Hypertension Research (2009) 32, 1112–1118

www.nature.com/hr

ORIGINAL ARTICLE

Association of *PLA2G7* polymorphisms with carotid atherosclerosis in hypertensive Japanese

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Although the plasma platelet-activating factor-acetylhydrolase (pPAF-AH) gene (*PLA2G7*) polymorphisms are reportedly associated with atherosclerotic diseases, their effects in hypertensive patients have not been well examined. Thus, we genotyped V279F, a loss-of-function mutation commonly seen in the Japanese, and I198T and A379V commonly seen in Caucasians, and investigated the (1) ethnic differences in the frequencies and (2) association of these variants with prevalence of carotid plaque in 733 treated hypertensive Japanese patients. The distribution of V279F (V allele 75.1% and F allele 24.9%) in hypertensive patients was similar to that previously reported in the healthy Japanese; however, allele frequencies of I198T (I allele 71.7% and T allele 28.3%) and A379V (A allele 84.7% and V allele 15.3%) were markedly different from those reported in Caucasians. In addition, V279F and I198T showed a strong linkage disequilibrium ($D'=1.0$, $r^2=0.89$). The phenotypes showed no difference among genotypes for each polymorphism except for the blood pressure level in I198T in women. Carotid plaque was significantly more prevalent in subjects with 279F and 198T than in those with the wild type among men but not women, whereas A379V did not affect it. In multivariate logistic regression analyses, 279F and 198T were detected as an independent risk factor even after adjustments for other atherosclerotic risk factors in men. Taken together, our data suggest an ethnic difference and the possible involvement of genetic polymorphisms of *PLA2G7* in the prevalence of carotid atherosclerosis in the hypertensive Japanese, especially in men.
Hypertension Research (2009) **32,** 1112–1118; doi:10.1038/hr.2009.151; published online 18 September 2009

Keywords: atherosclerosis; intima-media thickness; PAF-AH; *PLA2G7*; polymorphism

INTRODUCTION

The oxidation of low-density lipoprotein (LDL) is now recognized as a major initiator of atherosclerosis.[1,2] The LDL oxidation reportedly involves a platelet-activating factor (PAF), a potent lipid mediator implicated in inflammatory reactions.[3,4] Local inflammation in the vascular wall immediately triggers the production of chemical mediators, and at the same time induces leukocyte adhesion to the endothelium mainly through P-selectin.[3] Chemical mediators such as thrombin and bradykinin increase the expression of PAF on the endothelial surface and activate leukocytes. Activated leukocytes further stimulate the formation of oxidized lipid. Thus, local synthesis of PAF in the vascular wall may increase the production of oxidized LDL and modify the atherogenic process.

In plasma, PAF is hydrolyzed and inactivated by a specific enzyme, PAF-acetylhydrolase (PAF-AH; EC 3.1.1.47), a Ca^{2+}-independent phospholipase A_2.[5] Plasma PAF-AH (pPAF-AH) has a relatively broad substrate specificity for phospholipids, and can catalyze and inactivate not only PAF but also PAF-like oxidized lipids.[6] This enzyme activity has been reported to correlate with several inflammatory diseases such as asthma,[7,8] systemic lupus erythematosus[9] and juvenile rheumatoid arthritis.[10] Furthermore, previous reports have revealed that the polymorphism of pPAF-AH gene (*PLA2G7*) influences its activity. Miwa *et al.*[11] discovered an autosomal recessive heredity form of pPAF-AH deficiency in Japanese families. Stafforini *et al.*[12] investigated these pPAF-AH-deficient families and found a point mutation, T for G at position 994 in exon 9, which results in an amino acid substitution Phe for Val at residue 279 (994 G>T, V279F) at the active site of pPAF-AH. The activity of pPAF-AH was decreased about 50% in subjects with 279V/F[12] and completely abolished in subjects with 279F/F, 4% of healthy Japanese adults.[11] There have been several reports of a significant association between this polymorphism and atherosclerotic diseases such as coronary artery disease,[13] stroke[14] and atherosclerotic occlusive disease.[15] Although V279F is rare in Caucasians, other genetic mutations (R92H, I198T and A379V) have been described in European populations.[16] I198T and A379V were associated with atopy and asthma, probably due to a decrease in the affinity of pPAF-AH for substrate.[17] However, the frequencies and

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Received 14 January 2009; revised 5 August 2009; accepted 20 August 2009; published online 18 September 2009

the effects on atherosclerosis of these variants in the Japanese have not been fully examined.

