Elevated plasma transforming growth factor β1 levels predict the development of hypertension in normotensives: The 14-year follow-up study

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ABSTRACT

BACKGROUND

Transforming growth factor β1 (TGF-β1) is a multifunctional cytokine. There is growing evidence that TGF-β1 is involved in the pathogenesis of hypertension and the development of target organ damage in hypertensives. Although several studies have shown that TGF-β1 induced vascular hypertrophy and remodelling in various vascular diseases, there are no longitudinal data on hypertension in the epidemiological studies. The present study tested the hypothesis whether elevated TGF-β1 levels can predict the development of hypertension.

METHODS

In 2002-2004, 528 subjects received health examinations in Uku town, southwestern Japan. We examined blood pressure, body mass index and blood test. Data on fasting plasma TGF-β1 were obtained from 528 individuals. Of these, 149 normotensives (blood pressure <140/90 mmHg without anti-hypertensive medications) at baseline were followed-up for 14 years.

RESULTS

The ROC curve was used and the calculated cut-off value was 8.9ng/ml. Of 149 normotensives at baseline, 59 subjects developed hypertension. Plasma TGF-β1 levels were significantly associated with the development of hypertension after adjustment for confounding factors. To further examine the association between them, we performed logistic regression analysis. We divided the baseline plasma TGF-β1 levels into two groups using a cut-off value. The significant high odds ratio [3.582 (95% CI, 1.025-12.525)] for the development of hypertension was found in the highest group of TGF-β1 level vs. the lowest group after adjustment for confounders.

CONCLUSIONS

This is the first report demonstrating the causal relationship between them. Elevated plasma TGF-β1 levels predicted the development of hypertension in normotensives in a population of community-dwelling Japanese.

Key words: transforming growth factor β1; hypertension; cytokine; epidemiology; hypertrophy

INTRODUCTION

Transforming growth factor β1 (TGF-β1) is a multifunctional cytokine that has been linked to myocardial hypertrophy.[1] It has been recently revealed that plasma levels of TGF-β1 are markedly increased in patients with hypertension.[2] However, it is well known that one of the major complications of TGF-β receptor inhibitors for treatment of cancers is hypertension.[3-5] Several studies in humans and experimental models have shown that the increased myocardial TGF-β expression were associated with cardiac hypertrophy and fibrosis.[6-8] It has been also suggested that the levels of TGF-β were significantly associated with other organ damage including kidney.[9,10] Moreover, some life-style such as smoking habit is thought to increase plasma levels of TGF-β1.[11,12] However, no epidemiological prospective study has investigated the relationship between TGF-β1 and the development of hypertension in normotensives.

Therefore, we conducted a health examination of subjects (n=528) in a fishing community (Uku town, Nagasaki, Japan)[13-15], and performed an epidemiological study to examine the relationship between plasma TGF-β1 and hypertension.

METHODS

Study population. In 2002-2004, in a fishing community in southwestern Japan (Uku town), a total of 528 people received health examinations. This town is an isolated island near Fukue city, located in Nagasaki prefecture, and the total population is about 3,700. We have been performing an epidemiological study every year for recent 15 years. All participants approved the blood test. Eventually, complete data set for 528 subjects (190 males and 338 females) was available in this study.

Of 528 participants, 379 subjects were excluded due to no blood tests in 91, hypertensive medication in 201, and hypertensives in 87. Of 149 normotensives (blood pressure <140/90 mmHg without anti-hypertensive medications) at baseline, 9 subjects were unknown and moved, and 7 subjects were died. Finally, we included 133 subjects in the present study, who were followed-up for 14 years. Of these, 59 subjects developed hypertension. Sample size flow chart was shown in **Figure 1**.

Data collection. The medical history, smoking and alcohol statuses were ascertained by a questionnaire, which we carefully interviewed. Smoking

and alcohol were classified as current habitual use or not. Height and weight were measured, and body mass index (BMI: kilograms per meter squared) was calculated as an index of obesity. Blood pressure (BP) was measured twice with the subjects in the sitting and supine positions. Vigorous physical activity and smoking were avoided for at least 30 minutes before BP measurement. The supine BP with the fifth phase diastolic pressure was used for analysis.

Blood was drawn from the antecubital vein in the morning after 12-hour fast for determinations of lipids (total cholesterol, high density lipoprotein-cholesterol, and triglycerides), fasting plasma glucose (FPG), insulin, blood urea nitrogen (BUN), creatinine, uric acid, and TGF-β1. TGF-β1 was measured by Enzyme immune-assay (EIA method).[16] Intra- and inter-assay coefficients of variation of TGF-β1 in a commercially available laboratory (SRL Inc. Fukuoka, Japan) were 1.5% and 3.5%, respectively. All chemistries were measured at a commercially available laboratory (The Kyodo Igaku Laboratory, Fukuoka, Japan).

