elderly, after adjustment for other conventional risk factors (*37, 38*). A significant relationship between AI and cardiovascular events including stroke has also been reported (*39*). In younger subjects, decreased regional cerebral blood flow, neurogenic inflammation, and platelet activation have been proposed as pathophysiological mechanisms of migraine-related stroke (*40–42*). Different mechanisms, such as enhanced arterial stiffness, may be involved in the link between migraine and late-onset stroke in the elderly.

Medications from a broad range of classes have been demonstrated to be effective in preventing migraine attack (*33, 43*). Several antihypertensive drugs, such as β-blockers, calcium-channel antagonists, and angiotensin-converting enzyme inhibitors, also have preventive effects. In this study, approximately 30% of subjects were receiving antihypertensive treatment. Accordingly, the possibility could not be excluded that the medication might affect the assessment of vascular properties, as well as the diagnosis of migraine. Additionally, β-blockade increases AI mainly by reducing HR. Ergotamine, a vasoconstrictor for migraine treatment, also reduces arterial distensibility (*44*) and increases AI (*45*). However, the observed association between migraine and enhanced AI was still significant after adjustment for HR. The association was also significant in subjects without medication.

We used ID Migraine as a questionnaire to diagnose migraine. Although the questionnaire's validity and reproducibility were verified previously, it cannot differentiate migraine with aura from that without aura. Migraine can be divided into two major subtypes according to the existence or absence of aura (*1*). Aura is a combination of various reversible visual symptoms (flickering lights, spots or lines, loss of vision), sensory symptoms (pins and needles, numbness), and dysphasic speech disturbance that usually occur just before or at the onset of migraine headache. Several reports have shown that migraine with aura carries a higher risk for ischemic stroke than simple migraine (*13*). Further study is required to determine whether or not there is a subtype-specific association between migraine and enhanced arterial stiffness.

We observed consistent findings between subjects recruited from two distinct populations. Replication of the findings could strengthen our hypothesis that migraine is associated with enhanced arterial stiffness in elderly subjects. However, the prevalence of migraine was significantly different between the two populations, which could be due to several undefined biases in the study subjects. Accordingly, before it is appropriate to generalize about the observation in this study further confirmation would be necessary in a larger population adjusted for other potential confounding factors.

In summary, the present study showed that migraine is independently associated with enhanced arterial stiffness in community-dwelling elderly subjects, indicating the pathophysiological importance of the vasculature in linking migraine and stroke in the elderly. Migraine in the elderly requires careful attention as a clinical risk factor for future cardiovascular events.

Acknowledgements

We greatly appreciate the support of Y. Matsumoto, A. Matsumoto, and Ehime Elderly Health Promoting Society in recruiting the study subjects.

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Frequency of the G/G Genotype of Resistin Single Nucleotide Polymorphism at −420 Appears to Be Increased in Younger-Onset Type 2 Diabetes

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OBJECTIVE—Resistin is an adipocyte-secreted cytokine associated with insulin resistance in mice. We previously reported that the G/G genotype of a resistin single nucleotide polymorphism (SNP) at −420 increases type 2 diabetes susceptibility by enhancing its promoter activity. The aim of the present study was to determine the relevance of SNP −120 in a large number of subjects.

RESEARCH DESIGN AND METHODS— We examined 2,610 type 2 diabetic case and 2,502 control subjects. The relation between SNP −420 and the age of type 2 diabetes onset was further analyzed by adding 237 type 2 diabetic subjects with age of onset ≤40 years.

RESULTS—When analyzed without considering subject age, the SNP −420 genotype was not associated with type 2 diabetes. Since we reported that the onset of type 2 diabetes was earlier in G/G genotype, we analyzed the data using a trend test for age intervals of 10 years. The frequency of G/G genotype differed among age grades in type 2 diabetes ($P = 0.037$) and appeared to be higher in younger grades. In type 2 diabetes, G/G genotype was more frequent in subjects aged <40 years than in those aged ≥40 years (G/G vs. C/C, $P = 0.003$). In a total of 2,430 type 2 diabetic subjects with age of onset <60 years, the trend test showed that the G/G genotype had an increasing linear trend as the age grade of type 2 diabetes onset became younger ($P = 0.0379$). In control subjects, the frequency of C/G genotype showed an increasing linear trend with increasing age ($P = 0.010$).

CONCLUSIONS—The G/G genotype frequency of resistin SNP −420 appears to be increased in younger-onset type 2 diabetic subjects. *Diabetes* **56:2834–2838, 2007**

One characteristic of type 2 diabetes is insulin resistance in insulin target tissues (1). Type 2 diabetes is a probable polygenic disease, and its major genetic factors have yet to be identified (2). Single nucleotide polymorphisms (SNPs) such as peroxisome proliferator–activated receptor (PPAR)γ, KCNJ11, and TCF7L2 have been reported to be associated with type 2 diabetes (3). We reported that SNP at −420 in the resistin gene (*RETN*) (rs1862513) is associated with type 2 diabetes (4).

In mice, resistin is secreted from adipocytes and antagonizes insulin action both in vitro and in vivo (5,6). Serum resistin is increased in obese diabetic mice and is reduced by PPARγ ligands (6). Transgenic mice overexpressing *retn* in the liver have high serum resistin and are insulin resistant (7). The $retn^{-/-}$ mice show lower fasting blood glucose (8). Therefore, the role of resistin as an adipocyte-secreted cytokine inducing insulin resistance appears to be established in rodents.

In humans, *RETN* is rarely expressed in adipose tissues and is expressed at high levels in monocytes or macrophages, in contrast to its dominant expression in adipose tissues in mice (9,10). Macrophages infiltrating into adipose tissues could account for the observed insulin resistance in obese mice, suggesting a possible role of resistin in insulin resistance in humans (11,12). The role of *RETN* in human type 2 diabetes or obesity has been controversial in studies of the association of SNPs or serum resistin (4,13–16). The discrepancy among previous reports may be resolved by considering the SNP −420 genotype or by analyzing a larger number of samples.

We reported that the G/G genotype of *RETN* promoter SNP −420 is associated with type 2 diabetes susceptibility (4). Sp1 and Sp3 transcription factors specifically bind to the DNA element including −420G, resulting in an enhanced promoter activity. *RETN* mRNA in monocytes is positively associated with its simultaneous serum levels and is highest in subjects with G/G genotype (17). Serum resistin is higher in type 2 diabetic subjects than in control subjects and highest in subjects with G/G genotype, followed by C/G and C/C. Therefore, the specific recognition of −420G by Sp1/3 appears to increase *RETN* promoter

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Received for publication 17 August 2006 and accepted in revised form 8 August 2007.

Published ahead of print at http://diabetes.diabetesjournals.org on 13 August 2007. DOI: 10.2337/db06-1157.

Additional information for this article can be found in an online appendix at http://dx.doi.org/10.2337/db06-1157.

PPAR, peroxisome proliferator–activated receptor; SNP, single nucleotide polymorphism.

TABLE 1
G/G genotype was not associated with type 2 diabetes when age was not considered

Genotype or allele	Type 2 diabetic subjects	Control subjects	Comparison	χ^2	P	OR (95% CI)
n	2,610	2,502	—	—	—	—
CC	1,169	1,080	CC/CG/GG	1.44	0.486	—
CG	1,144	1,123	GG vs. CC	0.87	0.351	0.92 (0.77–1.10)
GG	297	299	CG vs. CC	1.04	0.308	0.94 (0.84–1.06)
			GG vs. CG	0.08	0.784	0.98 (0.81–1.17)
			GG + CG vs. CC	1.37	0.242	0.94 (0.84–1.05)
			GG vs. CG + CC	0.40	0.525	0.95 (0.80–1.12)
G-allele	1,738 (33.3)	1,721 (34.4)	G- vs. C-allele	1.38	0.241	—

Data are n or n (%) unless otherwise indicated. χ^2 test was used for statistical analysis. ORs calculated by defining the G-allele as a susceptibility allele.

activity, which could induce insulin resistance and human type 2 diabetes through enhanced monocyte mRNA and serum levels of resistin. Therefore, we analyzed the relevance of *RETN* SNP −420 in a large number of samples.

RESEARCH DESIGN AND METHODS

We recruited native Japanese subjects—2,610 type 2 diabetic case and 2,502 control subjects—from six prefectures located in Honshu and Shikoku in Japan. These samples are assumed not to be heterogeneous since Matsumoto et al. (18) showed that the Japanese population is homogenous, except for the Ainus from Hokkaido and the Okinawans from Miyako, using genetic markers of human immunoglobulin. Diabetes was diagnosed based on American Diabetes Association criteria (19). The control subjects were chosen based on either no history of diabetes and A1C levels <5.6% or normal glucose tolerance as evidenced by a 75-g oral glucose tolerance test. To analyze the relation between SNP −420 and age of type 2 diabetes onset, 237 type 2 diabetic patients with onset age ≤40 years were added. The clinical characteristics of the 2,610 type 2 diabetic case and 2,502 control subjects and additional 237 type 2 diabetic subjects are summarized in Supplementary Table 1 (available in an online appendix at http://dx.doi.org/10.2337/db06-1157). The average age of the control subjects was significantly older than the age of onset of type 2 diabetes in *panel 1* (Student's t test, $P < 0.0001$). Of subjects in *panel 1*, we typed SNP −420 in 397 type 2 diabetic patients and 406 control subjects as *panels 1* and *2* and 154 case and 143 control subjects as *panel 3* in a previous article (4).

All subjects were informed of the purpose of the study, and informed consent was obtained. The study was approved by the ethics committee of Ehime University (including Chiba Central Medical Center), Ehime Prefectural Hospital, Kobe University, the University of Tokyo, the University of Tokushima, and Kyoto Prefectural University of Medicine.