Hypertension is well recognized as a major cause of atherosclerotic complications. At high blood pressure, excessive production of angiotensin II[18] and hemodynamic changes such as increased pulsatile stretch[19] are reported to produce oxidative stress. pPAF-AH may be expressed to compensate for the increase in PAF and PAF-like oxidized lipids under these conditions. In fact, increased pPAF-AH activity was reported in patients with essential hypertension.[20] However, no study has examined the effect of *PLA2G7* polymorphism on atherosclerosis in hypertensive patients. Therefore, in this study, we investigated the frequency and association with carotid atherosclerosis of three functional *PLA2G7* polymorphisms (I198T, V279F and A379V) in Japanese patients with essential hypertension.

METHODS

This study was conducted in accordance with the Declaration of Helsinki. The study protocol was approved by the Ethics Review Committee of the National Cardiovascular Center. Written informed consent was obtained from all patients.

Hypertensive patients

A total of 953 hypertensive patients (522 men and 431 woman) were initially recruited from the Division of Hypertension and Nephrology at the National Cardiovascular Center as reported previously.[21,22] Patients with secondary hypertension, severe hyperlipidemia (total cholesterol $>7.8\,mmol\,l^{-1}$ (300 mg per 100 ml) and/or triglyceride $>4.5\,mmol\,l^{-1}$ (400 mg per 100 ml)), severe diabetes (HbA1c $>10.0\%$ and/or under insulin treatment) and severe renal failure (serum creatinine $>177\,mmol\,l^{-1}$ (2.0 mg per 100 ml)) were excluded. Therefore, 733 outpatients with essential hypertension (395 men, 338 women, average age 65.0 ± 10.5 years old) were analyzed. All of the participants were Japanese. Hypertension was defined as systolic blood pressure of $\geqslant 140$ mm Hg, diastolic blood pressure of $\geqslant 90$ mm Hg, or the current use of antihypertensive medication. Hyperlipidemia was defined as LDL cholesterol $\geqslant 3.6\,mmol\,l^{-1}$ (140 mg per 100 ml) or current use of antihyperlipidemia medication. Diabetes was defined as fasting plasma glucose $\geqslant 7.0\,mmol\,l^{-1}$ (126 mg per 100 ml) or HbA1c $\geqslant 6.5\%$ or current use of the antidiabetic medication. Study subjects underwent routine laboratory tests including electrolytes, renal function, blood glucose and HbA1c.

Clinical parameters

At the time of the physical examination, blood pressure, body mass index, and a hematological and biochemical profile were determined. The measurements were performed in the morning after an overnight fast. Information on age, smoking and drinking status, and history was obtained through a questionnaire and interview. Blood pressure was measured after 15 min of quiet rest in the supported right arm of seated subjects with a mercury sphygmomanometer, the cuff size of which was adjusted to the arm's circumference. Three measurements made at intervals of more than 2 min were averaged. Total cholesterol, high-density lipoprotein (HDL) cholesterol and triglyceride levels were enzymatically determined using an autoanalyzer. The LDL cholesterol level was calculated using Friedewald's equation. The fasting plasma glucose and HbA1c levels were determined by standard laboratory methods.

