events/mortality.

Conclusions

In conclusion, TSP-2 can be a potential risk factor in a general population.

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Disclosures

Conflicts of interest: There are no conflicts of interest for this paper with respect to any of the authors.

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