Original Article

Arterial Stiffness, Physical Activity, and Atrial Natriuretic Peptide Gene Polymorphism in Older Subjects

Motoyuki IEMITSU[1),2)], Seiji MAEDA[1),2)], Takeshi OTSUKI[1)], Jun SUGAWARA[3)], Shinya KUNO[2)], Ryuichi AJISAKA[2)], and Mitsuo MATSUDA[1)]

An increase in arterial stiffness with advancing age is associated with several pathological states, including hypertension and arteriosclerosis. Regular exercise improves the aging-induced increase in arterial stiffness and has a protective effect against these diseases. However, not all individuals respond to exercise to the same extent. Atrial natriuretic peptide (ANP) is involved in the regulation of basal blood pressure, blood flow, and vascular tone. The present study was designed to clarify whether gene polymorphisms in ANP-related genes affect exercise-induced improvements in arterial stiffness. We performed a cross-sectional study of 291 healthy middle-aged and older Japanese subjects (63±1 years), examining the relationship between daily physical activity–induced improvements in arterial stiffness, estimated by brachial-ankle arterial pulse wave velocity (baPWV), and the gene polymorphisms of valine32methionine (V32M: 664G>A) in exon 1 of ANP and asparagine521aspartic acid (N521D: 1780A>G) in exon 8 of the ANP clearance receptor (NPR-C). The baseline baPWV was significantly lower in the active group, but no differences were seen in blood pressure. Active subjects with the ANP-VV genotype had significantly lower baPWV and higher plasma ANP levels compared with inactive subjects, but there were no variations related to the VM+MM genotype. Additionally, baPWV and plasma ANP levels were negatively correlated in ANP-VV genotype subjects, but were not correlated in VM+MM individuals. Our results suggest that ANP polymorphism in older Japanese subjects may affect the cardiovascular response to regular exercise. (*Hypertens Res* 2008; 31: 767–774)

***Key Words*: regular exercise, pulse wave velocity, genotype**

Introduction

The central arteries, the aorta and large arteries, have a buffering action to level off fluctuations in blood pressure created by cardiac pulsation, thereby supplying a relatively constant blood flow to the tissues and organs. Increases in arterial stiffness impair this buffering ability, leading to a rise in systolic blood pressure (SBP) and left ventricular afterload. In sedentary healthy humans, arterial stiffness of the central circulation increases with advancing age (*1*, *2*). The age-related increase in arterial stiffness is associated with the development of several pathological conditions, including hypertension, atherosclerosis, congestive heart failure, stroke, and aortic root regurgitation (*3*–*6*). Therefore, increased arterial stiffness may be an independent risk factor for all-cause mor-

From the [1)]Center for Tsukuba Advanced Research Alliance and [2)]Institute of Health and Sport Sciences, University of Tsukuba, Tsukuba, Japan; and [3)]Institute for Human Science and Biomedical Engineering, National Institute of Advanced Industrial Science and Technology, Tsukuba, Japan.

This work was supported by Grants-in-Aid for Scientific Research (17700484, 18300215, 18650186) and Special Coordination Funds from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

Address for Reprints: Seiji Maeda, Ph.D., Center for Tsukuba Advanced Research Alliance, University of Tsukuba, Tsukuba 305–8577, Japan. E-mail: smaeda@tara.tsukuba.ac.jp

Received April 5, 2007; Accepted in revised form November 27, 2007.

tality and the development of cardiovascular disorders (*3*, *4*). Several cross-sectional studies have clearly demonstrated that arterial stiffness is lower in physically active individuals compared with sedentary individuals (*2*, *7–9*). Furthermore, aerobic exercise training intervention has been shown to attenuate age-related increases in arterial stiffness (*9*, *10*). Thus, habitual exercise prevents and/or reverses the increases in arterial stiffness with advancing age.

The natriuretic peptide system is thought to play roles in the regulation of blood pressure, body fluid homeostasis, and vascular remodeling (*11–13*). The natriuretic peptides consist of three endogenous ligands, atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and C-type natriuretic peptide (CNP) (*11*, *14*), and their activity is mediated by at least three different receptors: natriuretic peptide receptor (NPR)-A, NPR-B, and ANP clearance receptor (NPR-C) (*11*, *15*). ANP, primarily secreted from the atrium of the heart, regulates basal blood pressure, blood flow, vascular volume and tone, and arterial stiffness (*12*, *16–18*). Variations in ANP genetic sequence were recently found to be associated with differences in the development of cardiovascular disease and related conditions such as hypertension, stroke, and hypercholesterolemia (*19–22*). However, the relationship between genetic variations in ANP and exercise-induced improvements in arterial stiffness remains unclear.