Genotyping of polymorphisms

Three polymorphisms of *PLA2G7* (I198T, V279F and A379V) were genotyped using the TaqMan PCR system as described previously.[23] The sequences of PCR primers and probes for the TaqMan PCR method were as follows: I198T; primers, 5′-GAAGGGAAGGAGCATGCATAAA-3′ and 5′-TCAGGGTTCTA AGGTAGAGCCAA-3′, probes, Fam-TGCAGAAATAGGGGAC-MGB (for the I allele) and Vic-CAGAAACAGGGGACAA-MGB (for the T allele), V279F; primers, 5′-GGGAAAAAATAGCAGTAATTGGACA-3′ and 5′-ACTCCAAGAG ATCCCTTCTTCACT-3′, probes, Fam-CAACGGTTATTCAGAC-MGB (for the V allele) and Vic-AGCAACGTTTATTCAGA-MGB (for the F allele), A379V; primers, 5′-ACATGCTCAAATTAAAGGGAGACAT-3′ and 5′-AGAATGCTAAT GAAGCTTTGTTGCT-3′, probes, Fam-ATTCAAATGTAGCTATTGAT-MGB (for the V allele) and Vic-TTCAAATGCAGCTATTGA-MGB (for the A allele).

Carotid artery ultrasonography

Ultrasonography of both carotid arteries was performed and it measured the mean intima-media thickness (C-IMT) and maximum IMT (C-IMT*max*) as described previously.[24,25] A plaque was defined as a local thickening of the vascular wall (IMT $\geqslant 1.2$ mm) in both common carotid arteries and bifurcations (near and far walls). Two independent sonographers who were masked from the clinical data performed the measurements.

Statistical analysis

The Hardy–Weinberg equilibrium was assessed by χ^2 analysis. Linkage disequilibrium (LD) was calculated using the SNP Alyze version 2.1 (DYNACOM, Mohara, Japan). To measure LD between polymorphisms, Lewontin's D' and r^2 values were calculated. An analysis of variance was used to compare the mean values among genotypes for each polymorphism. Frequencies of carotid plaques were compared by χ^2 analysis or the Cochran–Armitage test. The association with the prevalence of plaques was examined in each gender through logistic regression analysis considering the potential confounding risk variables including age, body mass index, current smoking, duration of hypertension, hyperlipidemia, diabetes, antihypertensive agents and lipid-lowering therapy. For multivariate risk predictors, the adjusted odds ratios were given with 95% confidence intervals. All statistical analyses were performed using JMP IN Version 5.1.1. J (SAS Institute, Cary, NC, USA). $P<0.05$ was considered statistically significant.

RESULTS

Clinical characteristics of hypertensive patients with or without carotid plaques

Table 1 shows the clinical characteristics of hypertensive patients. Age, smoking ratio, duration of hypertension and HbA1c were significantly higher in patients with plaques than in those without, in both genders. Complicated ratios of obesity, hyperlipidemia and diabetes that cause atherosclerosis did not differ between the two groups in men. However, in women, hyperlipidemia was more frequent in patients with plaques. Obesity and diabetes also tended to be frequent. For the agents used, ratios of angiotensin II receptor blockers and/or angiotensin-converting enzyme inhibitors, and diuretics did not differ between the groups. In contrast, calcium channel blockers and α-blockers were highly used in hypertensive patients with plaques. The number of antihypertensive agents was also greater only in men. Lipid-lowering agents were highly used in patients with plaques in women.

Genotype distributions and LD

Three polymorphisms of *PLA2G7* (V279F, I198T and A379V) were genotyped in all hypertensive patients. Genotype distributions and characteristics for each polymorphism are shown in Table 2. The control for deviation from the Hardy–Weinberg equilibrium gave nonsignificant results for any polymorphism. Allele frequencies for V279F were V allele 75.1% and F allele 24.9%, not so different from the values reported in the healthy Japanese.[26] However, the frequencies for I198T (I allele 71.7% and T allele 28.3%) and A379V (A allele 84.7% and V allele 15.3%) were markedly different from those reported recently in hypercholesterolemic Sicilians (I198T (I allele 30.5% and T allele 69.5%) and A379V (A allele 33.9% and V allele 66.1%)).[27] I198T and V279F showed a strong LD ($D'=1.0$, $r^2=0.89$), whereas no association was found between I198T and A379V, or between V279F and A379V. For each polymorphism, no significant differences were observed in age, smoking ratio, BP, LDL-C and HbA1c between genotypes in men. However, in women, the systolic