The statistical power was calculated as follows (20). We assume that S and N are susceptibility and normal alleles, respectively. When the genotype relative risk is assumed to be 1.20 for SN and 1.44 for SS, the population frequency of S is 30% as SNP −420 and the prevalence of diabetes is 6.9% based on the International Diabetes Federation Diabetes e-Atlas (http://www.eatlas.idf.org/About_e_Atlas/); the penetrance for genotypes of SS, SN, and NN were calculated to be 0.088, 0.074, and 0.061, respectively. Under this condition, a significant difference in the allele frequency between 2,610 case and 2,502 control subjects can be detected with a power >99.6%.

SNP typing. Taqman analysis was used for typing SNP −420, as previously described (17,21). When required, PCR direct sequencing was performed, as described previously (4,22).

Statistical analysis. To analyze differences in SNP −420 frequencies among ages, trend testing using 10-year age intervals was used. Student's t test, ANOVA, or χ^2 test was used where indicated.

RESULTS

We analyzed *RETN* SNP −420 in 2,610 type 2 diabetic case and 2,502 control subjects recruited from six different prefectures in Japan. SNP −420 was in Hardy-Weinberg equilibrium in both case and control subjects. Neither the allele nor the genotype was associated with type 2 diabetes (Table 1).

Since we previously reported that the onset of type 2 diabetes was earlier in subjects with the G/G genotype (4), we examined the allele frequencies and genotype distribution of SNP −420 as a function of subject age. A trend test for 10-year intervals revealed that the G-allele frequency differed significantly among age grades in type 2 diabetic subjects ($P = 0.022$); the G-allele appears to be more frequent in younger type 2 diabetic subjects, especially those aged <40 years, although the increasing trend was not linear ($P = 0.458$) (Fig. 1). In contrast, this increase was not evident in control subjects.

The trend test also revealed that the frequency of the G/G genotype differed significantly among age grades in type 2 diabetic subjects ($P = 0.037$). The G/G genotype also appears to be more frequent in younger type 2 diabetic subjects, especially those below the age of 40 years, although the increasing trend was not linear ($P = 0.265$) (Fig. 2). In constrast, no difference was found in the frequency of the G/G genotype among age grades in control subjects ($P = 0.440$). There appeared to be no differences between male and female subjects (data not shown). Therefore, in type 2 diabetes, the frequency of both the G-allele and the G/G genotype appears to be higher in younger subjects.

Since the G-allele and G/G genotype frequency appear to be high in younger type 2 diabetic subjects, especially those aged <40 years, we compared the allele and genotype frequencies of SNP −420 between type 2 diabetic subjects aged <40 years and those aged ≥40 years (Table 2). The frequencies of either the G-allele or the G/G genotype were higher in the younger group (G-allele for younger group 43.0% vs. older group 33.0%, $P = 0.008$; odds ratio [OR] of G/G to C/C 2.47, $P = 0.003$). When both case and control subjects aged <40 years were analyzed, the frequencies of both the G-allele and the G/G genotype were higher in type 2 diabetic subjects (G-allele in type 2 diabetic subjects 43.0% vs. control subjects 33.3%, $P = 0.016$; OR of G/G to C/C 2.28, $P = 0.012$). Therefore, the G/G genotype at SNP −420 appeared to be associated with type 2 diabetes in younger subjects.

Finally, to examine the relation between SNP −420 and the age of type 2 diabetes onset, we added 237 type 2 diabetic subjects with onset age ≤40 years. To adjust the effect of aging on the increasing frequency of the G-allele, we analyzed a total of 2,430 type 2 diabetic subjects with age of onset <60 years. The trend test revealed that G-allele and G/G genotype had an increasing linear trend as the age grade of type 2 diabetes onset became younger ($P = 0.0492$ and $P = 0.0379$, respectively).

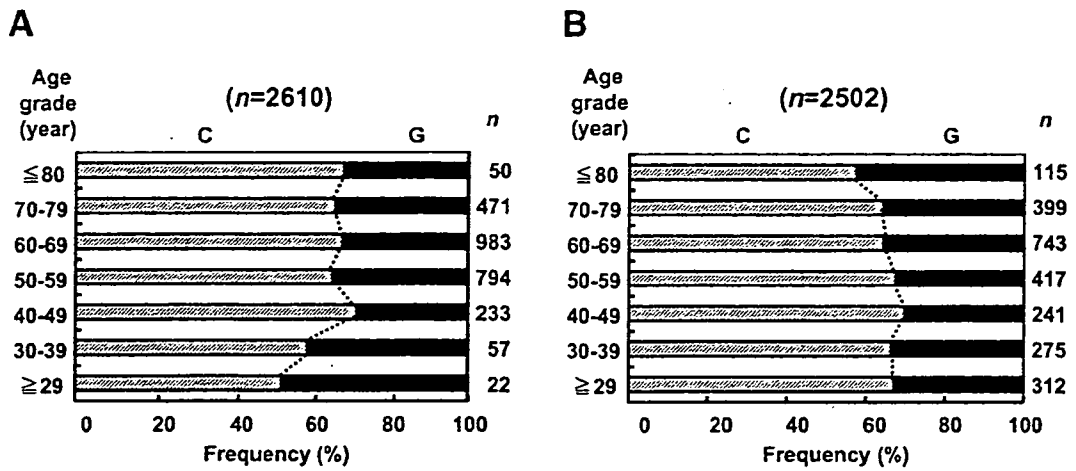

FIG. 1. The frequency of the G-allele of SNP −420 appears to be increased in younger type 2 diabetic subjects and showed an increasing linear trend in older control subjects. The allele frequencies of resistin SNP −420 stratified for 10-year age intervals for type 2 diabetic case (*A*) and control (*B*) subjects are shown. A trend test revealed that the frequency of the G-allele differed among age grades in type 2 diabetic subjects (P = 0.022), although the trend was not linear (P = 0.458). In control subjects, the frequency of the G-allele showed an increasing linear trend with increase in age (P = 0.008).

DISCUSSION

We report here that the G/G genotype at SNP −420 was associated with type 2 diabetes in younger subjects but not in total subjects by analyzing 2,610 type 2 diabetic case and 2,502 control subjects. Differences in G-allele frequencies among age grades in case and control subjects—namely, an increasing linear trend in control subjects in older grades—and an apparent increase in type 2 diabetic cases aged <40 years could result in no association between the SNP −420 genotype and type 2 diabetes in the total subjects. The association of SNP −420 with type 2 diabetes has been controversial, suggesting that a variety of factors could affect the results (4,13,14,16). This discrepancy may be resolved by considering age grades and increasing the number of samples, as suggested by the present study.

We have shown that the G/G genotype frequency was increased in younger type 2 diabetic subjects, in whom genetic factors are thought to have stronger effects on disease susceptibility. Conversely, this finding means that the G/G genotype frequency was decreased with increasing age. It is possible that resistin may become less of a significant risk factor as age increases or that type 2 diabetic patients with the G/G genotype may not live longer. It should be noted that P values observed were marginal and that the sample size, especially that of type 2 diabetic subjects with younger age of onset, was limited in this study. A larger number of samples should be analyzed for replication. When stratified by seven grades (2-kg/m^2 intervals) of BMI, no apparent linear trend of G-allele or G/G genotype was observed in control or type 2 diabetic subjects (data not shown). This supports that the trends in the age stratification are relevant although the effect of possible heterogeneity among areas cannot be completely excluded.

In contrast to type 2 diabetic subjects, a trend test revealed that in control subjects, the G-allele frequency had an increasing linear trend as the age grade became

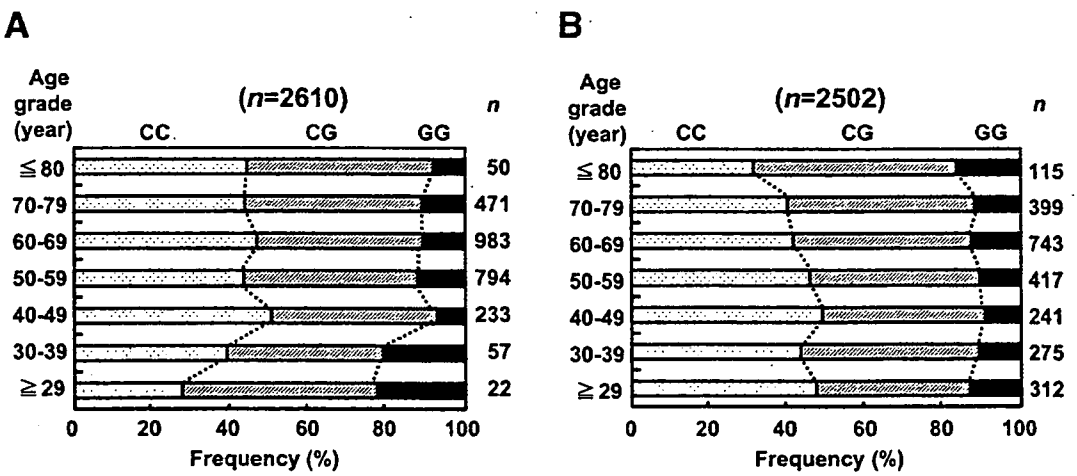

FIG. 2. The frequency of G/G genotype of SNP −420 appears to be increased in younger type 2 diabetic subjects, whereas that of C/G genotype showed an increasing linear trend in older control subjects. The genotype frequencies of resistin SNP −420 stratified by 10-year age intervals for type 2 diabetic case (*A*) and control (*B*) subjects are shown. A trend test revealed that the frequency of the G/G genotype differed among age grades in type 2 diabetic subjects (P = 0.037), though the trend was not linear (P = 0.265). In control subjects, the frequency of the G/G genotype did not differ among age grades (P = 0.440). The frequency of the C/G genotype showed an increasing linear trend with an increase in age in control subjects (P = 0.010), whereas that of the C/C genotype showed an decreasing linear trend (P = 0.002).