A 12-lead electrocardiogram (ECG) allowed estimation of left ventricular (LV) hypertrophy (high-voltage criteria in the Minnesota code:

$SV_1+RV_5 \geq 3.5$ mV; where SV_1 is the S-wave voltage in lead V_1 and RV_5 is the R-wave voltage in lead V_5). Two-dimensional directed and guided M-mode echocardiographic studies were performed in all subjects by experienced investigators. The LV mass was measured on the M-mode guided echocardiogram[17], which was derived from the formula described by American Society of Echocardiography (ASE)[18]: LV mass (gram) $=0.80\times1.04$ $([VSTd+LVIDd+PWTd]^3-[LVIDd]^3) +0.6$, where VSTd is the end-diastolic ventricular septal thickness; LVIDd is the LV end-diastolic internal dimension; and PWTd is the LV end-diastolic posterior wall thickness.

The mayor and the welfare section of Uku town and the Ethical Committee of Kurume University approved this study. All participants gave informed consent.

Statistical analysis. Because of skewed distributions, the natural logarithmic (ln) transformations were performed for triglycerides, FPG and insulin. Mean values with upper and lower 95% confidence intervals (CI) were exponentiated and presented as geometric mean ± standard deviation

(SD), where the SD was approximated as the difference of the exponentiated CI divided by 3.92, which is the number of SD in a 95% CI where data are normally distributed. Results are presented as mean ± SD. The medication for hypertension was coded as dummy variables. Univariate analysis was performed for determinants of plasma TGF-β1 levels in total subjects. To investigate independent determinants of TGF-β1 levels, multiple linear regression analysis adjusted for age and sex was performed in hypertensive subjects. Statistical significance was defined as $p<0.05$. All statistical analyses were performed with the use of the SAS system (release 9.4; SAS Institute, Cary, NC).

RESULTS

Cross-sectional study. Characteristics of 528 subjects are presented **Table 1**. Mean age was 64.1 years old. Almost half of them have taken anti-hypertensive treatment and smoking rates were relatively low (23.2% in males and 1.2% in females). The mean values of TGF-β1 were 5.7 ng/ml for males and 5.4 ng/ml for females. In the 528 subjects, systolic BP level ($p=0.002$) and smoking habit ($p=0.026$) was significantly associated with

TGF-β1. No significant association was observed between TGF-β1 and other parameters including hypertensives (data not shown).

Prospective study. Characteristics of 133 normotensives subjects are presented in **Table 2**. The mean values of TGF-β1 were 5.9 ng/ml for males and 4.7 ng/ml for females. To examine the association between TGF-β1 levels and the development of hypertension, we performed logistic regression analysis. Of 133 normotensives at baseline, 59 subjects developed hypertension during the 14-year follow-up. **Table 3** shows the results of univariate logistic regression analysis to predict the development of hypertension. Age (p=0.002), BMI (p=0.003), systolic BP (p=0.008), glucose (p=0.039), TGF-β1 (p=0.045), and LV mass index (p=0.035) were significantly associated with the development of hypertension.

As shown in box plots of plasma TGF-β1 levels stratified by with and without hypertension progression (**Figure 2**), mean TGF-β1 levels in subjects with hypertension were significantly higher than those without hypertension (p=0.030). Optimal plasma TGF-β1 cut-off value for the development of hypertension was examined using receiver operating

characteristic (ROC) analysis. The calculated cut-off value of TGF-β1 was 8.9 ng/ml with the C-statistics of 0.580. We divided the baseline plasma TGF-β1 levels into two groups using the cut-off value. The significant high odds ratio [3.582 (95% CI, 1.025-12.525)] for the development of hypertension was observed in the high group of plasma TGF-β1 level after adjustment for age, sex, systolic BP, BMI, glucose and LV mass index, as shown in **Table 4**.

DISCUSSION

This is the first prospective study demonstrating the causal relationship between the elevated TGF-β1 and the development of hypertension. Elevated plasma TGF-β1 levels could predict the development of hypertension in normotensives in a population of community-dwelling Japanese.

Cross-sectional study. Although the number of enrolled subjects in the previous studies was small, less than about 100,[2,9,11,17,19,20] the present study examined more than 500, which enables us to examine the distribution of

plasma TGF-β1 levels in a general population. The mean levels of TGF-β1 were 5.7 ng/ml for males and 5.4 ng/ml for females (**Table 1**).

TGF-β1 is involved in the pathogenesis of hypertension through several mechanisms[2], including stimulation of endothelin mRNA expression in the vascular endothelium[21], the increase of renin release from juxtaglomerular cells in the kidney, and the regulation of angiotensin II expression.[22] In univariate analysis in the present study, systolic BP was a significant parameter with TGF-β1 levels and the prevalence of hypertension was not related to TGF-ß1 levels (p=0.137) at baseline (data not shown), which were consistent with the previous study.[2] Although some anti-hypertensive drugs may affect TGF-β1 levels, we have no data regarding the issue. Further, because TGF-β receptor inhibitors can cause hypertension in the cancer fields,[3-5] the precise mechanisms should be clarified in future.