Table 1 Clinical characteristics of the hypertensive patients

	Patients without carotid plaques (men 142, women 139)	*Patients with carotid plaques (men 253, women 199)*	P-*value*
Age, year			
Men	58.2 ± 11.9	67.2 ± 9.4	<0.001
Women	64.3 ± 10.3	68.0 ± 8.6	0.003
BMI, kg m^{-2}			
Men	24.8 ± 3.8	24.1 ± 4.4	0.152
Women	23.6 ± 4.1	23.5 ± 4.4	0.787
Smoking, %			
Men	9.2	22.9	<0.001
Women	0	7.5	<0.001
Duration of HT, year			
Men	15.8 ± 10.2	18.9 ± 11.4	0.008
Women	15.2 ± 10.0	19.6 ± 10.4	<0.001
Systolic BP, mm Hg			
Men	140.6 ± 16.0	137.4 ± 16.8	0.083
Women	140.5 ± 18.3	144.0 ± 18.9	0.105
Diastolic BP, mm Hg			
Men	87.7 ± 10.1	82.1 ± 10.3	<0.001
Women	83.8 ± 11.3	82.6 ± 11.2	0.351
HDL cholesterol, mmol l^{-1}			
Men	1.3 ± 0.4	1.3 ± 0.4	0.337
Women	1.5 ± 0.4	1.5 ± 0.4	0.122
LDL cholesterol, mmol l^{-1}			
Men	3.2 ± 0.7	3.1 ± 0.7	0.071
Women	3.3 ± 0.7	3.3 ± 0.7	0.279
FPG, mmol l^{-1}			
Men	5.7 ± 1.1	5.9 ± 1.2	0.236
Women	5.4 ± 0.6	5.5 ± 1.0	0.119
HbA1c, %			
Men	5.5 ± 0.6	5.7 ± 0.6	0.005
Women	5.4 ± 0.5	5.6 ± 0.6	0.008
Other atherosclerotic risks			
Obesity, %			
Men	38.7	42.8	0.491
Women	26.6	35.7	0.079
Hyperlipidemia, %			
Men	36.6	40.3	0.471
Women	48.9	61.3	0.024
Diabetes, %			
Men	9.2	12.3	0.349
Women	2.9	7.5	0.068
Antihypertensive drugs			
ARBs and/or ACEIs, %			
Men	53.5	54.9	0.786
Women	49.6	45.7	0.480
CCBs, %			
Men	65.5	75.1	0.042
Women	63.3	72.9	0.062
β-Blockers, %			
Men	33.8	41.1	0.153
Women	27.3	33.2	0.255
α-Blockers, %			
Men	11.3	19.8	0.030
Women	5.8	10.1	0.160
Diuretics, %			
Men	18.3	21.7	0.419
Women	23.7	25.6	0.694
Number of drugs			
Men	1.8 ± 1.1	2.2 ± 1.3	0.008
Women	1.7 ± 1.1	1.9 ± 1.1	0.124
Lipid-lowering drugs, %			
Men	17.6	25.7	0.066
Women	29.5	42.7	0.013

Abbreviations: ACEIs, angiotensin-converting enzymes; ARBs, angiotensin II receptor blockers; BMI, body mass index; BP, blood pressure; CCBs, calcium channel blockers; FPG, fasting plasma glucose; HbA1c, hemoglobin.A1c; HDL, high-density lipoprotein; LDL, low-density lipoprotein.
Differences in continuous variables and frequencies between groups were analyzed with Student's *t*-test and χ^2-test, respectively.

blood pressure and diastolic blood pressure levels were significantly lower in subjects with T allele in I198T (I/T+T/T, 198T) than in those with 198I/I. V279F also showed a similar tendency, however, without statistical significance. The frequencies of obesity, hyperlipidemia and diabetes, and the kinds and numbers of antihypertensive agents and lipid-lowering agents also showed no significant differences (data not shown).