TABLE 2
G/G genotype at SNP −420 was associated with type 2 diabetes in younger subjects

Comparison between type 2 diabetic subjects aged <40 years (n = 79) with those aged ≥40 years (n = 2,531)						
Genotype or allele	<40 years old	≥40 years old	Comparison	χ^2	P	OR (95% CI)
CC	28	1,141	CC/CG/GG	8.96	0.011	—
CG	34	1,110	GG vs. CC	8.82	0.003	2.47 (1.34–4.58)
GG	17	280	CG vs. CC	0.74	0.390	1.25 (0.75–2.07)
			GG vs. CG	5.23	0.022	1.98 (1.09–3.60)
			GG + CG vs. CC	2.88	0.090	1.50 (0.94–2.39)
			GG vs. CG + CC	8.31	0.004	2.20 (1.27–3.82)
G-allele	68 (43.0)	1,670 (33.0)	G- vs. C-allele	6.96	0.008	—
Comparison between type 2 diabetic (n = 79) and control (n = 587) subjects aged <40 years						
Genotype or allele	Type 2 diabetic subjects	Control subjects	Comparison	χ^2	P	OR (95% CI)
CC	28	267	CC/CG/GG	6.27	0.044	—
CG	34	249	GG vs. CC	6.31	0.012	2.28 (1.18–4.40)
GG	17	71	CG vs. CC	0.96	0.327	1.30 (0.77–2.21)
			GG vs. CG	3.02	0.082	1.75 (0.93–3.32)
			GG + CG vs. CC	2.85	0.092	1.52 (0.93–2.48)
			GG vs. CG + CC	5.39	0.020	1.99 (1.10–3.60)
G-allele	68 (43.0)	391 (33.3)	G- vs. C-allele	5.84	0.016	—

Data are n or n (%) unless otherwise indicated. χ^2 test was used for statistical analysis. ORs calculated by defining the G-allele as a susceptibility allele.

older (P = 0.008) (Figs. 1*B* and 2*B*). The C/G genotype showed an increasing linear trend in older age grades (P = 0.010), whereas the C/C genotype showed a decreasing linear trend (P = 0.002). There appeared to be no sex differences (data not shown). These findings suggest that *RETN* may be a longevity gene like adiponectin (23) under certain conditions. We previously reported that serum resistin levels were highest in subjects with G/G genotype, followed by C/G and C/C (4,17). Therefore, moderately elevated serum resistin levels in C/G genotype, by reducing insulin signaling, may be beneficial for a longer life in nondiabetic control subjects. The lower serum resistin levels in C/C genotype may not be sufficient to have this effect. In fact, mutations in the insulin receptor homologous gene are known to result in longevity in *elegans* and *Drosophila* (24,25).

Recently, we reported that plasma resistin was correlated with insulin resistance in 2,078 subjects in the Japanese general population (21). Plasma resistin was highest in subjects with the G/G genotype of SNP −420, followed by C/G and C/C. The effect of SNP −420 on plasma resistin was independent of age, sex, and BMI. The 26% of total variance of plasma resistin could be explained by SNP −420, suggesting that not only SNP −420 but also other genetic and environmental factors could affect plasma resistin levels. The direct association between type 2 diabetes and SNP −420 may be more difficult to detect.

In summary, we analyzed *RETN* SNP −420 in 2,610 type 2 diabetic case and 2,502 control subjects. Although SNP −420 was not associated with type 2 diabetes when analyzed without considering subject age, the G/G genotype frequencies appear to be higher in younger subjects with type 2 diabetes. When 237 type 2 diabetic subjects with age of onset ≤40 years were added, in a total of 2,430 type 2 diabetic subjects with age of onset <60 years, the G/G genotype had an increasing linear trend as the age grade of type 2 diabetes onset became younger. Therefore, the G/G genotype frequency was increased in younger type 2 diabetic subjects. In contrast, the C/G genotype showed an increasing linear trend as the age grade became older in control subjects. It is not clear how resistin induces type 2 diabetes in younger subjects or whether it is beneficial for longer life. Further studies will be required to clarify these points.

ACKNOWLEDGMENTS

This work was supported by Grants for Scientific Research from the Ministry of Education, Culture, Science, Sports and Technology of Japan and by grants from Ehime University, Ehime, Japan. We thank our colleagues for collecting clinical data and samples, T. Takasuka and A. Murakami for technical assistance, and Drs. Nishida, Hashiramoto, Takata, Murase, and Nishimiya for suggestions.

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Plasma Resistin, Associated With Single Nucleotide Polymorphism −420, Is Correlated With Insulin Resistance, Lower HDL Cholesterol, and High-Sensitivity C-Reactive Protein in the Japanese General Population

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OBJECTIVE — Resistin, secreted from adipocytes, causes insulin resistance in rodents. We previously reported that the G/G genotype of a resistin gene promoter single nucleotide polymorphism (SNP) at −420 increases type 2 diabetes susceptibility by enhancing promoter activity. We report here on the relation between plasma resistin and either SNP −420 genotype or factors related to insulin resistance.

RESEARCH DESIGN AND METHODS — We cross-sectionally analyzed 2,078 community-dwelling Japanese subjects attending a yearly medical checkup. The SNP −420 genotype was determined by TaqMan analysis. Fasting plasma resistin was measured using an enzyme-linked immunosorbent assay kit.

RESULTS — Plasma resistin was associated with the SNP −420 genotype ($P < 0.0001$), which was highest in G/G followed by C/G and C/C. Plasma resistin was higher in elderly individuals, female subjects, nondrinkers, and subjects with high blood pressure ($P < 0.001$, 0.003, <0.001, and 0.001, respectively). Simple regression analysis revealed that age, female sex, homeostasis model assessment of insulin resistance (HOMA-IR) index, systolic blood pressure, low HDL cholesterol, and high-sensitivity C-reactive protein (hs-CRP) were positively correlated with plasma resistin ($P < 0.001$, 0.003, <0.001, 0.004, <0.001, and 0.003, respectively). Multiple regression analysis adjusted for age, sex, and BMI revealed that plasma resistin was an independent factor for HOMA-IR, low HDL cholesterol, and hs-CRP ($P = 0.001$, <0.001, and 0.006, respectively).

CONCLUSIONS — Plasma resistin was associated with SNP −420 and was correlated with insulin resistance, low serum HDL cholesterol, and high hs-CRP in the Japanese general population.

Diabetes Care 30:1501–1506, 2007

Resistin, secreted from adipocytes of mice, antagonizes insulin action in vitro and in vivo (1,2). Serum resistin is increased in obese diabetic mice and is reduced by insulin sensitizers, peroxisome proliferator–activated receptor γ ligands (1,2). Overexpression of resistin gene in the liver increases serum resistin and insulin resistance (3), whereas its disruption reduces fasting plasma glucose (FPG) (4). Therefore, an elevation in serum resistin appears to cause insulin resistance in rodents, although some other studies are not in agreement with this conclusion (5).

Type 2 diabetes is characterized by insulin resistance in insulin target tissues (6). Major genetic factors of type 2 diabetes, a probable polygenic disease, remain to be identified, whereas it has been reported that some single nucleotide polymorphisms (SNPs) are associated with type 2 diabetes (7). We recently reported that the G/G genotype of a human resistin gene (*RETN*) SNP at −420 (rs1862513) was associated with type 2 diabetes susceptibility (8). Of the frequent SNPs in the linkage disequilibrium area including SNP −420, only SNP −420 was significantly associated with type 2 diabetes. In vitro, Sp1/3 transcription factors specifically recognized G at −420 and enhanced resistin promoter activity. Subjects with G/G genotype had the highest serum resistin, followed by C/G and C/C (8,9). Thus, the association between SNP −420 and serum resistin in the general population merits further investigation.

It remains controversial whether cir-

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Received for publication 20 September 2006 and accepted in revised form 10 March 2007.

Published ahead of print at http://care.diabetesjournals.org on 23 March 2007. DOI: 10.2337/dc06-1936.

H.O. and Y.T. contributed equally to this work.

Abbreviations: CRP, C-reactive protein; CVD, cardiovascular disease; FPG, fasting plasma glucose; HOMA-IR, homeostasis model assessment of insulin resistance; hs-CRP, high-sensitivity CRP; IRI, immunoreactive insulin; SNP, single nucleotide polymorphism; SBP, systolic blood pressure.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

culating resistin levels are associated with insulin resistance, type 2 diabetes, or adiposity in humans (9–17). It has been reported that resistin is increased in type 2 diabetes (9,13) and in obesity (10,12). McTernan et al. (15) and Youn et al. (17) reported that resistin is increased in type 2 diabetes but not associated with BMI, although the role of obesity was not the primary focus of the former's study. Silha et al. (16), but not Lee et al. (14), found an association between resistin and insulin resistance. No association was detected between resistin and either type 2 diabetes or obesity (14). The discrepancy among previous reports may be resolved by analyzing a larger number of samples.

Metabolic syndrome, a cluster of abnormalities including central obesity, glucose intolerance or diabetes, hypertension, and dyslipidemia (high triglyceride levels and/or low HDL cholesterol), increases the risk of cardiovascular disease (CVD) (18). Because underlying insulin resistance could be fundamental for this syndrome, the relation between resistin and metabolic syndrome factors should be assessed.