TGF-β plays a causal role in myocardial fibrosis and diastolic through fibroblast activation in pressure-overloaded hearts.[8,23,24] Schultz et al.[25] demonstrated that TGF-β1 is an important mediator of the hypertrophic growth response of the heart to angiotensin II. In the linear

regression analysis in the present study, there was a marginal (p=0.053) positive correlation between TGF-β1 and LV mass index (data not shown in Table). The elevated TGF-β1 is related to cardiac hypertrophy in hypertensive subjects.[26] Although the cross-sectional analyses were not able to indicate whether the elevated TGF-β1 levels were the causes or the results of cardiac hypertrophy, the elevated TGF-β1 may be a useful biomarker for left ventricular hypertrophy in hypertensive subjects.

Current smoking is significantly associated (p=0.026) with TGF-β1 in univariate linear regression analysis. In the same districts, we also have demonstrated that current smoking is significantly associated with plasma heat shock protein 27 levels.[27] Nicotine has been reported to promote TGF-β1 and basic fibroblastic growth factor release in bovine aortic endothelial cells.[28] Also, it has been reported that cigarette smoking condensate induced cell adhesion molecules in human endothelial cells through protein kinase C activation[29], which is a common pathway for TGF-β1 synthesis. Ematjes et al.[11] showed that smoking may be associated with higher production of TGF-β1.

Prospective study. This is the first longitudinal study to demonstrate the hypothesis that high levels of plasma TGF-β1 levels could predict the development hypertension, in subjects without hypertension at baseline. Although a prospective study from Italy has demonstrated that higher TGF-β1 levels are associated with the development of hypertensive renal disease, they did not demonstrate the association of TGF-β1 with the development of hypertension.[30] Rather than the development of hypertension, there are some manuscripts regarding the excess risk of renal diseases.[31,32] The mechanisms are not still clear; however, TGF-β1 is a fibrogenic cytokine that plays a key role in the pathogenesis of renal diseases, and TGF-β1 blockade may prevent renal disease progression.[33,34] Otherwise, it may be caused by the racial difference, both of which[33,34] were analyzed in African Americans. In the present study, we demonstrated not only our hypothesis but also the extent and the magnitude of the association; that is, subjects with plasma TGF-β1 levels ≥9.0 ng/mL were shown to have a 3.58-fold higher risk of developing hypertension in 14 years than those with TGF-β1 levels ≤8.9 ng/mL.

It is interesting to note that LV mass index is marginally (p=0.053)

and significantly (p=0.035) associated with TGF-β1 levels in our cross-sectional and longitudinal studies, which are consistent with the previous studies.[19,35,36] In the present study, multiple linear regression analysis showed that LVH in electrocardiogram and LVMI in echocardiogram were significantly associated with TGF-β1 levels in hypertensive subjects adjusted by confounding factors (Data not shown).[26] Almendral JL et al.[16] reported the significant association ($p<0.01$) between TGF-β1 and LV mass in hypertensive patients.

We did not show the dose-dependency of TGF-β1 levels to predict the development of hypertension, which suggests that a cutoff value may exist. Accordingly, we calculated cutoff values by receiver-operating characteristic curves, which was 8.9 ng/mL. Taken together, these findings indicate that this cutoff value may be relevant when considering the association of TGF-β1 levels with the development of hypertension. Subjects with baseline TGF-β1 levels ≥9.0 ng/dL were 3.58 times more likely to develop hypertension after 14 years compared with subjects with TGF-β1 levels ≤8.9 ng/dL. Our findings may be pertinent in the context of a pathophysiological role of TGF-β1 in the general population.

Study limitations and perspective

There were several limitations in this study. First, this study was conducted in Japan, where the incidence of hypertension is relatively high compared with Caucasians. Second, 16 subjects (10.7%) were lost to follow-up, because of death, moved or unknown to participate in re-examination. Therefore, we limited our analyses to subjects who completed baseline and follow-up examinations. Third, the sample size was relatively small, although it was larger than those in the previous studies.[2,9,11,17,19,20] A final limitation is that we could not clarify the types of antihypertensive drugs used during the 14-year period, because antihypertensive drugs were changed frequently by primary physicians to control BP or for adverse effects.

Cross-sectional study showed that LV mass index and LVH in electrocardiogram were significantly associated with TGF-β1 levels in hypertensive subjects. Prospective study showed that the elevated TGF-β1 predicted the development of hypertension in normotensives at baseline. Thus, it would be useful to examine whether TGF-β1 blockers could be

effective for hypertensives to decrease LV hypertrophy and hopefully further to improve LV diastolic dysfunction, and even for normotensives to prevent the development of hypertension in subjects with high plasma TGF-β1 levels.

In conclusion, the present study demonstrated that elevated TGF-β1 levels predicted the development of hypertension after 14 years in subjects without hypertension at baseline in a general population.

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Disclosure:

The authors declared no conflicts of interest.