Because ANP is implicated in the regulation of arterial stiffness, we hypothesized that genetic variations in ANP could affect the exercise-induced reduction in arterial stiffness in human subjects. Thus, we examined whether polymorphisms in the ANP genes affected the degree of change in arterial stiffness following exercise in middle-aged and older human subjects. Additionally, as another target gene, we examined gene polymorphisms in NPR-C, the ANP clearance receptor. In the present study, we tested our hypothesis by assessing the daily physical activity, arterial stiffness, and genotypes of single-nucleotide polymorphisms (SNPs) of valine32methionine (V32M: 664G>A) in exon 1 of ANP on chromosome 1 and asparagine521aspartic acid (N521D: 1780A>G) in exon 8 of NPR-C on chromosome 5 in healthy middle-aged and older Japanese subjects. In the present study, the SNPs were picked up from the JSNP (a database of Japanese Single Nucleotide Polymorphisms) public database (*23*, *24*), which constitutes a potent infrastructure for the next step toward personalized medicine, *i.e.*, whole-genome association studies of common diseases or drug sensitivities in Japanese individuals. We measured brachial-ankle arterial pulse wave velocity (baPWV) as an index of arterial stiffness (*25*). Finally, the plasma ANP level was determined in different study groups.

Methods

Subjects

Two hundred and ninety-one middle-aged and older Japanese subjects (85 males and 206 females) between 51 and 78 years of age participated in this cross-sectional study (mean: 63±1 years). Subjects were divided into inactive lifestyle (Inactive) and active lifestyle (Active) groups, with the dividing line set at the median value (216 kcal/d) of mean energy expenditure per day. Subjects were recruited for the present study by advertisement. All subjects were free of any overt signs and symptoms of chronic diseases. None of the participants had a history of smoking, and none were currently taking medications. SBP, diastolic blood pressure (DBP), heart rate (HR), baPWV, daily physical activity, polymorphisms in ANP and NPR-C genes, and plasma ANP levels were determined in all subjects in the same season. Serum cholesterol, triglycerides, and insulin, and plasma glucose levels were also measured. All of the female subjects were postmenopausal women.

The study was approved by the Ethical Committees of the Institute of Health and Sport Sciences and the Institute of Clinical Medicine of the University of Tsukuba. All subjects gave written informed consent to participate before inclusion in the study.

Measurement of Daily Physical Activity

Daily physical activity was measured using a uniaxial accelerometer (Life-Corder; Suzuken Co., Nagoya, Japan) as described in previous studies (*8*). All subjects wore the uniaxial accelerometer on the waist continuously for 14 d, except for sleeping and bathing, and data from a continuous 7-d period was used to assess physical activity. The total energy expenditure (kcal) was calculated as the sum of the energy measured by both the uniaxial accelerometer and the questionnaire of activities with the Compendium of Physical Activity (*26*).

Measurement of Arterial Pulse Wave Velocity

baPWV (using formPWV/ABI; Colin Medical Technology, Komaki, Japan) was measured as previously described (*8*, *25*) The baPWV value reflects the degree of stiffness in the central arteries, and baPWV correlates well with the aortic pulse wave velocity (PWV) measured using a catheter tip with pressure manometer (*25*).

SNP Genotyping

Genomic DNA was extracted from plasma buffy coats and buccal cells using the QIAamp DNA Blood Maxi Kit (QIAGEN, Tokyo, Japan). ANP and NPR-C SNP genotypes were determined by real-time PCR with TaqMan probes using an ABI Prism 7700 Sequence Detector (Perkin-Elmer Applied Biosystems, Foster, USA) as previously described with minor modifications (*8*). The gene-specific primers and TaqMan probes for each SNP were synthesized using Primer Express v.1.5 software (Perkin-Elmer Applied Biosystems) according to the published DNA sequences for each SNP as

Table 1. Characteristics of Subjects in the Active and Inactive Groups

	Active	Inactive
Number of subjects	146	145
Age, years	63±1*	64±1
Body weight, kg	58±1*	54±1
Height, cm	158±1*	154±1
Body mass index, kg/m^2	23.9±0.2	23.5±0.2
Systolic blood pressure, mmHg	125±1	128±2
Diastolic blood pressure, mmHg	78±1	77±1
Heart rate, beats/min	60±1*	63±1
Total cholesterol, mg/dL	215±3	224±3
HDL cholesterol, mg/dL	62±1	63±1
LDL cholesterol, mg/dL	127±3*	134±2
Triglycerides, mg/dL	96±4	101±5
Insulin, μU/mL	5.3±0.3	6.0±0.3
Glucose, mg/dL	99±1	98±1
Pulse wave velocity, cm/s	1,453±18*	1,510±21
Daily physical activity, kcal/day	360±12*	148±4

HDL, high-density lipoprotein; LDL, low-density lipoprotein. Values are means±SEM. *$p<0.05$ *vs*. Inactive.

follows: V32M in exon 1 of ANP (NCBI accession #rs5063) and N521D in exon 8 of NPR-C (NCBI accession #rs2270915). The sequences of the oligonucleotides used are as follows: ANP forward: 5′-TCAGACCAGAGCTAATCC CATGTA-3′; ANP reverse: 5′-GGCCCTACCTTGAAATCC ATCAG-3′; ANP/G probe: 5′-AATGCCGTGTCCAAC-3′; ANP/A probe: 5′-CAATGCCATGTCCAAC-3′; NPR-C forward: 5′-CCATTGAGAGGCGAACCCA-3′; NPR-C reverse: 5′-TCTTCCCGTAATTCCCGATGTTTTC-3′; NPR-C/A probe: 5′-AAGAAGAAAGTAACCTTG-3′; NPR-C/G probe: 5′-AAGAAGAAAGTGACCTTG-3′.