To determine the relation between plasma resistin and either SNP −420 or factors related to insulin resistance, we cross-sectionally analyzed 2,078 subjects. Plasma resistin was associated with SNP −420 and was correlated with homeostasis model assessment of insulin resistance (HOMA-IR), lower HDL cholesterol, and high-sensitivity C-reactive protein (hs-CRP).

RESEARCH DESIGN AND METHODS

— All subjects were native to Japan. We analyzed community-dwelling subjects attending a yearly medical checkup in a rural town located in Ehime prefecture, Japan, in 2002. Of the 2,889 subjects who agreed to participate, 2,078, for whom overnight fasting plasma samples (>11 h) were available, were analyzed for plasma resistin levels. Because of the availability of plasma samples, immunoreactive insulin (IRI) and hs-CRP were measured in 2,017 and 1,875 subjects, respectively. Of the 2,078 subjects, 157 with A1C levels <5.6%, FPG levels <110 mg/dl, no history of diabetes, and no evidence of diabetes within first-degree relatives were used as nondiabetic control subjects in a previous study (9). There was no overlapping of samples between the present study and the other previous study (8). Of the 2,078 subjects, 151 were considered diabetic because they were being treated with antihyperglycemic agents or had FPG levels of ≥126 mg/dl. The association between SNP −420 and diabetes was not significant, possibly because of the lack of power using the small numbers of diabetic subjects. The plasma samples were immediately separated, frozen, and stored at −80°C. The baseline characteristics of the study subjects, such as alcohol habituation, history or symptoms of CVD, and medication, were investigated in an individual interview using a structured questionnaire. The clinical characteristics of these subjects are summarized in Table 1. All subjects were informed of the purpose of the study and their consent was obtained. The study was approved by the ethics committee of the Ehime University Graduate School of Medicine. Definitions used are as follows: obesity, BMI ≥25 kg/m^2; impaired glucose tolerance, FPG ≥110 mg/dl (6.1 mmol/l) and/or under medication of antihyperglycemic agents; high blood pressure, systolic blood pressure (SBP) ≥140 mmHg and/or diastolic pressure ≥90 mmHg and/or under medication with antihypertensive agents; hypertriglyceridemia, triglyceride levels ≥150 mg/dl (1.69 mmol/l) and/or under medication with antihyperlipidemic agents; and low HDL cholesterol, HDL cholesterol <40 mg/dl (1.04 mmol/l). CVD includes stroke, myocardial infarction, and angina pectoris. Because Japanese individuals are generally leaner than Caucasians, BMI ≥25 kg/m^2 was used as the standard cutoff value for the diagnosis of obesity (19). Waist circumference data were not available in this study. Blood pressure was measured using an automatic cuff-oscillometric device with an appropriately sized cuff on the left arm (BP-103i; Colin, Aichi, Japan) after a resting period of at least 5 min in the sitting position.

Table 1—*Characteristics of the population studied*

Characteristics	
n (males/females)	2,078 (914/1,164)
Age (years)	62 ± 13
BMI (kg/m^2)	23.4 ± 3.2
SBP (mmHg)	139 ± 22
DBP (mmHg)	82 ± 12
Total cholesterol (mg/dl)	203 ± 35
HDL cholesterol (mg/dl)	62 ± 16
Triglycerides (mg/dl)	114 ± 78
FPG (mg/dl)	98 ± 22
IRI (μU/ml)*	6.7 ± 5.0
HOMA-IR†	1.6 ± 1.4
Resistin (ng/ml)	11.5 ± 6.6
hs-CRP (mg/dl)‡	0.075 ± 0.086
Current smoking (%)	16.3
Current drinking (%)	28.6
History of CVD (%)§	7.3
Medication (%)	
Hypertension	25.8
Diabetes	3.5
Hyperlipidemia	5.7
SNP −420 genotype (CC/CG/GG)	938/902/238

Data are means ± SD or *n* (%) unless otherwise noted. *n = 2,017; †HOMA-IR calculated as fasting blood serum × IRI/405; ‡n = 1,875; §CVD includes stroke, myocardial infarction, and angina pectoris. DBP, diastolic blood pressure.

SNP typing

SNP −420 was typed by TaqMan analysis (Applied Biosystems). The probes used were VIC 5′-CATGAAGACGGAGGC C-3′ for −420C and FAM 5′-ATGAAGA GGGAGGCC-3′ for −420G. Forward and reverse primers were 5′-CCACCTCC TGACCAGTCTCT-3′ and 5′-AGCCTTC CCACTTCCAACAG-3′, respectively. When required, PCR direct sequencing was performed as previously described (8,20).

Measurement of plasma resistin and hs-CRP levels

Plasma resistin was measured using a human resistin enzyme-linked immunosorbent assay kit (LINCO Research) following the manufacturer's protocol as

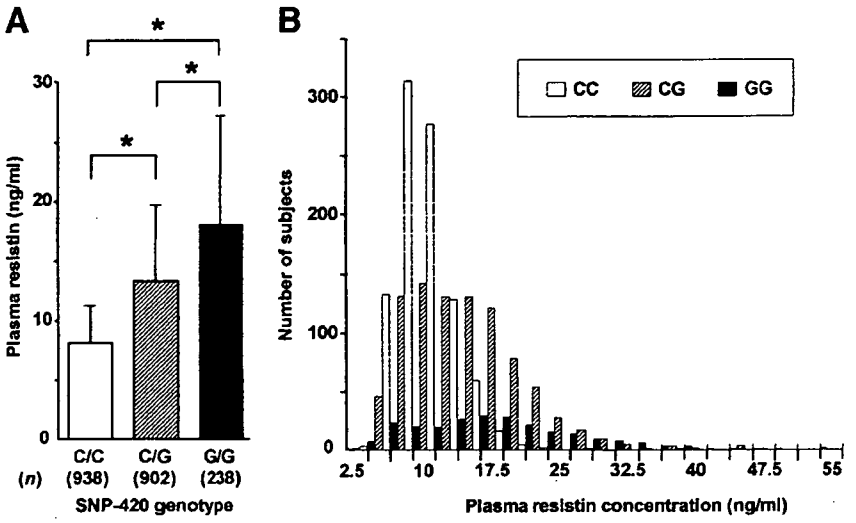

Figure 1—*Fasting plasma resistin was highest in subjects with the G/G genotype of resistin SNP −420, followed by C/G and C/C in the Japanese general population* (n = 2,078). *Fasting plasma samples from each subject were measured as described (see* RESEARCH DESIGN AND METHODS). A: *Fasting plasma resistin increased with an increased number of G allele. Data are means ± SD for each of the SNP −420 genotypes. ANOVA was used for the statistical analyses* ($F = 368.6$, $P < 0.0001$). *The calculated power based on the observed effect and the sample sizes with* $\alpha = 0.05$ *was 0.999. Scheffe's test was then used in post hoc analyses, and* $P < 0.0001$ (*). B: *The plasma resistin at the peak of the numbers of subjects with each genotype appears to be in the order G/G > C/G > C/C. Number of subjects are calculated for each 2.5 ng/ml range of plasma resistin in each of the SNP −420 genotypes. The range of plasma resistin in which the number of subjects was highest in each genotype was 15–17.5 (G/G), 7.5–10 (C/G), and 5–7.5 ng/ml (C/C).*

described (8). The linearity was maintained <0.16 ng/ml. Inter- and intra-assay coefficients of variation (CVs) were 6.9 and 1.7% (low levels) and 7.2 and 8.1% (high levels), respectively. The kit used had a good correlation with the other kit ($r = 0.978$; $y = 2.216x + 8.0$, where y is this kit and x is BioVender's kit). Plasma hs-CRP concentration was measured using a previously validated assay system (Dade Behring) (21). Inter- and intra-assay CVs were 3.2 and 6.7%, respectively.

Statistical analysis

To examine effects of SNP −420 on plasma resistin, a multiple regression analysis involving SNP −420, age, sex, and BMI as independent variables and plasma resistin as a dependent variable was used. In this analysis, the genotypes for SNP −420, C/C, C/G, and G/G were denoted by two dummy variables (c1 and c2 [0 and 0, 1 and 0, and 0 and 1, respectively]). To examine the relation of plasma resistin with age, sex, BMI, SBP, HDL cholesterol, triglyceride levels, FPG, IRI, HOMA-IR, or hs-CRP, simple regression analysis involving plasma resistin as a dependent variable was performed. A multiple regression analysis was then performed using only the significant factors. HOMA-IR, HDL cholesterol, hs-CRP, or SBP was analyzed as a dependent variable, and plasma resistin, age, sex, and BMI were involved as independent variables. CVD was involved as a dependent variable in logistic regression analysis. ANOVA was used where indicated. All analyses were performed with SPSS version 14.0J (SPSS, Chicago, IL). Bonferroni's correction was applied to the initial analyses of the relation between plasma resistin and either categories (raw P value ×9 for ANOVA) or continuous parameters (raw P value ×10 for simple regression analysis) and the subsequent multiple and logistic regression analyses using factors selected from these results (raw P value ×5). The proportion of variance of plasma resistin explained by SNP −420 was assessed based on results of a simple regression analysis. Power was calculated based on the observed effect and sample sizes using general linear model for ANOVA (simple and multiple regression analyses with $\alpha = 0.05$). Null hypotheses were rejected at $P < 0.05$.