REFERENCES

1. Border WA, Nobel NA. Transforming growth factor-β in tissue fibrosis. *N Engl J Med*. 1994; 331:1286-1292.
2. Derhaschnig U, Shehata M, Herkner H, Bur A, Woisetschlager C, Laggner AN, Hirschl MM. Increased levels of transforming growth factor-β1 in essential hypertension. *Am J Hypertens*. 2002;15: 207-211.
3. Bhola NE, Balko JM, Dugger TC, Kuba MG, Sánchez V, Sanders M, Stanford J, Cook RS, Arteaga CL. TGF-β inhibition enhances chemotherapy action against triple-negative breast cancer. *J Clin Invest*. 2013; 123:1348-1358.
4. Zhou L, McMahon C, Bhagat T, Alencar C, Yu Y, Fazzari M, Sohal D, Heuck C, Gundabolu K, Ng C, Mo Y, Shen W, Wickrema A, Kong G, Friedman E, Sokol L, Mantzaris I, Pellagatti A, Boultwood J, Platanias LC, Steidl U, Yan L, Yingling JM, Lahn MM, List A, Bitzer M, Verma A. Reduced SMAD7 leads to overactivation of TGF-beta signaling in MDS that can be reversed by a specific inhibitor of TGF-beta receptor I kinase. *Cancer Res*. 2011; 71:955-963.
5. Senturk S, Mumcuoglu M, Gursoy-Yuzugullu O, Cingoz B, Akcali KC,

Ozturk M. Transforming growth factor-beta induces senescence in hepatocellular carcinoma cells and inhibits tumor growth. *Hepatology*. 2010; 52:966-974.

6. Villarreal FJ, Dillmann WH. Cardiac hypertrophy-induced changes in mRNA levels for TGF-beta 1, fibronectin, and collagen. *Am J Physiol*. 1992; 262:H1861-H1866.
7. Takahashi N, Calderone A, Izzo NJ, Maki TM, Marsh JD, Colucci WS. Hypertrophic stimuli induce transforming growth factor-β1 expression in rat ventricular myocytes. *J Clin Invest.* 1994; 94:1470-1476.
8. Tomita H, Egashira K, Ohara Y, Takemoto M, Koyanagi M, Katoh M, Yamamoto H, Tamaki K, Shimokawa H, Takeshita A. Early induction of transforming factor-β via angiotensin II type 1 receptors contributes to cardiac fibrosis induced by long-term blockade of nitric oxide synthesis in rats. *Hypertension.* 1998; 32:273-279.
9. Laviades C, Varo N, Diez J. Transforming growth factor β in hypertensives with cardiorenal damage. *Hypertension*. 2000; 36:517-522.
10. Bobik A. Hypertension, transforming growth factor-β, angiotensin II

and kidney disease. *J of Hypertens.* 2004; 22:1265-1267.

11. Esmatjes E, Flores L, Lario S, Claria J, Cases A, Inigo P, Campistol JM. Smoking increases serum levels of transforming growth factor-β in diabetic patients. *Diabetes Care.* 1999; 22:1915-1916.
12. Peterson MC. Circulating transforming growth factor-β-1: a partial molecular explanation for associations between hypertension, diabetes, obesity, smoking and human disease involving fibrosis. *Med Sci Monit.* 2005; 11: RA229-232.
13. Fukami A, Adachi H, Hirai Y, Enomoto M, Otsuka M, Kumagai E, Nakamura S, Yoshimura A, Obuchi A, Nohara Y, Nakao E, Hori K, Fukumoto Y. Association of serum eicosapentaenoic acid to arachidonic acid ratio with microalbuminuria in a population of community-dwelling Japanese. *Atherosclerosis*. 2015; 239:577-582.
14. Obuchi A, Adachi H, Enomoto M, Fukami A, Kumagai E, Nakamura S, Yoshimura A, Nohara Y, Nakao E, Umeki Y, Fukumoto Y, Imaizumi T. High plasma fetuin-A levels are associated with metabolic syndrome among males but not females in a Japanese general population. *Diabetes Res Clin Pract*. 2014; 106:128-135.

15. Esaki E, Adachi H, Hirai Y, Yamagishi S, Kakuma T, Enomoto M, Fukami A, Kumagai E, Ohbu K, Obuchi A, Yoshimura A, Nakamura S, Nohara Y, Fujiyama T, Fukumoto Y, Imaizumi T. Serum vaspin levels are positively associated with carotid atherosclerosis in a general population. *Atherosclerosis*. 2014; 233:248-252.
16. Almendral JL, Shick V, Rosendorff C, Atlas SA. Association between transforming growth factor-β1 and left ventricular mass and diameter in hypertensive patients. *J Am Soc Hypertens.* 2010; 4:135-141.
17. Yasunari K, Maeda K, Watanabe T, Nakamura M, Yoshikawa J, Asada A. Comparative effects of valsartan versus amlogipine on left ventricular mass and reactive oxygen species formation by monocytes in hypertensive patients with left ventricular hypertrophy. *J Am Coll Cardiol.* 2004; 43:2116-2123.
18. Lang RM, Badano LP, Mor-Avi V, Afilalo J, Armstrong A, Ernande L, Flachskampf FA, Foster E, Goldstein SA, Kuznetsova T, Lancellotti P, Muraru D, Picard MH, Rietzschel ER, Rudski L, Spencer KT, Tsang W, Voigt JU. Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of

Echocardiography and the European Association of Cardiovascular Imaging. *Eur Heart J Cardiovasc Imaging*. 2015; 16:233-270.