PCR 96-well plates were read on an ABI-7700 using the end-point analysis mode of the SDS v1.7a software package (Perkin-Elmer Applied Biosystems). Genotypes were determined automatically by the single processing algorithms in the software package.

Measurements of Serum Cholesterol, Triglycerides, and Insulin Levels, and Plasma ANP and Glucose Levels

Fasting serum concentrations of cholesterol, triglycerides, and insulin, and plasma concentrations of glucose were determined using standard enzymatic techniques. Plasma ANP concentrations were determined using a sandwich-EIA Kit (Phoenix Pharmaceuticals Inc., Belmont, USA) for ANP. The reported cross-reactivity for the antibody was 0% for BNP and 0% for CNP.

Table 2. Distribution of Gene Polymorphisms of ANP (V32M) and NPR-C (N521D) and Allele Frequency in the Study Subjects

		Total	Male	Female
Genotypes, % (*n*)				
ANP	VV	82.8 (241)	87.1 (74)	81.1 (167)
	VM	15.8 (46)	10.6 (9)	18.0 (37)
	MM	1.2 (4)	2.1 (2)	0.8 (2)
NPR-C	NN	63.6 (185)	67.9 (57)	61.8 (128)
	ND	32.6 (95)	29.8 (25)	33.8 (70)
	DD	2.8 (11)	1.8 (2)	3.1 (9)
Allele frequency				
ANP (M allele)		0.09	0.08	0.10
NPR-C (D allele)		0.20	0.17	0.21

ANP, atrial natriuretic peptide; NPR-C, ANP clearance receptor. The genotype frequencies did not deviate from Hardy-Weinberg equilibrium. No difference was found between genders.

Statistical Analysis

The ANP and NPR-C allelic frequencies were calculated using a gene-counting method, and Hardy-Weinberg equilibrium was confirmed using a χ^2 test. Student's *t*-test for unpaired values was used to evaluate differences between active and inactive groups or differences among the different genotype groups. Values of $p<0.05$ were considered statistically significant. Furthermore, the PWV comparisons between the genotype groups in each active and inactive group were assessed by a covariance analysis (ANCOVA) model that included age, SBP, and sex as covariates. Unless indicated otherwise, values of $p<0.05$ were considered statistically significant. Because of the multiple comparisons of genotypes, we applied Bonferroni correction. Since we examined three genotypes, we divided 0.05 by 3 to get 0.0167. Thus, values of $p<0.0167$ were considered statistically significant. Values are expressed as the means±SEM.

Results

Comparison of Characteristics in Low and High Physical Activity Groups

We examined 291 individuals in a cross-sectional study comparing physical activity levels, arterial stiffness, and ANP and NPR-C genotype, and the subject characteristics are shown in Table 1. In the active group, the mean baPWV, a measure that directly correlates with arterial stiffness, was significantly lower than that of the inactive group. There were no significant differences in body mass index (BMI), SBP, DBP, total cholesterol, high-density lipoprotein (HDL), triglycerides, insulin, and glucose between the active and inactive groups. HR and low-density lipoprotein (LDL) were significantly lower in the active group than in the inactive group.

Table 3. Genotypes of ANP (V32M) and NPR-C (N521D) and Subject Characteristics

	ANP (V32M)		NPR-C (N521D)	
	VV	VM+MM	NN	ND+DD
Age, years	64±1	60±1*	63±1	64±1
Body weight, kg	56±1	56±1	56±1	55±1
Height, cm	156±1	157±1	156±1	155±1
Body mass index, kg/m^2	23.7±0.2	23.3±0.4	23.7±0.2	23.5±0.3
Systolic blood pressure, mmHg	127±1	120±2*	126±1	127±2
Diastolic blood pressure, mmHg	78±1	73±1*	77±1	77±1
Heart rate, beats/min	62±1	61±1	61±1	62±1
Total cholesterol, mg/dL	220±2	216±5	222±3	216±3
HDL cholesterol, mg/dL	62±1	65±2	63±1	62±1
LDL cholesterol, mg/dL	131±2	125±5	132±2	126±3
Triglycerides, mg/dL	99±3	91±7	97±3	97±5
Insulin, μU/mL	5.8±0.3	5.4±0.4	5.3±0.2	6.4±0.5
Glucose, mg/dL	99±1	97±2	99±1	97±2
Daily physical activity, kcal/d	251±9	250±23	257±11	245±14
Pulse wave velocity, cm/s	1,497±15	1,380±26*	1,470±17	1,485±23

ANP, atrial natriuretic peptide; NPR-C, ANP clearance receptor; HDL, high-density lipoprotein; LDL, low-density lipoprotein. Values are means±SEM. *$p<0.05$ *vs*. VV genotype of ANP (V32M).