RESULTS

SNP −420 was associated with plasma resistin in the Japanese general population

We first assessed plasma resistin based on each genotype of SNP −420 in 2,078 subjects (Fig. 1*A*). Fasting plasma resistin was highest in subjects with the G/G genotype, followed in order by those with C/G and those with C/C ($F = 368.6$, $P < 0.0001$, power = 0.999). This association was consistent when analyzed in either male ($F = 150.6$, $P < 0.0001$) or female ($F = 221.3$, $P < 0.0001$) subjects. When 50, 20, and 5% of the subjects were randomly selected and compared using the SPSS program, these P values were consistently low ($P < 0.0001$). Therefore, plasma resistin was associated with SNP −420 in this population.

We then examined the number of subjects in each 2.5 ng/ml range of plasma resistin concentration based on the SNP −420 genotype (Fig. 1*B*). The plasma resistin at the highest number of subjects with each genotype appears to be in the order of G/G > C/G > C/C. The range of plasma resistin was broadest in subjects with G/G, followed in order by C/G and C/C (1.9–52.7, 2.2–46.2, and 2.2–35.2 ng/ml, respectively), suggesting that factors other than SNP −420 genotype may affect plasma resistin.

To examine isolated effects of SNP −420 on plasma resistin, a multiple regression analysis involving SNP −420, age, sex, and BMI as independent variables was used. The SNP −420 genotype including G alleles (G/G vs. C/C, $P < 0.001$, power = 0.999 and C/G vs. C/C, $P < 0.001$, power = 0.999), higher age ($P < 0.001$, power = 0.999), and female

sex ($P = 0.001$, power = 0.894), but not higher BMI ($P = 0.195$, power = 0.254), was positively correlated with plasma resistin. The standardized coefficient (β) of the G/G genotype compared with C/C was highest ($\beta = 0.480$), followed by that of C/G compared with C/C ($\beta = 0.384$) (age, $\beta = 0.100$; female sex, $\beta = 0.060$; and BMI, $\beta = 0.024$). Therefore, SNP −420 genotype was the strongest determinant of plasma resistin among these factors. The contribution of this genotype to the observed total variance of resistin (R^2) was 26.1%.

Plasma resistin was higher in elderly individuals, female subjects, nondrinkers, and subjects with high blood pressure

We then examined mean plasma resistin in each category without considering the SNP −420 genotype. Plasma resistin was higher in elderly individuals (aged ≥65 years) (mean ± SD 12.2 ± 7.1 vs. 10.9 ± 6.1; ANOVA $P < 0.001$, power = 0.994), female subjects (11.9 ± 6.6 vs. 11.0 ± 6.6; $P = 0.003$, power = 0.852), nonhabitual alcohol drinkers (12.0 ± 6.7 vs. 10.4 ± 6.3; $P < 0.001$, power = 0.999), subjects with high blood pressure (11.9 ± 6.9 vs. 11.0 ± 6.2; $P = 0.001$, power = 0.905), those with low HDL cholesterol (13.0 ± 8.3 vs. 11.4 ± 6.5; $P = 0.014$, power = 0.693), and those with a history of CVD (12.5 ± 6.7 vs. 11.4 ± 6.6; $P = 0.045$, power = 0.516). Age, sex, alcohol drinking, and high blood pressure remained significant after Bonferroni's correction. Obesity ($P = 0.613$, power = 0.080), IGT ($P = 0.733$, power = 0.063), or hypertriglyceridemia ($P = 0.497$, power = 0.104) was not associated with plasma resistin.

Age, female sex, SBP, low HDL cholesterol, HOMA-IR, and hs-CRP were correlated with plasma resistin

We then examined which factors are correlated with plasma resistin (Table 2). Simple regression analysis revealed that age, female sex, SBP, low HDL cholesterol, IRI, HOMA-IR, and hs-CRP were correlated with plasma resistin. Each of these *P* values remains significant after Bonferroni's correction. BMI, triglyceride levels, and FPG were not correlated with plasma resistin. Therefore, with possible effects of age and sex, high plasma resistin was correlated with insulin resistance, low HDL cholesterol, high SBP, and high hs-CRP.

Table 2—*Age, female sex, SBP, low HDL cholesterol, HOMA-IR, and hs-CRP were correlated with plasma resistin*

Independent variable for simple regression	Unstandardized regression coefficient	Standardized regression coefficient	*P*
Age (years)	0.055	0.104	<0.001*
Sex (male)	−0.877	−0.066	0.003*
BMI (kg/m^2)	0.060	0.029	0.186
SBP (mmHg)	0.019	0.063	0.004*
HDL cholesterol (mg/dl)	−0.033	−0.077	<0.001*
Triglyceride level (mg/dl)	0.001	0.014	0.533
FPG (mg/dl)	−0.003	−0.010	0.658
IRI (μU/ml)†	0.120	0.090	<0.001*
HOMA-IR‡	0.401	0.082	<0.001*
hs-CRP (mg/dl)§	4.999	0.068	0.003*

Simple regression analysis was performed involving plasma resistin (ng/ml) as a dependent variable and each factor as an independent variable. Sex: male = 1; female = 0. *P values remained significant after Bonferroni's correction (raw *P* value ×10); †IRI, $n = 2{,}017$; ‡HOMA-IR, calculated as FPG × IRI/405; §hs-CRP, $n = 1{,}875$. Each calculated power based on the observed effect size and the sample size with $\alpha = 0.05$ was age (0.997), sex (0.852), BMI (0.262), SBP (0.822), HDL cholesterol (0.942), triglyceride level (0.096), FPG (0.073), IRI (0.982), HOMA-IR (0.959), and hs-CRP (0.839).

Plasma resistin was correlated with HOMA-IR, low HDL cholesterol, or hs-CRP, independent of age, sex, and BMI

To examine isolated effects of plasma resistin on each factor, a multiple regression analysis adjusted for age, sex, and BMI was performed (Table 3). Factors significantly associated with plasma resistin in Table 2, namely, HOMA-IR, HDL cholesterol, hs-CRP, and SBP, were individually analyzed as a dependent variable. Among these factors, only HOMA-IR, low HDL cholesterol, and hs-CRP were correlated with plasma resistin, with the caution that plasma resistin has a relatively small effect on these parameters based on the regression coefficients. Therefore, plasma resistin, associated with SNP −420, was correlated with HOMA-IR, low HDL cholesterol, and hs-CRP, independent of age, sex, and BMI.

CONCLUSIONS — Our cross-sectional study that included 2,078 subjects from the Japanese general population shows that plasma resistin was associated with SNP −420. Plasma resistin was higher in the elderly, female subjects, nondrinkers, and subjects with high blood pressure. Multiple regression analysis adjusted for age, sex, and BMI revealed that plasma resistin was an independent factor for HOMA-IR, low HDL cholesterol, and hs-CRP.

We found that SNP −420 was associated with plasma resistin in the order G/G > C/G > C/C in a large number of

Table 3—*Plasma resistin was correlated with either HOMA-IR, low HDL cholesterol, or hs-CRP, independent of age, sex, and BMI*

Dependent variable (individually analyzed)	Unstandardized regression coefficient of plasma resistin	Standardized regression coefficient of plasma resistin	*P*
HOMA-IR*	0.013	0.065	0.001†
HDL cholesterol (mg/dl)	−0.190	−0.081	<0.001†
hs-CRP (mg/dl)	0.001	0.061	0.006†
SBP (mmHg)	0.062	0.018	0.346

All characteristics were adjusted for age, sex, and BMI. Multiple regression analysis involving age, sex (male = 1, female = 0), BMI, and plasma resistin (ng/ml) as independent variables was performed, and HOMA-IR, HDL, hs-CRP, and SBP were individually analyzed as a dependent variable. Each calculated power based on the observed effect size and the sample size with $\alpha = 0.05$ was HOMA-IR (0.908), HDL cholesterol (0.973), hs-CRP (0.780), or SBP (0.156). Logistic regression analysis involving CVD as a dependent variable and age, sex, BMI, and plasma resistin as independent variables showed that CVD was not correlated with plasma resistin (unstandardized regression coefficient, 0.010; $P = 0.424$). *HOMA-IR calculated as FPG × IRI/405; †*P* remained significant after Bonferroni's correction (raw *P* value ×5).

samples. This finding provides strong evidence for a tight correlation between a functional promoter SNP and its gene product as the final output in humans. The association is also supported by studies in which smaller numbers of samples were used, namely by Cho et al. (11) and ourselves (8). Haplotypes including SNP −420 also show this similar tendency in Japanese subjects (22). A total of four independent groups reported that the activity of the mutant RETN promoter including −420G is higher than that of the wild type including −420C in vitro (8,11,22,23). Therefore, we propose that SNP −420 is a determinant of plasma resistin. Because only SNP −420 was typed in this study, the other SNPs in *RETN* should be analyzed to further examine this hypothesis.

Our findings have shown that plasma resistin was positively associated with HOMA-IR, independent of age, sex, and BMI. To our knowledge, the positive correlation between circulating resistin and HOMA-IR in humans is supported in 2 of >10 previous studies, whereas the role of resistin as a factor inducing insulin resistance has been established in mice (16,24). The lower power with small numbers of subjects may account for this difference. The broader range of the assay used in this study could also be a contributing factor. It should be noted that serum resistin probably exists as a hexamer (major form) or trimer (a more biologically active form) in mice, which may also affect the assay results (25). The existence of multimers in human serum has recently been implicated (26).

We have shown that plasma resistin was inversely associated with serum HDL cholesterol, independent of age, sex, and BMI. Resistin was reported to be associated with low HDL cholesterol in a smaller numbers of subjects (27,28). Overexpression of resistin in the liver using adenovirus in mice shows enhanced insulin resistance, low serum HDL cholesterol, and high triglyceride levels, which resembles the metabolic syndrome in humans (29). Insulin is known to upregulate lipoprotein lipase, a critical factor producing HDL cholesterol through lipoprotein metabolism. Therefore, insulin resistance caused by elevated plasma resistin could result in reduced serum HDL cholesterol.