19. Tanaka H, Oki T, Tabata T, Yamada H, Harada K, Kimura E, Oishi Y, Ishimoto T, Ito S. Losartan improves regional left ventricular systolic and diastolic function in patients with hypertension: Accurate evaluation using a newly developed color-coded tissue Doppler imaging technique. *J Card Fail.* 2004; 10:412-420.
20. Haramaki R, Tamaki K, Fujisawa M, Ikedo H, Haramaki N, Okuda S. Steroid therapy and urinary transforming growth factor-β1 in IgA nephropathy. *Am J Kidney Dis.* 2001; 38:1191-1198.
21. Kurihara H, Yoshizumi M, Sugiyama T, Takaku F, Yanagisawa M, Masaki T, Hamaoki M, Kato H, Yazaki Y. Transforming growth factor-β stimulates the expression of endothelin mRNA by vascular endothelial cells. *Biochem Biophys Res Comm.* 1989; 159:1435-1440.
22. Border WA, Noble NA. Interactions of transforming growth factor-beta and angiotensin II in renal fibrosis. *Hypertension.* 1998; 31:181-188.
23. Kuwahara F, Kai H, Tokuda K, Kai M, Takeshita A, Egashira K, Imaizumi T. Transforming growth factor-β function blocking prevents

myocardial fibrosis and diastolic dysfunction in pressure-overloaded rats. *Circulation.* 2002; 106:130-135.

24. Kai H, Kuwahara F, Tokuda K, Imaizumi T. Diastolic dysfunction in hypertensive hearts: Roles of perivascular inflammation and reactive myocardial fibrosis. *Hypertensive Res.* 2005; 28:483-490.

25. Schultz JE, Witt SA, Glascock BJ, Nieman ML, Reiser PJ, Nix SL, Kimball TR, Doetschman T. TGF-β1 mediates the hypertrophic cardiomyocyte growth induced by angiotensin II. *J Clin Invest.* 2002; 109:787-796.

26. Molloy TJ, Okin PM, Devereux RB, Kligfield P. Electrocardiographic detection of left ventricular hypertrophy by the simple QRS voltage-duration product. *J Am Coll Cardiol*. 1992; 20:1180-1186.

27. Nakayoshi T, Adachi H, Ohbu-Murayama K, Enomono M, Fukami A, Kumagai E, Nakamura S, Nohara Y, Nakao E, Sasaki K, Fukumoto Y. Plasma heat shock protein 27 is increased in renal dysfunction and habitual smoking in a Japanese general population. *J Cardiol*. 2016; 67: 110-114.

28. Cucina A, Corvino V, Sapienza P, Borrelli V, Lucarelli M, Scarpa S,

Strom R, D'Angele LS, Cavallaro A. Nicotine regulates basic fibroblastic growth factor and transforming factor β1 production in endothelial cells. *Biochem Biophys Res Comm.* 1999; 257:306-312.

29. Shen Y, Rattan V, Sultana C, Kalra VK. Cigarette smoke condensate-induced adhesion molecule expression and transendothelial migration of monocytes. *Am J Physiol.* 1996; 270:H1624-1633.

30. Scaglione R, Argano C, Parrinello G, Colomba D, Di Chiara T, Ferrante A, Di Garbo V, Avellone G, Licata G. Relationship between transforming growth factor beta1 and progression of hypertensive renal disease. *J Hum Hypertens.* 2002; 16:641-645.

31. August P, Sharma V, Ding R, Schwartz JE, Suthanthiran M. Transforming growth factor beta and excess burden of renal disease. *Trans Am Clin Climatol Assoc*. 2009; 120:61-72.

32. Suthanthiran M, Gerber LM, Schwartz JE, Sharma VK, Medeiros M, Marion R, Pickering TG, August P. Circulating transforming growth factor-beta1 levels and the risk for kidney disease in African Americans. *Kidney Int*. 2009; 76:72-80.

33. Border WA, Noble NA. Transforming growth factor beta in tissue

fibrosis. *N Engl J Med*. 1994; 331:1286-1292.