Association of *PLA2G7* polymorphisms with carotid atherosclerosis

We next investigated the degree of carotid atherosclerosis for each *PLA2G7* polymorphism. When the absolute values of C-IMT or C-IMT*max* were compared by ANOVA (analysis of variance), the subjects with T/T of I198T had significantly greater C-IMT than those with I/I (Figure 1). Similarly, subjects with F/F of V279F had significantly greater C-IMT than those with V/F or V/V. The associations with C-IMT*max* were not significant in all polymorphisms, although I198T and V279F showed tendencies similar to C-IMT. Furthermore, as shown on the upper side of Table 3, the prevalence of carotid plaque was significantly associated with I198T or V279F but not with A379V in the codominant genetic model. A significant increase in the prevalence of carotid plaque was observed in subjects with 198T and with F allele in V279F (V/F+F/F, 279F) compared with those with wild type (198I/I and 279V/V) in men. In women, these variants also tended to associate with carotid plaques, without, however, being statistically significant. A379V did not affect the prevalence of plaques in either gender. In the multivariate logistic regression analysis, an association between the two variants (198T and 279F) and plaques remained even after adjusting for age (Model 1), age and other atherosclerotic risk factors (Model 2), and age, other atherosclerotic risk factors and factors contributing to carotid plaques such as antihypertensive and lipid-lowering drugs (Model 3) in men (Table 4). Also, in women, 279F was detected as an independent risk factor for carotid plaques after adjusting for other variables (Model 2 and Model 3).

DISCUSSION

In this study, we found apparent differences of allele frequency between the Caucasians and the Japanese in I198T and A379V of the

Table 2 Genotype distributions and characteristics of pPAF-AH polymorphisms

Genotype	N	Age	Smoking	BMI	Systolic BP	Diastolic BP	LDL-C	HbA1c
Men								
I198T								
I/I	238	64.1	18.1	24.4	139.1	84.0	119.9	5.7
I/T	138	64.1	18.8	24.2	137.7	84.2	119.4	5.6
T/T	19	62.0	10.5	24.8	138.6	84.0	120.3	5.4
I/T+T/T (198T)	157	63.8	17.8	24.3	137.8	84.2	119.5	5.6
V279F								
V/V	258	63.9	17.8	24.2	138.2	83.7	120.0	5.6
V/F	125	64.3	18.4	24.5	139.3	85.0	119.2	5.6
F/F	12	60.9	16.7	24.7	137.3	82.2	119.8	5.5
V/F+F/F (279F)	137	64.0	18.2	24.5	139.2	84.8	119.2	5.6
A379V								
A/A	331	63.7	17.5	24.4	138.8	84.3	119.8	5.6
A/V	62	64.8	19.4	23.9	137.3	83.6	120.4	5.6
V/V	2	72.5	50.0	25.4	140.5	70.5	90.2	6.0
A/V+V/V (379V)	64	65.1	20.3	23.9	137.4	83.1	119.4	5.6
Women								
I198T								
I/I	220	66.2	5.9	23.7	144.2	84.2	128.5	5.5
I/T	102	67.1	1.0	23.3	139.7	80.6	126.6	5.5
T/T	16	65.4	6.3	22.9	140.1	85.1	128.8	5.5
I/T+T/T (198T)	118	66.9	1.7	23.3	139.7*	81.2*	126.9	5.5
V279F								
V/V	239	65.7	5.4	23.6	143.7	83.8	128.5	5.5
V/F	90	67.1	2.2	23.6	140.1	81.4	126.4	5.5
F/F	9	65.7	0.0	22.2	138.9	84.0	128.8	5.6
V/F+F/F (279F)	99	67.0	2.0	23.5	140.0	81.6	126.6	5.5
A379V								
A/A	270	66.8	4.8	23.4	142.1	83.3	128.8	5.5
A/V	67	65.3	3.0	24.2	144.6	82.3	125.4	5.5
V/V	1	59.0	0.0	29.1	144.0	75.0	81.2	6.1
A/V+V/V (379V)	68	65.2	2.9	24.3	144.5	83.1	124.8	5.5