We found that plasma resistin was positively associated with hs-CRP. Shetty et al. (28) and McTernan et al. (15) reported that resistin is positively correlated with C-reactive protein (CRP) in a cross-sectional analysis of 77 subjects having diabetes or its risk and 45 type 2 diabetic subjects, respectively. Al-Daghri et al. (30) showed that resistin is associated with CRP in subjects with type 2 diabetes or coronary artery disease in the Saudi Arabian population. Reilly et al. (31) reported that plasma resistin is correlated with inflammatory markers and is predictive of coronary atherosclerosis in humans, independent of CRP. In vitro, resistin increases the expression of critical factors involved in atherosclerotic lesion, such as vascular cell adhesion molecule-1, intracellular adhesion molecule-1, and monocyte chemoattractant protein-1 (32,33). Resistin also enhances human aortic smooth muscle cell proliferation (34). Therefore, resistin could enhance vascular inflammation resulting in elevated serum hs-CRP, whereas an inflammatory cascade has been proposed to lead to hyperresistinemia in humans (35).

In summary, SNP −420 was associated with plasma resistin in the Japanese general population. Plasma resistin was correlated with insulin resistance, lower HDL cholesterol, and high hs-CRP. It is not clear what genetic or environmental factors other than SNP −420, age, and sex affect plasma resistin and how resistin induces insulin resistance in humans. Further studies will be required to clarify these points.

Acknowledgments— This work was supported by grants for research of metabolic disorders from Ehime University, Kurozumi Medical Foundation, and Astellas Foundation and for scientific research from the Ministry of Education, Culture, Sports, Science and Technology of Japan; the Ministry of Health, Labour and Welfare of Japan; and the Japan Arteriosclerosis Prevention Fund.

We thank M. Murase, T. Nishimiya, Dr. Hashiramoto, and Dr. Takata for suggestions. We also thank C. Hiraoka, A. Murakami, and T. Takasuka for technical assistance.

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Original Article

Relationship between Cardio-Ankle Vascular Index (CAVI) and Carotid Atherosclerosis in Patients with Essential Hypertension

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Aortic stiffness measured by aorta-iliac or carotid-femoral pulse wave velocity (PWV) predicts all-cause and cardiovascular mortality. Brachial-ankle PWV (baPWV) has been developed as a more convenient assessment of arterial stiffness. However, the problem with clinical use of baPWV is that the index itself is closely dependent on blood pressure. Recently, a new method, termed the cardio-ankle vascular index (CAVI), has been proposed in Japan to overcome the disadvantages associated with measuring PWV. However, its clinical usefulness has not yet been fully clarified. In the present study, we compared the usefulness of CAVI with that of ultrasound for evaluating atherosclerosis in patients with essential hypertension. CAVI was measured in 70 hypertensive patients. The intima-media thickness (IMT), cross-sectional distensibility coefficient (CSDC), stiffness parameter β, and mean diastolic (V_d) and systolic (V_s) flow velocities were evaluated by carotid ultrasound. The V_d/V_s ratio, an index of peripheral arterial resistance, was also calculated. CAVI was positively correlated with IMT (r=0.360, p=0.0022) and stiffness β (r=0.270, p=0.0239) and negatively correlated with V_d/V_s (r=−0.471, p<0.0001) and CSDC (r=−0.315, p=0.0079). Stepwise regression analysis revealed that age (r=0.475, p<0.0001) and pulse pressure (r=0.492, r<0.0001) were independent determinants of CAVI. These results suggest that CAVI is a useful clinical marker for evaluating atherosclerosis and arteriolosclerosis in patients with essential hypertension. (*Hypertens Res* 2007; 30: 335–340)

***Key Words*: cardio-ankle vascular index, pulse wave velocity, intima-media thickness, arterial stiffness, atherosclerosis**

Introduction

Aortic stiffness is an independent predictor of all-cause and cardiovascular mortality, fatal and nonfatal coronary events, and fatal strokes in patients with essential hypertension (*1*, *2*). Arterial stiffness can be evaluated by measuring pulse wave velocity (PWV) between two sites in the arterial tree (*3*). However, aortic PWV measurement is technically difficult and has low reproducibility (*4*). Brachial-ankle PWV (baPWV), which provides a more convenient assessment of arterial stiffness, has been developed in Japan (*5*, *6*). BaPWV is also closely related to risk factors and organ damage associated with cardiovascular disease (*7–9*). However, the problem with the clinical use of baPWV is that the index itself is closely dependent on blood pressure levels (*10–12*). To overcome this disadvantage, a novel stiffness diagnostic parameter called the cardio-ankle vascular index (CAVI) has been developed in Japan. This stiffness parameter has been reported to be independent of blood pressure levels (*10*, *11*, *13*). CAVI is measured from an ECG, phonocardiogram (PCG), brachial artery waveform, and ankle artery waveform

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Received August 14, 2006; Accepted in revised form December 12, 2006.

and calculated using a specific algorithm (*13*). However, its clinical usefulness has not yet been fully clarified in patients with essential hypertension.

An alternative method for evaluating arterial stiffness is the relative change in lumen diameter during the cardiac cycle adjusted for driving pulse pressure, expressed as arterial distensibility. Carotid distensibility is measured by ultrasound imaging. An ultrasound imaging of the common carotid artery (CCA) has been developed for *in vivo* evaluation of early atherosclerotic lesions (*14–16*). Hypertensive patients exhibit markedly increased intima-media thickness (IMT), a higher prevalence of plaques and increased peripheral vascular resistance in the CCA compared to normotensive individuals (*17*).

In the present study, we measured CAVI in hypertensive patients and noted a significant relationship between the index and morphological, functional and hemodynamic changes in the CCA.

Methods

Study Subjects

Seventy consecutive patients with essential hypertension were enrolled in this study. Hypertension was defined as the use of antihypertensive medications or a systolic blood pressure (SBP) >140 mmHg or diastolic blood pressure (DBP) >90 mmHg. The SBP and DBP were the average of three measurements taken with a brachial sphygmomanometer with the patient in the seated position. Patients with congestive heart failure, previous myocardial infarction, angina pectoris, atrial fibrillation, diabetes mellitus (fasting glucose level >126 mg/dl), chronic renal failure (serum creatinine >1.5 mg/dl), history of stroke, malignant tumor or autoimmune diseases were excluded. The ethics committee of the Ehime University School of Medicine provided approval for this study. Informed consent was obtained from all patients prior to participation.

Blood Sampling

Serum creatinine, fasting glucose, total cholesterol (TC), triglyceride (TG), high-density lipoprotein-cholesterol (HDL-C), and HbA1c were measured using a 200FR analyzer (Toshiba, Tokyo, Japan).

Ultrasound Evaluation

Ultrasound evaluation of the CCA was performed with a SONOS 5500 (PHILIPS Co., Tokyo, Japan) using a 7.5-MHz probe equipped with a Doppler system, as described previously (*17*). After the subjects had rested in the supine position for at least 10 min, their neck was placed in a slightly hyperextended position and then optimal bilateral visualization of the carotid artery was performed. Based on multiple visualizations, plaque formation was identified as the presence of wall thickening at least 50% greater than the thickness of the surrounding wall (*18*). To evaluate the distribution of atherosclerosis in the carotid arteries, we used a plaque scoring method, plaque score was calculated as the sum of the areas of bilateral thickness greater than 1.1 mm as described previously (*19*). The IMT of the far wall was measured in the CCA at sites 1 and 2 cm proximal to the bulb from the anterior, lateral, and posterior approaches, and the results were averaged in order to obtain the mean IMT values. No measurements were carried out at the level of discrete plaques.

Two-dimensional guide M-mode tracing of the right CCA 2 cm proximal to the bulb was recorded with simultaneous ECG and PCG. M-mode images were obtained in real time using a frame grabber. The axial resolution of the M-mode system was 0.1 mm. The internal diameters of the CCA at end-diastole (D_d) and peak-systole (D_s) were determined by continuous tracing of the intimal-luminal interface of the near and far wall of the CCA during three cycles, and the results were then averaged. The cross-sectional distensibility coefficient (CSDC) and carotid arterial stiffness index β were calculated by the following formulae:

$$\mathrm{CSDC} = (D_s^2 - D_d^2)/\{D_d^2 \times (\mathrm{SBP} - \mathrm{DBP})\}$$

$$\beta = \ln(\mathrm{SBP}/\mathrm{DBP}) \times \{D_d/(D_s - D_d)\}$$

SBP and DBP were measured at the brachial artery by an automated sphygmomanometer (BP-103 iII; Omlon-Colin Co., Ltd., Tokyo, Japan) immediately after the evaluation of carotid ultrasound.

Doppler evaluation was performed by scanning the right CCA in the anterior projection. Using color flow mapping, the sample volume was located at the center of the vessel. Flow velocity–time integrals of the systolic and diastolic phases were computed automatically by electronic integration of the instantaneous flow velocity curves, followed by calculation of the systolic (V_s) to diastolic flow velocity (V_d) ratios to assess hemodynamics in the CCA.

Measurement of CAVI

The patients were placed in the supine position for at least 10 min, and then ECG and PCG were monitored. PWV from the heart to the ankle was obtained by measuring the length from the aortic valve to the ankle (VaSera VS-1000; Fukuda Denshi, Tokyo, Japan) (*13*). The formula used to calculate CAVI was as follows:

$$\mathrm{CAVI} = a\{(2\rho/\Delta P) \times \ln(\mathrm{SBP}/\mathrm{DBP})\mathrm{PWV}^2\} + b,$$

where ΔP is SBP – DBP, ρ is blood density, and a and b are constants to match aortic PWV.