34. Huang Y, Border WA, Noble NA. Perspectives on blockade of TGFbeta overexpression. *Kidney Int*. 2006; 69:1713-1734.

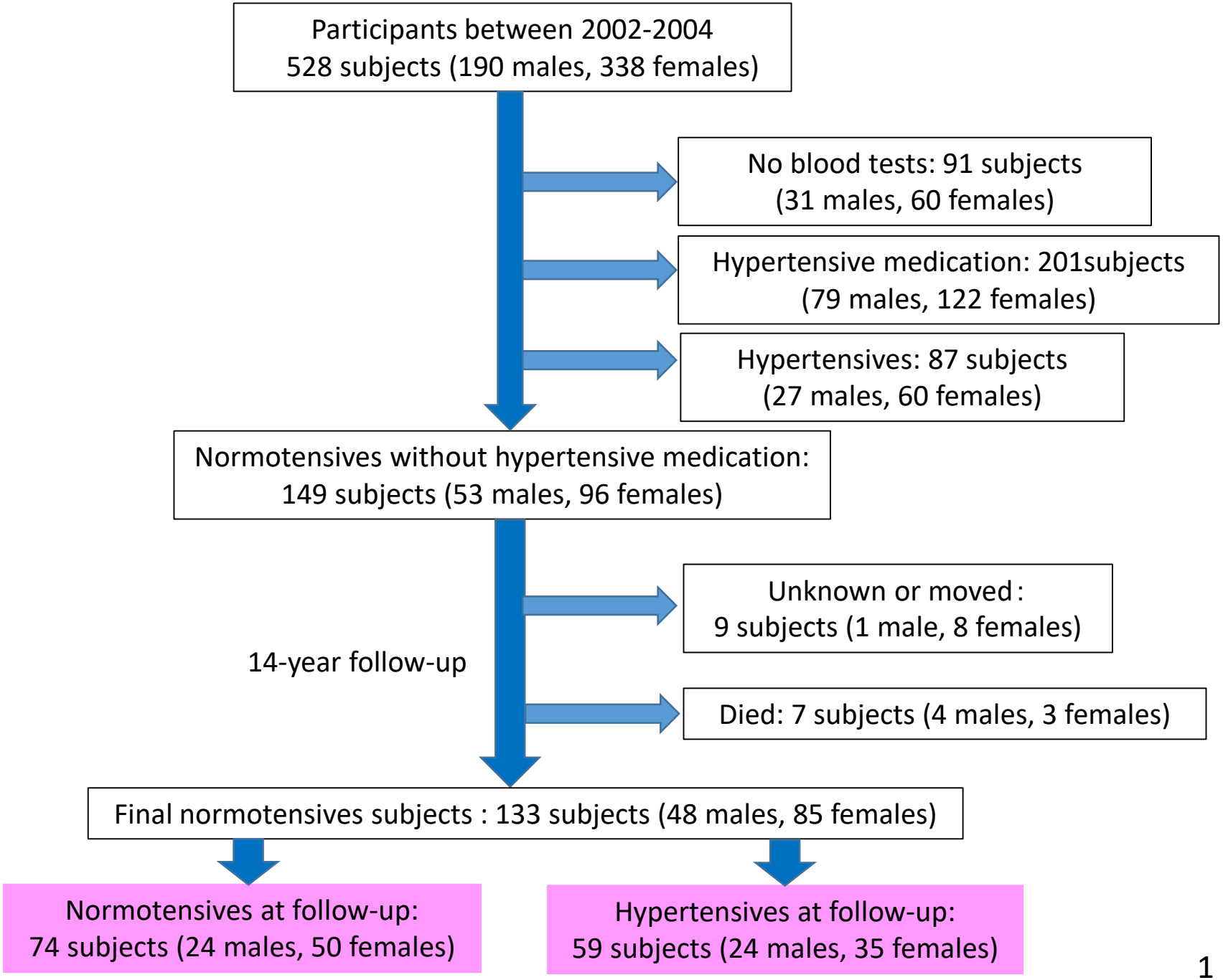
35. Villar AV, Cobo M, Llano M, Montalvo C, González-Vílchez F, Martín-Durán R, Hurlé MA, Nistal JF. Plasma levels of transforming growth factor-beta1 reflect left ventricular remodeling in aortic stenosis. *PLoS One*. 2009; 4: e8476.

36. Xu HY, Hou XW, Wang LF, Wang NF, Xu J. Association between transforming growth factor beta1 polymorphisms and left ventricle hypertrophy in essential hypertensive subjects. *Mol Cell Biochem*. 2010; 335:13-17.

Figure legends

Figure 1. Flow chart of enrolled subjects.

Figure 2: Plasma TGF-β1 levels stratified by without and with hypertension progression.


Participants between 2002-2004
528 subjects (190 males, 338 females)
No blood tests: 91 subjects
(31 males, 60 females)
Hypertensive medication: 201subjects
(79 males, 122 females)
Hypertensives: 87 subjects
(27 males, 60 females)
Normotensives without hypertensive medication:
149 subjects (53 males, 96 females)
14-year follow-up
Unknown or moved :
9 subjects (1 male, 8 females)
Died: 7 subjects (4 males, 3 females)
Final normotensives subjects : 133 subjects (48 males, 85 females)
Normotensives at follow-up:
74 subjects (24 males, 50 females)
Hypertensives at follow-up:
59 subjects (24 males, 35 females)