Abbreviations: BMI, body mass index; BP, blood pressure; FPG, fasting plasma glucose; HbA1c, hemoglobin.A1c; HDL, high-density lipoprotein; LDL, low-density lipoprotein.
Data represent mean values.
*$P<0.05$ *vs.* 198I/I.

PLA2G7 polymorphism. The allele frequencies in Japanese hypertensive patients were I allele 71.7% and T allele 28.3% (I198T), and A allele 84.7% and V allele 15.3% (A379V). A recent report indicated that the allele frequencies of these variants in hypercholesterolemic Sicilians were I allele 30.5% and T allele 69.5% (I198T), and A allele 33.9% and V allele 66.1% (A379V),[27] which are markedly different from the present results. In V279F, which has not been found in the Caucasians,[28,29] 29.3% of subjects were heterozygotes (215 patients) and 2.9% were homozygotes (21 patients). These values are similar to previously reported values in the healthy Japanese (heterozygotes 21.0–32.3%, homozygotes 0.9–4.2%),[26] suggesting that V279F may not affect the occurrence of hypertension, although more detailed studies with a case–control design are required.

Another important finding of this study is the significant association between polymorphisms of 0*PLA2G7* and carotid atherosclerosis. The values of C-IMT and the plaque prevalence were significantly different among genotypes in I198T or V279F, whereas C-IMT*max* showed no difference. As the differences in the C-IMT*max* values in each subject were relatively large compared with those in C-IMT, such discrepancies in association with polymorphisms may be observed. In our hypertensive patients, although the blood pressure was relatively well controlled, the prevalence of carotid plaques was significantly higher in subjects with 198T and 279F than in wild-type (198I/I and 279V/V) subjects in men. Furthermore, these two mutations were detected as independent factors for the occurrence of plaques even after adjustments with other atherosclerotic risk factors and as factors contributing to carotid plaques (Table 3). Several previous studies have suggested that 279F is a potential risk factor for atherosclerosis. Yamada *et al.*[14] reported that 279F was highly frequent in Japanese men with myocardial infarction (279V/F 33.0%, 279F/F 2.1%) compared with controls (279V/F 21.0%, 279F/F 2.2%). An increased occurrence of 279F was also reported in Japanese patients with stroke[13] and atherosclerotic occlusive disease.[15] The increased prevalence of carotid plaque in subjects with 279F observed in this study was consistent with these results. It is natural to conclude that these findings are caused by the loss of function of pPAF-AH, because pPAF-AH activity is completely abolished in individuals with 279F/F and suppressed in those with 279V/F.[12] Furthermore, it has been reported that age-dependent increase in pPAF-AH activity was diminished in 279V/F subjects,[30] suggesting that the reactive pPAF-AH induction caused by an increase in PAF is suppressed in subjects with 279V/F. These factors lead to the lack of an anti-inflammatory effect in response to injurious stimuli in the vascular wall and, therefore, may increase the risk of atherosclerosis.

I198T, another variant associated with the prevalence of carotid plaques, has been shown to reduce the substrate affinity of pPAF-AH, similar to A379V,[17] whereas these variants did not affect pPAF-AH activity.[27] However, the ranges of substrate (PAF) concentrations used in the previous study were far above the physiological level measured in plasma. Considering our results that A379V showed no correlation with the development of plaques, the functional abnormality of I198T and A379V may have small effects *in vivo*. In contrast, the strong LD with V279F ($D'=1.0$, $r^2=0.89$) may rather contribute to the association of I198T with carotid plaques in this study. In fact, all 21 subjects with 279F/F had the 198T/T genotype. Contrary to our results, Campo *et al.*[27] recently reported that R92H, I198T and A379V did not affect plasma PAF-AH activity and that these variants were not associated with carotid atherosclerosis in hypercholesterolemic Sicilian subjects. However, the population analyzed in their study was relatively small (190 subjects) and was not divided by gender. Furthermore, a significant LD was found between I198T and A379V, which was not observed in our subjects. These results suggest that the allele frequency and LD differ among races, which can lead to markedly different results.