Comparison of Characteristics between Each Genotype

We analyzed the ANP and NPR-C genotypes of the study subjects (Table 2), and no significant differences in the frequency of these polymorphisms were found between genders. Additionally, the allelic frequencies did not deviate from the expected Hardy-Weinberg equilibrium.

We next compared the characteristics of subjects with different gene polymorphisms (Table 3). In the genotypes of V32M of ANP, SBP, DBP, and baPWV were significantly lower in the VM+MM group than the VV group. There were no significant differences in daily physical activity, body weight, height, BMI, HR, total cholesterol, HDL, LDL, triglycerides, insulin, and glucose between these groups. In the genotypes of N521D of NPR-C, there were no significant differences in any parameters between the NN and ND+DD groups.

Comparison of PWV and Plasma ANP Levels between Genotypes and Physical Activity

The baPWV values of subjects with the VV genotype of V32M of ANP or the NN genotype of N521D of NPR-C were significantly lower in the active group compared with the inactive group, but no significant differences were seen in the baPWV of subjects with the VM+MM of ANP or ND+DD of NPR-C genotypes (Fig. 1). Additionally, the plasma ANP concentrations of subjects with the VV genotype of V32M of ANP or the NN genotype of N521D of NPR-C were higher in the active group compared with the inactive group, and there was no significant difference in plasma ANP levels for individuals with the VM+MM of ANP or ND+DD of NPR-C genotypes regardless of physical activity (Fig. 2). To further explore a possible relationship between arterial stiffness and plasma ANP levels, we performed regression analyses between baPWV and plasma ANP level. There were negative and significant correlations between baPWV and plasma ANP level in all the study subjects ($y=-4.9x+1,669$, $r=0.26$, $p<0.01$). In the genotypes of V32M of ANP, there was a negative and significant correlation between baPWV and the plasma ANP level of the individuals with the VV genotype (Fig. 3A). There was no significant correlation observed for the VM+MM genotype (Fig. 3B), however. Additionally, baPWV and the plasma ANP level of individuals with both genotypes of N521D of NPR-C were significantly correlated (Fig. 3C and D). Interestingly, the slopes of the regression lines were significantly different between the genotypes of ANP ($p<0.05$), but not significantly different between the genotypes of NPR-C. Moreover, we performed a multivariate analysis between baPWV and body weight, height, SBP, DBP, HR, total cholesterol, HDL, LDL, triglycerides, insulin, glucose, daily physical activity, sex, and plasma ANP level by using stepwise multivariable regression models in each genotype of ANP. In the stepwise multivariable regression models, baPWV in the VV group remained, and was associated with DBP and the plasma ANP level. On the other hand, in the VM+MM group, baPWV remained, and was associated with SBP, DBP and body weight.

Discussion

The principle findings of the present cross-sectional study were the relationships among arterial stiffness, physical activ-