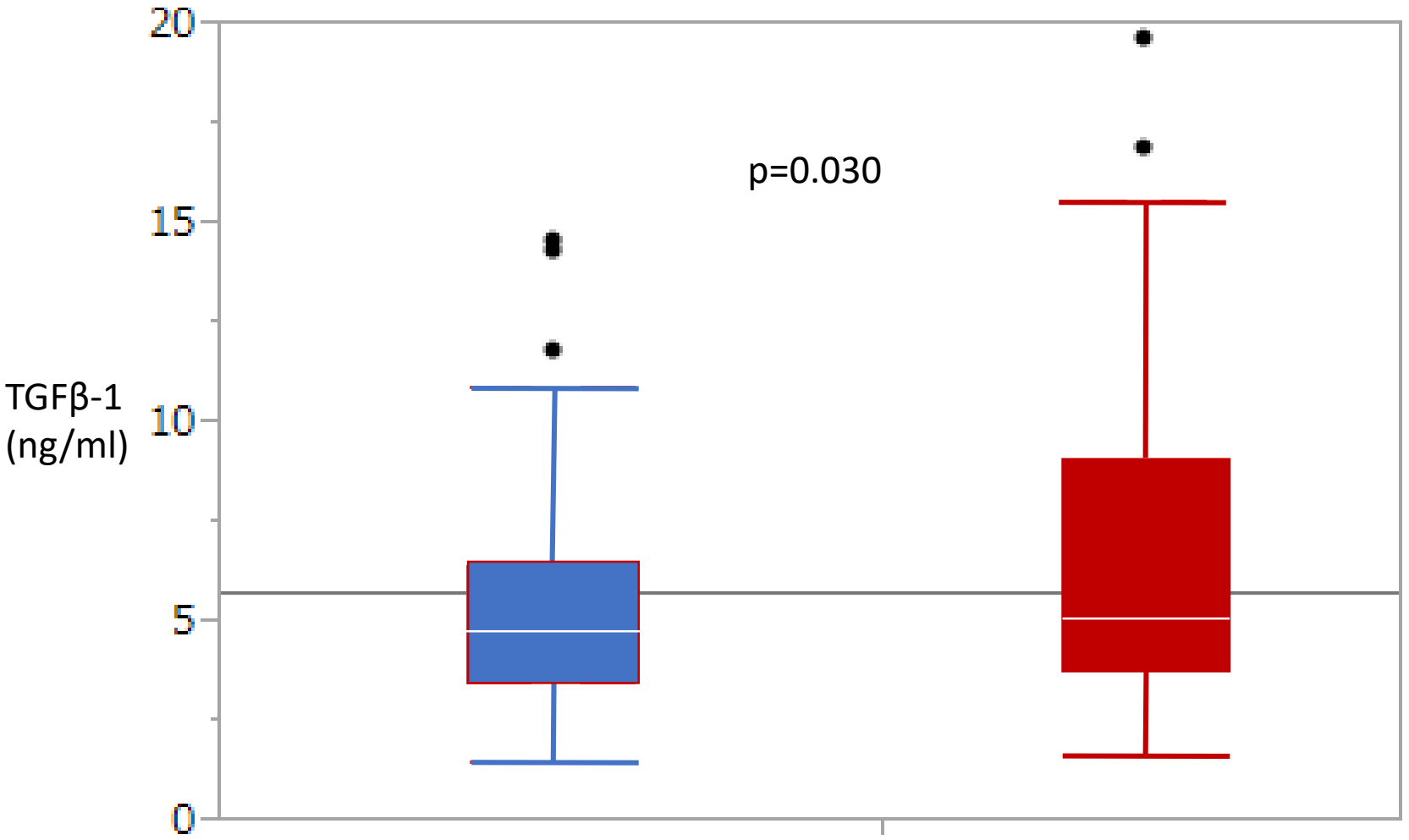

20
15
10
5
0
TGFβ-1
(ng/ml)
p=0.030
HT progression (-)
HT progression (+)
Median(IQR)
4.70（3.45-6.43）
5.05（3.78-8.93）

Table 1. Characteristics of 528 Subjects at Baseline in 2002-2004

Parameters	Males (n=190)	Females (n=338)	Total (n=528)
Age, y	65.8±8.7	63.1±9.7	64.1±9.4
Body mass index (kg/m^2)	23.7±3.2	23.6±3.4	23.6±3.3
Systolic BP (mmHg)	137.0±18.9	139.4±21.3	138.6±20.5
Diastolic BP (mmHg)	83.4±11.9	81.5±11.4	82.2±11.6
Total cholesterol (mg/dl)	195.4±33.5	210.6±33.3	205.1±34.1
HDL-cholesterol (mg/dl)	57.3±14.2	62.4±14.5	60.6±14.6
Triglycerides (mg/dl) *	96.3 (37-777)	84.5 (20-284)	88.6 (20-777)
Glucose (mg/dl)	100.7±16.9	93.3±10.6	95.9±13.6
Insulin (μU/ml)*	4.9 (1-101)	4.8 (1-23)	4.9 (1-101)
BUN (mg/dl)	18.5±4.5	16.8±4.3	17.4±4.7
Creatinine (mg/dl)	0.9±0.2	0.7±0.2	0.8±0.2
eGFR (ml/min/1.73m^2)	70.4±18.1	71.1±17.3	70.9±17.6
Uric acid (mg/dl)	5.9±1.4	4.5±1.0	5.0±1.4
TGF-β1 (ng/ml)*	5.7 (1.6-46.5)	5.4 (1.4-48.3)	5.5 (1.4-48.3)
sV_1+rV_5 (mm)	27.3±10.7	26.2±9.4	26.6±9.9
LV mass index (g/m^2)	101.4±21.6	98.8±24.7	99.7±23.7
Alcohol intake (%)	53.2	3.3	21.2
Smoking (%)	23.2	1.2	9.0
Medication for hypertension (%)	49.5	44.4	46.2
Prevalence of hypertension (%)	66.3	65.7	65.9

Data are expressed as mean±SD, unless otherwise indicated.