Despite several reports indicating a significant correlation between pPAF-AH and atherosclerosis, the actual role of pPAF-AH on atherogenesis still remains unclear because of its complicated effect. As pPAF-AH degrades PAF and proinflammatory oxidized phospholipids, it may be a potent anti-atherogenic enzyme. In contrast, pPAF-AH also generates bioactive oxidized free fatty acids and lysophosphatidylcholine, which stimulate inflammatory reactions that could promote atherogenesis. However, previous studies using animal models of vascular injury have clearly indicated that pPAF-AH functions as an anti-atherogenic factor. The administration of a recombinant pPAF-AH reduced the size of myocardial infarctions and neutrophil infiltration in rabbits with coronary ligation.[28] Adenovirus-mediated

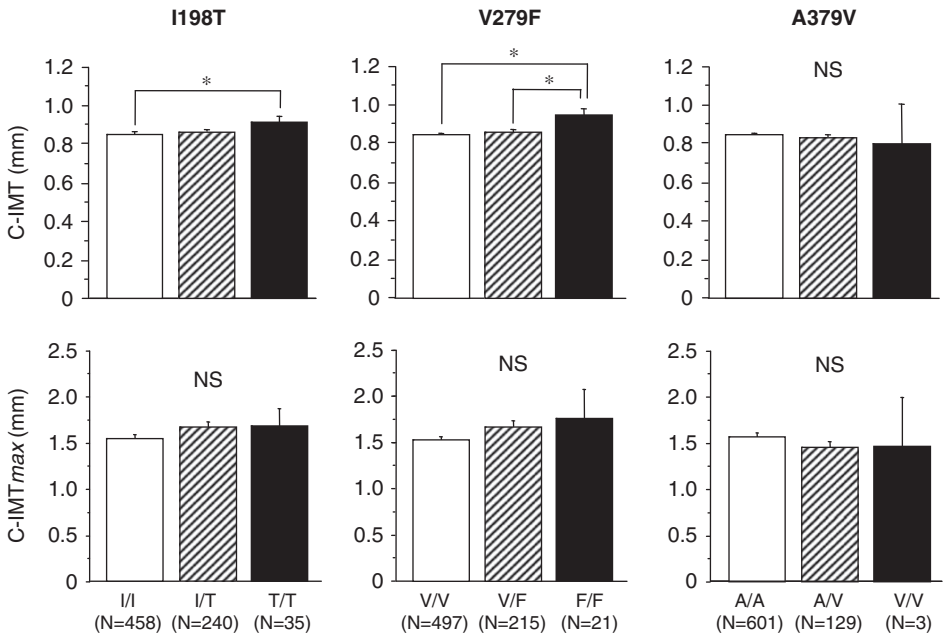

Figure 1 Comparison of mean intima-media thickness (C-IMT) or maximum IMT (C-IMT*max*) in the *PLA2G7* polymorphisms. *P*-values were evaluated with analysis of variance (ANOVA) followed by Bonferroni *post hoc* test. **P*<0.05. NS, not significant.