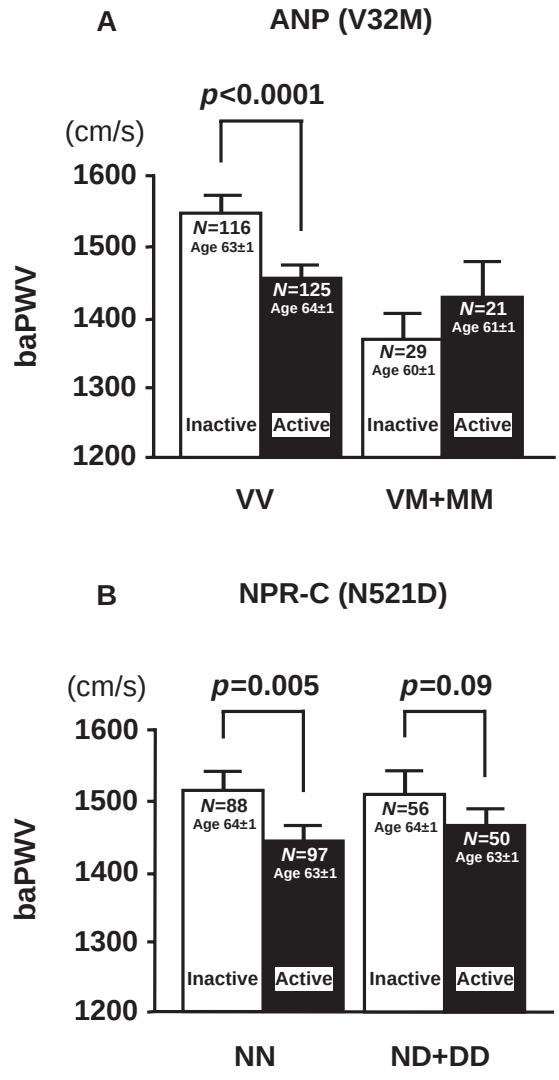

Fig. 1. *Brachial-ankle PWV (baPWV) values for each genotype of the ANP (V32M) (A) and NPR-C (N521D) (B) gene polymorphisms of subjects in the cross-sectional study. Subjects were divided into inactive lifestyle (Inactive: open bar) or active lifestyle (Active: solid bar) groups using the median value (216 kcal/d) of energy expenditure per day as a cut-off. Differences in PWV between each genotype in the Active and the Inactive groups were assessed by a covariance analysis (ANCOVA) model that included age, systolic blood pressure (SBP), and sex as covariates. Data are expressed as the means±SEM.*

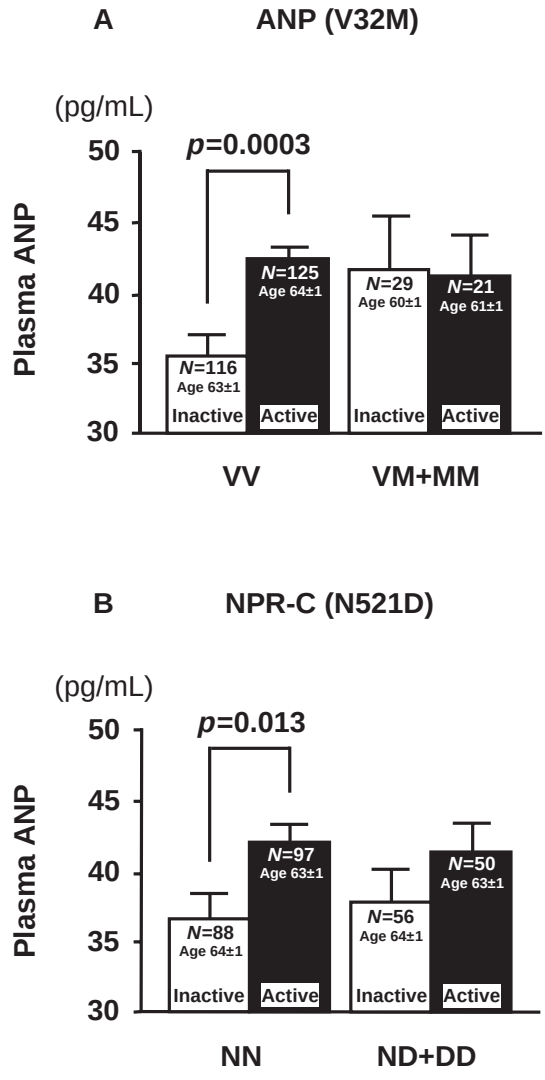

Fig. 2. *Plasma ANP levels of each genotype of the ANP (V32M) (A) and NPR-C (N521D) (B) gene polymorphisms of subjects in the cross-sectional study. Subjects were divided into inactive lifestyle (Inactive: open bar) or active lifestyle (Active: solid bar) groups using the median value (216 kcal/d) of energy expenditure per day as a cut-off. Data are expressed as the means±SEM.*

ity, and polymorphisms in ANP genes in Japanese middle-aged and older human subjects. Interestingly, using baPWV as a measure of arterial stiffness, increased physical activity in individuals with the VV genotype at V32M (664G/A) of the ANP gene decreased arterial stiffness, but there was no effect of exercise on arterial stiffness in individuals with the VM+MM genotype. In contrast, when polymorphisms of NPR-C were examined, N521D (1780A/G) had no effect on the changes in arterial stiffness following the prescribed exercise. Thus, genetic polymorphisms in components of the ANP pathway may differentially affect changes in age-related arterial stiffness in response to habitual exercise.

Several studies have reported that arterial stiffness is lower in physically active individuals compared with sedentary individuals (*2*, *7*–*9*). In regard to the mechanism, it is well known that habitual exercise induces activation of vasodilating factors, *e.g.*, nitric oxide, in older humans (*27*). Rogers *et al*. (*28*) reported that exercise training increased plasma ANP