*Log-transformed values were used in analyses. Hypertensives were defines as systolic BP≥140 and/or diastolic BP≥90mmHg and/or treatment.

Abbreviations: BP; blood pressure, HDL; high density lipoprotein, BUN; blood urea nitrogen, eGFR; estimated glomerular filtration rate, TGF-β1; transforming growth factor β1, LV; left ventricular

Table 2. Characteristics of 133 Normotensive Subjects

Parameters	Males (n=48)	Females (n=85)	Total (n=133)
Age, y	62.2±9.9	57.9±9.9	59.4±10.1
Body mass index (kg/m^2)	23.9±3.3	23.1±3.2	23.4±3.2
Systolic BP (mmHg)	121.5±9.8	119.7±11.1	120.3±10.6
Diastolic BP (mmHg)	75.1±7.7	73.8±8.1	74.3±7.9
Total cholesterol (mg/dl)	198.1±36.5	210.6±36.3	206.1±36.7
HDL-cholesterol (mg/dl)	56.1±16.4	63.4±13.7	60.8±15.1
Triglycerides (mg/dl) *	107.4 (43-777)	76.5 (33-252)	86.5 (33-777)
Glucose (mg/dl)	94.8±10.3	91.4±12.3	92.6±11.7
Insulin (μU/ml)*	4.5 (1-38)	4.6 (1-15)	4.5 (1-38)
BUN (mg/dl)	18.4±4.5	16.9±4.7	17.5±4.6
Creatinine (mg/dl)	0.9±0.2	0.7±0.2	0.8±0.2
eGFR (ml/min/1.73m^2)	72.6±15.5	67.9±16.5	69.6±16.3
Uric acid (mg/dl)	5.6±1.2	4.2±0.9	4.7±1.2
TGF-β1 (ng/ml)*	5.9 (1.6-46.5)	4.7 (1.4-16.9)	5.1 (1.4-46.5)
sV_1+rV_5 (mm)	22.7±8.1	23.5±6.2	23.2±6.9
LV mass index (g/m^2)	103.9±19.9	94.1±19.8	97.6±20.3
Alcohol intake (%)	45.8	3.5	18.8
Smoking (%)	33.3	1.2	12.8

Data are expressed as mean±SD, unless otherwise indicated.

*Log-transformed values were used in analyses.

Abbreviations: BP; blood pressure, HDL; high density lipoprotein, BUN; blood urea nitrogen, eGFR; estimated glomerular filtration rate, TGF-β1; transforming growth factor β1, LV; left ventricular

Table 3. Univariate Logistic Regression Analysis for Predictors of the Development of Hypertension in 133 Normotensives

	Odds ratio	95%C.I.	*p*
Age	1.061	1.022-1.101	**0.002**
Sex (Males=0, Females=1)	0.700	0.344-1.426	0.326
Body mass index	1.191	1.060-1.338	**0.003**
Systolic BP (mmHg)	1.047	1.012-1.084	**0.008**
Diastolic BP (mmHg)	1.030	0.986-1.077	0.186
Glucose (mg/dl)	1.042	1.002-1.083	**0.039**
eGFR (ml/min/1.73m^2)	0.983	0.962-1.005	0.132
TGF-β1 (ng/ml)*	1.948	1.015-3.740	**0.045**
sV_1+rV_5 (mm)	1.020	0.970-1.072	0.439
LV mass index (g/m^2)	1.018	1.001-1.034	**0.035**
Alcohol intake (No=0, Yes=1)	1,782	0.741-4.285	0.197
Smoking (No=0, Yes=1)	1.133	0.408-3.143	0.811

*Log-transformed values were used in analyses.

Abbreviations: BP; blood pressure, HDL; high density lipoprotein, BUN; blood urea nitrogen, eGFR; estimated glomerular filtration rate, TGF-β1; transforming growth factor β1, LV; left ventricular

Table 4. Relative Risks of Development of Hypertension by the Two Groups of Plasma TGF-β1 Levels

	Low group of TGF-β1	High group of TGF-β1	*p*
Models			
TGF-β1, ng/ml	1.4-8.9	9.0-46.5	
Number	113	20	
Number of the development of hypertension (%)	44 (38.9)	15 (75.0)	
Model 1	1.000	4.705 (1.597-13.860)	0.005
Model 2	1.000	4.283 (1.256-14.606)	0.020
Model 3	1.000	3.582 (1.025-12.525)	0.046

Model 1 : Unadjusted
Model 2: Adjusted for age, sex, systolic blood pressure, body mass index and glucose
Model 3: Adjusted for age, sex, systolic blood pressure, body mass index, glucose and LV mass index