This equation was derived from Bramwell-Hill's equation and the stiffness parameter β. CAVI reflects the stiffness of the aorta, femoral artery and tibial artery as a whole, and is theoretically not affected by blood pressure (*13*). All these

Table 1. Characteristics of the Subjects

N (male/female)	70 (46/24)
Age (years)	61±12
BMI (kg/m^2)	25.3±3.7
Systolic blood pressure (mmHg)	137±17
Diastolic blood pressure (mmHg)	85±13
Pulse rate (/min)	66±12
Total cholesterol (mg/dl)	201±36
Triglyceride (mg/dl)	139±76
HDL-C (mg/dl)	55±17
Fasting plasma glucose (mg/dl)	102±15
HbA1c (%)	5.2±0.3
Serum creatinine (mg/dl)	0.80±0.22
CAVI	8.34±1.35
Mean IMT (mm)	0.78±0.17
Plaque score	2.28±3.17
CSDC ($\times 10^{-3}$/mmHg)	3.54±1.57
Stiffness β	7.41±5.17
V_d/V_s	0.53±0.08

BMI, body mass index; HDL-C, high-density lipoprotein-cholesterol; CAVI, cardio-ankle vascular index; IMT, intima-media thickness; CSDC, cross-sectional distensibility coefficient; V_s, systolic mean velocity; V_d, diastolic mean velocity; V_d/V_s, relative diastolic flow velocity.

measurements and calculation were made together and automatically in VaSera. The blood pressure was measured at the brachial artery. The average coefficient of variation for this measurement has been reported to be 3.8% (*13*).

Statistics

All values were expressed as the mean±standard deviation. Pearson's correlation coefficient was used to assess the association between continuous variables. Unpaired *t*-test was used to analyze the comparisons between means. We used stepwise multiple regression analysis to evaluate the independent determinants of CAVI. A *p*-value of <0.05 was considered to be statistically significant.

Results

Characteristics of the Study Participants

The mean age of the participants was 61±12 years. Forty-nine patients (70%) were treated with antihypertensive drugs, including calcium channel blockers (34 patients), angiotensin II receptor blockers/angiotensin converting enzymes (27 patients), β-blockers (6 patients), diuretics (2 patients) and α-blocker (1 patient). Eight (11.4%) patients were treated with statins and 8 (11.4%) were treated with anti-platelet drugs. The clinical characteristics and data of CAVI and carotid parameters of the study subjects are summarized in Table 1.

Table 2. Correlation between CAVI and Other Clinical Parameters (Pearson's Correlation Coefficients)

	r	*p* value
Age	0.609	<0.0001
Systolic blood pressure	0.279	0.0192
Diastolic blood pressure	0.175	0.1469
Pulse pressure	0.620	<0.0001
Total cholesterol	0.043	0.7241
Triglyceride	0.071	0.5608
HDL-C	0.101	0.4032
HbA1c	0.275	0.0022
Serum creatinine	0.133	0.2716

CAVI, cardio-ankle vascular index; HDL-C, high-density lipoprotein-cholesterol.

There were no differences in clinical characteristics, CAVI or carotid parameters between the antihypertensive drug–treated patients (n=49) and non-treated patients (n=21), with the exception of DBP (antihypertensive drug–treated patients: 84±11; non-treated patients: 92±11; p<0.0085).

Correlation between CAVI and Clinical Variables

We examined the relationships between CAVI and pro-atherosclerotic factors such as age, SBP and DBP, pulse pressure, serum creatinine, HbA1c, TC, TG and HDL-C. The univariate linear regression analysis showed that CAVI was strongly correlated with age (r=0.609, p<0.0001) and pulse pressure (r=0.620, p<0.0001), weakly correlated with SBP (r=0.279, p=0.0192) and HbA1c (r=0.275, p=0.0022), and not correlated at all with DBP (r=0.175, p=0.1469), serum creatinine (r=0.133, p=0.2716), TC (r=0.043, p=0.7241), TG (r=0.071, p=0.5608) or HDL-C (r=0.101, p=0.4032) (Table 2). There were no correlations between CAVI and TC, TG or HDL-C even in the 62 patients who did not take statins.

A stepwise multiple regression analysis was performed to evaluate the independent determinants of CAVI using age, SBP, pulse pressure and HbA1c as covariates. Pulse pressure and age were found to be independent determinants of CAVI (partial correlation coefficients: β=0.492 and p<0.0001 for pulse pressure, β=0.475 and p<0.0001 for age).

Correlation between CAVI and Carotid Ultrasound Parameters

There was a significant positive correlation between CAVI and IMT (r=0.360, p=0.0022) (Fig. 1a), but not between CAVI and plaque score (r=0.116, p=0.3409) (Fig. 1b). There was also a weak positive correlation between CAVI and stiffness β (r=0.270, p=0.0239) (Fig. 1c) and a weak negative correlation between CAVI and CSDC (r=−0.315, p=0.0079) or V_d/V_s (r=−0.471, p<0.0001) (Fig. 1d and e, respectively). The independent determinant factor of CAVI

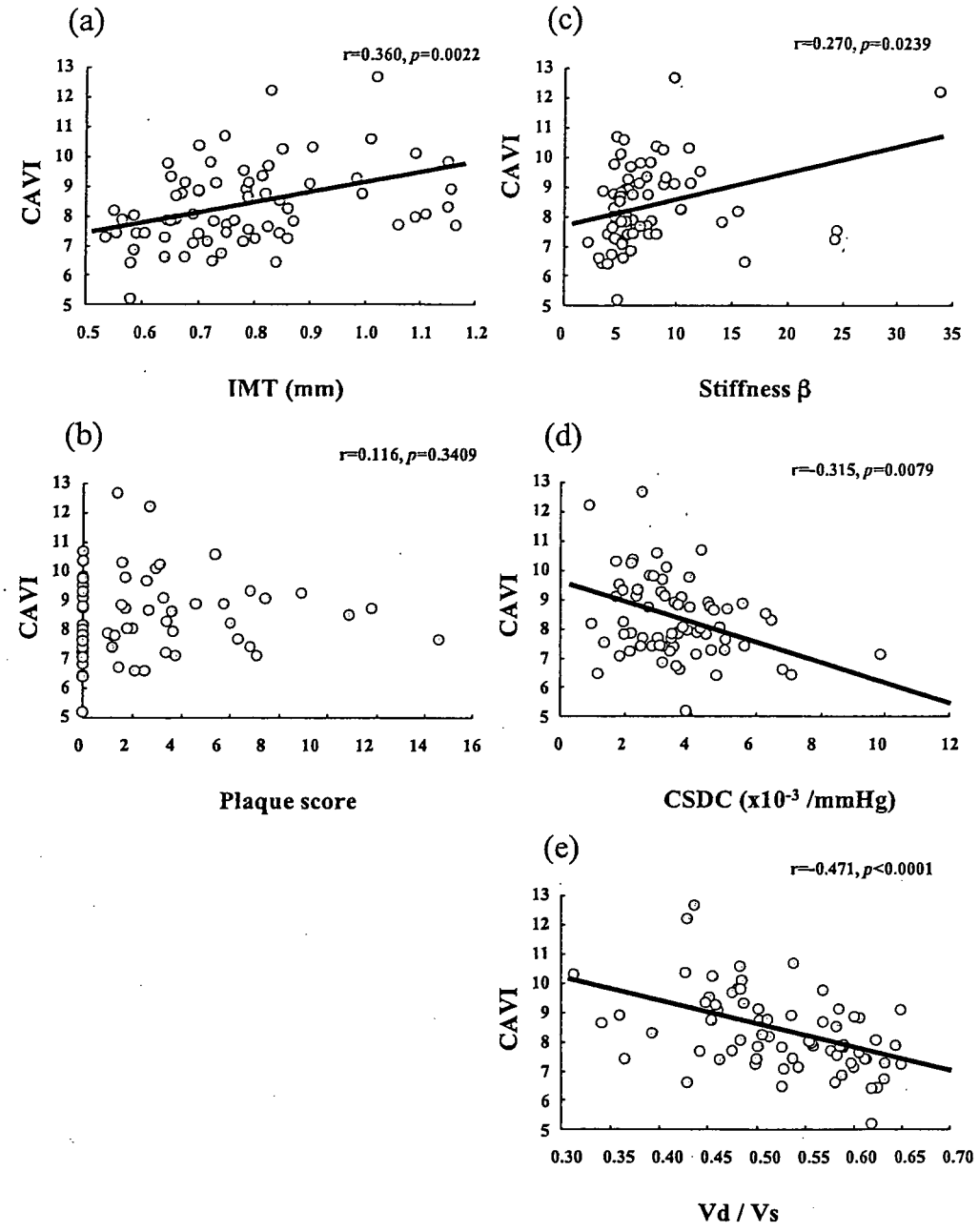

Fig. 1. *Relationship between CAVI and carotid parameters. CAVI was correlated significantly with IMT (a), stiffness* β *(c), CSDC (d) and* V_d/V_s *(e) but not with plaque score (b).*

was V_d/V_s (β=−0.471, p<0.0001) estimated by a stepwise regression analysis using IMT, PS, CSDC, β and V_d/V_s as covariates.