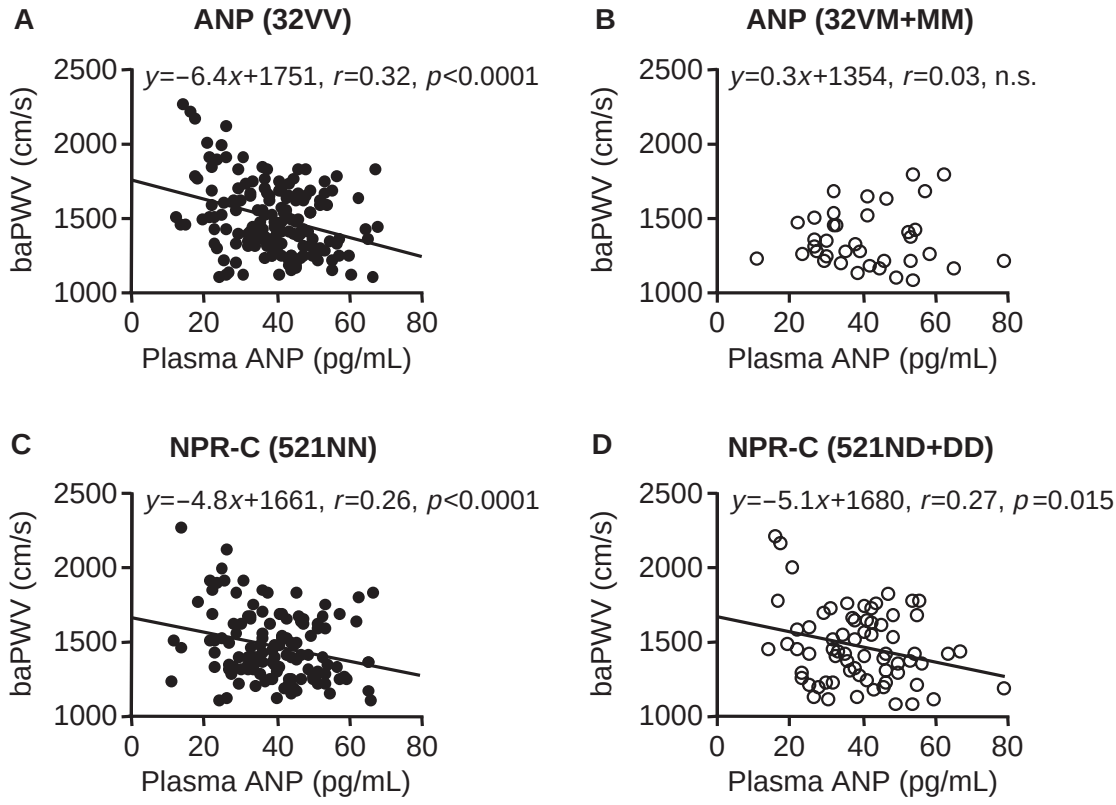

Fig. 3. *The relations between brachial-ankle PWV (baPWV) and plasma ANP levels of subjects with each genotype of the ANP (V32M) (A: VV; B: VM+MM) and NPR-C (N521D) (C: NN; D: ND+DD) gene polymorphisms.*

levels at rest. In healthy humans, ANP regulates basal blood pressure, blood flow, and vascular volume and tone (*12*, *13*, *16*, *17*). After binding to its specific receptor(s), ANP increases cellular concentrations of cyclic guanosine monophosphate (cGMP) by activation of a receptor-coupled guanylate cyclase (*29*). We showed that plasma ANP levels negatively correlate with arterial stiffness. Thus, ANP may participate in a mechanism of physical activity–related change in PWV. Interestingly, the plasma ANP levels of individuals with the VV genotype of V32M of ANP did correlate with arterial stiffness, but this was not seen in subjects with the VM+MM genotype. Additionally, this correlation was independent of the examined polymorphism in NRP-C. The V32M polymorphism is located in exon 1 of the ANP gene, and it resides in a structural region of the gene. Thus, variations in this location could affect the regulation of gene expression, mRNA stability, and/or ANP translation. Alternatively, changes in the coding region of ANP could alter its receptor binding characteristics and downstream activity. Collectively, polymorphisms in different components of the ANP system may differentially affect various functional aspects of this system, such as changes in the plasma ANP level, differences in ligand-binding affinity, and intracellular cGMP production *via* the ANP pathway. However, there is no clear reason to link the genotype of the ANP gene to physical activity–related changes in baPWV. Future studies should clarify this issue and examine the functional effect of the ANP genotype on the relationship between arterial stiffness and regular exercise.

Arterial infusion of exogenous ANP decreased PWV without affecting mean blood pressure and HR, and infusion of A71915, a selective NRP-A antagonist, increased PWV in an animal study *in vivo* (*18*). These data suggest that the ANP system is associated with regulation of basal arterial stiffness. In the present study, regular exercise abrogated the age-related increase in arterial stiffness of subjects with the VV genotype of V32M of ANP. The mechanism by which this polymorphism affects the ability of exercise to decrease arterial stiffness remains unclear, but this finding further supports the importance of ANP in modulating the arterial-buffering function. In contrast, changes in arterial distensibility following the introduction of an exercise regimen were not affected by polymorphisms in NPR-C. Additionally, the negative correlation between arterial stiffness and plasma ANP levels was seen in both NPR-C genotypes. Thus, the examined NPR-C polymorphism does not seem to affect the relationship between changes in plasma ANP and reductions in age-related arterial stiffness following regular exercise.

In the present study, we selected the polymorphisms of V32M in the ANP gene and N521D in NPR-C to investigate

the effect of genotypes in the ANP system on the relationship between arterial stiffness and regular exercise. However, there may be other important polymorphisms in other regions of the ANP gene or NPR-C gene. We performed our linkage analysis by using a linkage disequilibrium map from the International HapMap Project (http://www.hapmap.org/index.html.en) (*30*) to research common genetic variants of V32M in the ANP gene or N521D in the NPR-C gene. However, there was no SNP which linked genetic variants to V32M in the ANP gene. A previous report identified associations between polymorphism in the exon 3 region of the ANP gene, *e.g.*, stop152arginine (2238T>C: rs5065), and cardiovascular disease (*20*). We confirmed the effect of this polymorphism in the present study, but the allele frequency of stop152arginine was 98% in stop codon genotype. We did not observe any relationship between this polymorphism and the effect of exercise on changes in arterial stiffness in middle-aged and older subjects (data not shown). Additionally, the linkage to N521D in the NPR-C gene was detected at rs2246427 A>G and rs3811953 G>A; however, there were two SNPs in the intron region. Therefore, it is considered that the two polymorphisms selected in the present study, V32M in the ANP gene and N521D in the NPR-C gene, may be important genotypes in the ANP system that effect the relationship between arterial stiffness and regular exercise. However, more genotypes in different positions of the ANP gene or NPR-C gene should be evaluated for their role in regulating vascular tone under sedentary and active conditions. In addition, our study examined only Japanese subjects, and thus our data cannot be used to address differences in ANP and NPR-C polymorphisms in other ethnic groups.

The present results showed that age-related changes in SBP, DBP and baPWV were lower in patients with the VM+MM genotype of ANP than in those with the VV genotype. Although the comparisons of SBP, DBP and baPWV between the genotype groups were assessed by an ANCOVA model that included age as a covariate, these differences were not changed. However, the difference of age may have affected these relationships between polymorphism and SBP, DBP or baPWV. Thus, future studies should clarify this issue and examine the effect of age on SBP, DBP and baPWV in different genotypes in ANP. Secondly, the sample size in the present study was relatively small. In the future, it will be important to investigate various polymorphisms in ANP and NPR-C using a much larger number of Japanese subjects in the current experimental setting. Finally, although the present study revealed that ANP polymorphism affected exercise-induced improvements in arterial stiffness, these results may have been affected by diets. Future studies will be needed to clarify this issue and to examine the effect of diet on the relationship between polymorphisms in ANP and NPR-C and arterial stiffness and regular exercise.

In conclusion, we investigated the relationship of polymorphisms in ANP and NPR-C with arterial stiffness and regular exercise. We identified the V32M molecular variant in exon 1 of the ANP gene as a polymorphism affecting the improvement of arterial stiffness following habitual exercise. Furthermore, the V32M polymorphism of ANP affected the relationship between arterial stiffness and circulating ANP level. However, the N521D polymorphism in exon 8 of NPR-C did not affect the exercise-associated changes in arterial stiffness. Thus, molecular variations in the ANP gene affect the cardiovascular response to regular exercise in middle-aged and older subjects. These effects could be important for health promotion and the development of strategies to prevent cardiovascular diseases.

References

1. Avolio AP, Deng FQ, Li WQ, *et al*: Effects of aging on arterial distensibility in populations with high and low prevalence of hypertension: comparison between urban and rural communities in China. *Circulation* 1985; **71**: 202–210.
2. Vaitkevicius PV, Fleg JL, Engel JH, *et al*: Effects of age and aerobic capacity on arterial stiffness in healthy adults. *Circulation* 1993; **88**: 1456–1462.
3. Blacher J, Asmar R, Djane S, London GM, Safar ME: Aortic pulse wave velocity as a marker of cardiovascular risk in hypertensive patients. *Hypertension* 1999; **33**: 1111–1117.
4. Laurent S, Boutouyrie P, Asmar R, *et al*: Aortic stiffness is an independent predictor of all-cause and cardiovascular mortality in hypertensive patients. *Hypertension* 2001; **37**: 1236–1241.
5. Arnett DK, Evans GW, Riley WA: Arterial stiffness: a new cardiovascular risk factor? *Am J Epidemiol* 1994; **140**: 669–682.
6. Rowe JW: Clinical consequences of age-related impairments in vascular compliance. *Am J Cardiol* 1987; **60**: 68G–71G.
7. Mohiaddin RH, Underwood SR, Bogren HG, *et al*: Regional aortic compliance studied by magnetic resonance imaging: the effects of age, training, and coronary artery disease. *Br Heart J* 1989; **62**: 90–96.
8. Iemitsu M, Maeda S, Otsuki T, *et al*: Polymorphism in endothelin-related genes limits exercise-induced decreases in arterial stiffness in older subjects. *Hypertension* 2006; **47**: 928–936.
9. Tanaka H, DeSouza CA, Seals DR: Absence of age-related increase in central arterial stiffness in physically active women. *Arterioscler Thromb Vasc Biol* 1998; **18**: 127–132.
10. Tanaka H, Dinenno FA, Monahan KD, Clevenger CM, DeSouza CA, Seals DR: Aging, habitual exercise, and dynamic arterial compliance. *Circulation* 2000; **102**: 1270–1275.
11. Nakao K, Itoh H, Saito Y, Mukoyama M, Ogawa Y: The natriuretic peptide family. *Curr Opin Nephrol Hypertens* 1996; **5**: 4–11.
12. Richards AM, Nicholls MG, Ikram H, Webster MW, Yandle TG, Espiner EA: Renal, haemodynamic, and hormonal effects of human alpha atrial natriuretic peptide in healthy volunteers. *Lancet* 1985; **1**: 545–549.
13. van der Zander K, Houben AJ, Kroon AA, de Leeuw PW: Effects of brain natriuretic peptide on forearm vasculature: comparison with atrial natriuretic peptide. *Cardiovasc Res*