Discussion

Aortic PWV is an independent predictor of cardiovascular risk in the general population and an independent predictor of cardiovascular mortality in patients with essential hypertension (*1–3*). Recently a new index, baPWV, has been developed to provide a more convenient assessment of arterial stiffness (*5, 6*). However, this method is influenced by both blood pressure and the autonomic nervous system (*12*). To overcome these disadvantages, CAVI, which is not influ-

enced by blood pressure, has been developed in Japan (*11–13*). Shirai *et al.* (*13*) and Wakabayashi *et al.* (*20*) reported that CAVI was associated with SBP but not with DBP in dialysis patients and type 2 diabetes patients, respectively. In the present study, CAVI was weakly related to SBP. It has previously been established that CAVI measurement is not affected by blood pressure levels, although CAVI may be affected by the presence of long-term hypertension. CAVI might be able to evaluate the risk of blood pressure during long term for arteriosclerosis properly (*13*).

The present study is the first report of the relationships between CAVI and carotid ultrasound parameters in patients with essential hypertension. The results showed that CAVI was related to carotid IMT, CSDC, stiffness β and V_d/V_s. Atherosclerosis involves a combination of fatty degeneration (atherosis) and vessel stiffening (sclerosis) of the arterial wall (*21*). Arterial stiffness is usually assessed in the aorta by measuring carotid-femoral PWV, but it can also be assessed in the CCA by measuring the distensibility coefficient. Atherosis is commonly assessed by IMT and the presence of plaques in the carotid artery (*22*). A significant relationship between PWV and IMT has been demonstrated, especially in the general population (*22, 23*). However, these studies showed that the strength of the correlation between aortic and carotid stiffness became weaker as the number of cardiovascular risk factors increased (*23*). In the present study, CAVI was related to IMT but not to plaque score. Yambe *et al.* reported that baPWV was positively correlated with both IMT ($r=0.32$, $p<0.01$) and plaque score in hypertensive patients (*14*). However, the correlation between baPWV and plaque score was very weak ($r=0.24$, $p<0.01$). Tamaki *et al.* reported that baPWV was associated with the existence of plaque, but not with the severity of plaque in patients with cerebral thrombosis (*24*). In another study, plaque score was reported to be more closely related to serum CRP level than to IMT (*25*). CRP level has also been shown to be correlated with visceral fat accumulation and therefore linked to the metabolic syndrome and type 2 diabetes (*26, 27*). Wakabayashi *et al.* reported that CRP was significantly associated with CAVI in patients with type 2 diabetes (*20*). These reports and our results suggest that the correlation between CAVI and plaque score may be stronger in patients with type 2 diabetes than in patients with hypertension. Indeed, Masugata *et al.* reported that baPWV was associated with plaque score in type 2 diabetes ($r=0.37$, $p=0.001$) (*28*). Another reason for the lack of a significant relationship between CAVI and plaque score may have been that about one-half of the patients 33 (47%) had a "zero" plaque score, which reduced the power of the statistical analysis to demonstrate a significant relationship.

The progression of arteriolosclerosis, as in the hyaline degeneration of arterioles, increases arterial stiffness and small arteriolar resistance leading to a decrease in diastolic flow velocity. We reported previously that relative diastolic blood flow, V_d/V_s, in the CCA of hypertensive patients was correlated with the intra-renal pulsatility index and resistive index evaluated by a Doppler flow method (*18*). This finding indicated that V_d/V_s is a useful index for evaluating peripheral resistance and arterial stiffness. It is interesting to note that, in the present study, the strongest and most independent association between CAVI and a carotid parameter was the association with V_d/V_s, a hemodynamic parameter ($r=0.471$, $p<0.0001$).

We have shown previously that there is a correlation between stiffness β, CSDC, V_d/V_s and hypertensive target organ damage. Hypertensive patients with left ventricular hypertrophy had a higher stiffness β and lower CSDC and V_d/V_s than normotensive subjects (*17*). We have also reported a negative correlation between V_d/V_s and the severity of asymptomatic cerebral deep gray matter lesions, "etat crible," estimated by brain MRI (*29*). In the present study, we found a significant correlation between CAVI and stiffness β, CSDC, and V_d/V_s, in addition to IMT, suggesting that CAVI may serve as a useful clinical marker of arteriosclerosis and atherosclerosis.

BaPWV has been reported to be associated with waist circumference, HDL-C, TG, uric acid, fasting glucose, fasting insulin and HbA1c, in addition to SBP and DBP (*30*). The present study in hypertensive patients showed that CAVI was associated with HbA1c but not with HDL-C and TG, despite the exclusion of diabetic patients from the study. CAVI may therefore be useful for evaluating the atherosclerotic state, especially in patients with impaired glucose tolerance and type 2 diabetes patients as well as hypertensive patients.

There were several limitations in our study, namely that the study population was relatively small and that we could not eliminate the effect of medications on CAVI level. Another limitation of this study is that brachial SBP and DBP were used to calculate the carotid CSDC and stiffness β instead of carotid SBP and DBP, respectively. Physiologically, mean blood pressure and DBP are nearly identical in the carotid and brachial arteries, whereas SBP and pulse pressure are significantly higher in the brachial arteries than the carotid arteries, although the differences are minimized with aging (*31*). This may be a reason that CAVI was associated with stiffness β and CSDC, although these associations were relative weak.

In conclusion, we demonstrated that CAVI was associated with carotid IMT, CSDC, strain β and V_d/V_s in patients with essential hypertension. CAVI may serve as a useful clinical marker for arteriolosclerosis and atherosclerosis in patients with essential hypertension.

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Clin Exp Nephrol (2007) 11:26–33
DOI 10.1007/s10157-006-0445-4

ORIGINAL ARTICLE

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CCAAT/enhancer-binding protein-δ is induced in mesangial area during the early stages of anti-Thy1.1 glomerulonephritis and regulates cell proliferation and inflammatory gene expression in cultured rat mesangial cells

Received: June 8, 2006 / Accepted: November 16, 2006

Abstract
Background. Interleukin (IL)-6, cyclooxygenase (COX)-2, and monocyte chemoattractant protein (MCP)-1 contribute to renal injury. The promoter regions of these genes contain CCAAT/enhancer-binding protein (C/EBP)-binding sites. In this study, we investigated the role of C/EBP-δ in mesangial cells (MCs).
Methods. In an in vivo study, anti-Thy 1.1 glomerulonephritis rats were generated and C/EBP-δ, IL-6, COX-2, and MCP-1 expressions were assessed by immunohistochemistry. In an in vitro study, cultured MCs were transfected with non-silencing (NS) short interfering RNA (siRNA) or C/EBP-δ siRNA. Subsequently, after stimulation with IL-1β, *C/EBP-δ*, *IL-6*, *COX-2*, and *MCP-1* mRNA expression levels were evaluated using real-time polymerase chain reaction (PCR). IL-6 concentration in the culture medium was determined by enzyme-linked immunosorbent assay. In addition, cell proliferative activity against IL-1β or platelet-derived growth factor-BB was assessed by bromodeoxyuridine incorporation.
Results. In the in vivo study, C/EBP-δ, IL-6, COX-2, and MCP-1 were expressed in the mesangial region of anti-Thy 1.1 glomerulonephritis rats on day 1. In the in vitro study, IL-1β increased *C/EBP-δ* mRNA levels in NS siRNA-transfected MCs (7.3-fold), but no increase was evident in C/EBP-δ siRNA-transfected MCs. *IL-6*, *COX-2*, and *MCP-1* mRNA levels in C/EBP-δ siRNA-transfected MCs were all lower than those in NS siRNA-transfected MCs (decreases of 57.7%, 85.7%, and 69.3%, respectively). The IL-6 concentration in the culture medium from C/EBP-δ siRNA transfected MCs (7.37 ± 4.3 pg/ml) was also lower than that in the culture medium from NS siRNA-transfected MCs (25.2 ± 3.4 pg/ml). Cell proliferative activity in C/EBP-δ siRNA-transfected MCs was lower than that in NS siRNA transfected MCs.
Conclusions. C/EBP-δ was induced in the mesangial region during the early stages of anti-Thy1.1 glomerulonephritis. C/EBP-δ contributes to inflammatory gene expression and MC proliferation.

Key words CCAAT/enhancer-binding protein-δ · Cyclooxygenase-2 · Interleukin-6 · Mesangial cell · Monocyte chemoattractant protein-1 · Short interfering RNA

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Introduction

Mesangial proliferative glomerulonephritis represents an important cause of chronic renal failure and mainly affects young people. Mesangial cell proliferation is evident in many types of glomerulonephritis which are also characterized by the expression of various inflammatory cytokines and inducible enzymes, such as interleukin (IL)-6, cyclooxygenase (COX)-2, and monocyte chemoattractant protein (MCP)-1. IL-6 is released by mesangial cells (MCs) and induces MC proliferation.[1] Indeed, glomerular expression of IL-6 is found in human mesangial proliferative glomerulonephritis[2] and IL-6 has been implicated in the cytokine network controlling glomerular inflammation.[1] COX-2 is an inducible enzyme often found at sites of inflammation and results in the production of the paracrine mediator prostaglandin E2, which exerts antiproliferative effects on rat mesangial cells.[3] COX-2 is strongly expressed in the glomeruli of clinical and experimental glomerulonephritis.[4] MCP-1 is a member of a group of small chemotactic cytokines called chemokines and mediates monocyte/macrophage infiltration. Glomerular MCP-1 is found in murine models of crescentic nephritis[5] and lupus nephritis.[6]

CCAAT/enhancer-binding proteins (C/EBPs) are a family of leucine zipper transcription factors and have at least seven known members including CCAAT/enhancer-binding protein-δ (C/EBP-δ).[7] C/EBP-δ is present at relatively low levels in normal physiological conditions but is strongly induced during acute inflammation. The promoter regions of the genes for *IL-6*,[8] *COX-2*[9] and *MCP-1*[10] contain