1999; **44**: 595–600.
14. Kangawa K, Tawaragi Y, Oikawa S, *et al*: Identification of rat gamma atrial natriuretic polypeptide and characterization of the cDNA encoding its precursor. *Nature* 1984; **312**: 152–155.
15. Fuller F, Porter JG, Arfsten AE, *et al*: Atrial natriuretic peptide clearance receptor. Complete sequence and functional expression of cDNA clones. *J Biol Chem* 1988; **263**: 9395–9401.
16. Hughes A, Thom S, Goldberg P, Martin G, Sever P: Direct effect of alpha-human atrial natriuretic peptide on human vasculature *in vivo* and *in vitro*. *Clin Sci* 1988; **74**: 207–211.
17. Schmitt M, Broadley AJ, Nightingale AK, *et al*: Atrial natriuretic peptide regulates regional vascular volume and venous tone in humans. *Arterioscler Thromb Vasc Biol* 2003; **23**: 1833–1838.
18. Schmitt M, Qasem A, McEniery C, *et al*: Role of natriuretic peptides in regulation of conduit artery distensibility. *Am J Physiol Heart Circ Physiol* 2004; **287**: H1167–H1171.
19. Dedoussis GV, Maumus S, Skoumas J, *et al*: Natriuretic peptide Val7Met substitution and risk of coronary artery disease in Greek patients with familial hypercholesterolemia. *J Clin Lab Anal* 2006; **20**: 98–104.
20. Gruchala M, Ciecwierz D, Wasag B, *et al*: Association of the ScaI atrial natriuretic peptide gene polymorphism with nonfatal myocardial infarction and extent of coronary artery disease. *Am Heart J* 2003; **145**: 125–131.
21. Ramasawmy R, Kotea N, Lu C, Sayada C, Baligadoo S, Krishnamoorthy R: A new polymorphic restriction site at the human atrial natriuretic peptide (hANP) gene locus. *Hum Genet* 1993; **91**: 509–510.
22. Zhang S, Mao G, Zhang Y, *et al*: Association between human atrial natriuretic peptide Val7Met polymorphism and baseline blood pressure, plasma trough irbesartan concentrations, and the antihypertensive efficacy of irbesartan in rural Chinese patients with essential hypertension. *Clin Ther* 2005; **27**: 1774–1784.
23. Haga H, Yamada R, Ohnishi Y, Nakamura Y, Tanaka T: Gene-based SNP discovery as part of the Japanese Millennium Genome Project: identification of 190,562 genetic variations in the human genome. *J Hum Genet* 2002; **47**: 605–610.
24. Hirakawa M, Tanaka T, Hashimoto Y, Kuroda M, Takagi T, Nakamura Y: JSNP: a database of common gene variations in the Japanese population. *Nucleic Acids Res* 2002; 30: 158–162.
25. Yamashina A, Tomiyama H, Takeda K, *et al*: Validity, reproducibility, and clinical significance of noninvasive brachial-ankle pulse wave velocity measurement. *Hypertens Res* 2002; **25**: 359–364.
26. Ainsworth BE, Haskell WL, Leon AS, *et al*: Compendium of physical activities: classification of energy costs of human physical activities. *Med Sci Sports Exerc* 1993; **25**: 71–80.
27. Maeda S, Tanabe T, Otsuki T, *et al*: Moderate regular exercise increases basal production of nitric oxide in elderly women. *Hypertens Res* 2004; **27**: 947–953.
28. Rogers PJ, Tyce GM, Bailey KR, Bove AA: Exercise-induced increases in atrial natriuretic factor are attenuated by endurance training. *J Am Coll Cardiol* 1991; **18**: 1236–1241.
29. D'Souza SP, Davis M, Baxter GF: Autocrine and paracrine actions of natriuretic peptides in the heart. *Pharmacol Ther* 2004; **101**: 113–129.
30. The International HapMap Consortium: The International HapMap Project. *Nature* 2003; **426**: 789–796.