Table 3 Prevalence of carotid plaques in relation to *PLA2G7* polymorphisms

Genotype	*Patients with carotid plaques/total (N)*	*Frequency (%)*	P-*value*
ALL			
I198T			
I/I	266/458	58.1	
I/T	162/240	67.5	
T/T	24/35	68.6	0.014 (Trend *P*)
V279F			
V/V	288/497	57.9	
V/F	149/215	69.3	
F/F	15/21	71.4	0.004 (Trend *P*)
A/A	368/601	61.2	
A/V	82/129	63.6	
V/V	2/3	66.7	0.599 (Trend *P*)
Men			
I198T			
I/I	142/238	59.7	
I/T+T/T (198T)	111/157	70.7	0.025
V279F			
V/V	154/258	57.9	
V/F+F/F (279F)	99/137	72.3	0.014
A379V			
A/A	209/331	63.1	
A/V+V/V (379V)	44/64	68.8	0.393
Women			
I198T			
I/I	124/220	56.4	
I/T+T/T (198T)	75/118	63.6	0.201
V279F			
V/V	134/239	56.1	
V/F+F/F (279F)	65/99	65.7	0.104
A379V			
A/A	159/270	58.9	
A/V+V/V (379V)	40/68	58.8	0.992

Trend *P*-values in all subjects were evaluated with Cochran–Armitage test. Differences between groups in men or women were analyzed with χ^2-test.

pPAF-AH gene transfer prevented neointima formation in apolipoprotein E-deficient mice.[29] Hypertension may also cause inflammation of the vascular wall and induce expression of PAF, and therefore the association of the loss-of-function mutation in *PLA2G7* with carotid plaques in hypertensives supports the hypothesis that pPAF-AH is an anti-atherogenic factor. As a loss-of-function mutation such as V279F has not been identified in the Caucasians, the *PLA2G7* polymorphism may be a potential risk factor for atherosclerosis specific to the Japanese.

There are some limitations to this study. This study had a cross-sectional design, even though we analyzed a relatively large population. Our subjects were treated with antihypertensive and lipid-lowering drugs, some of which were intensively administered to patients with plaques (Table 1). These agents were reported to suppress the progression of atherosclerosis beyond their essential pharmacological effects, especially inhibitors of the renin–angiotensin system

Table 4 Logistic regression analyses of factors affecting the prevalence of carotid plaques

	Men		*Women*	
	OR (95% CI)	P-*value*	*OR (95% CI)*	P-*value*
Model 1				
198T (I/T+T/T)	1.32 (1.05–1.66)	0.016	1.15 (0.91–1.44)	0.240
279F (V/F+F/F)	1.34 (1.06–1.70)	0.011	1.20 (0.95–1.51)	0.126
Model 2				
198T	1.37 (1.08–1.74)	0.010	1.23 (0.97–1.57)	0.082
279F	1.38 (1.08–1.75)	0.009	1.31 (1.03–1.67)	0.026
Model 3				
198T	1.40 (1.10–1.79)	0.007	1.23 (0.96–1.56)	0.100
279F	1.37 (1.10–1.42)	0.006	1.30 (1.02–1.67)	0.034

Abbreviations: ACEIs, angiotensin-converting enzymes; ARBs, angiotensin II receptor blockers; BMI, body mass index; CI, confidential interval; OR, odds ratio.
Polymorphisms were analyzed by a dominant model. I198T (T/T, 1; I//T, 1; I/I, 0), V279F (F/F, 1; V/F, 1; V/V; 0).
Model 1: adjusted with age.
Model 2: adjusted with age, BMI, duration of HT, smoking, hyperlipidemia and diabetes.
Model 3: adjusted with age, BMI, duration of HT, smoking, hyperlipidemia, diabetes, ACEIs and/or ARBs, and lipid-lowering drugs.

(angiotensin II receptor blocker and angiotensin-converting enzyme inhibitor)[31,32] and statins.[33] However, even after adjusting for these factors, 198T and 279F were independently associated with plaques in men. In addition, menopausal status may influence our results in women because estrogen has been shown to increase the plasma PAF-AH activity.[34] However, we could not analyze the effect, as we did not collect the information on menopause. To further clarify these issues in detail, a cohort study in a large healthy population is required.

In conclusion, we showed the ethnic differences in allele frequency between the Caucasians and the Japanese in I198T and A379V of pPAF-AH. We also found that the loss-of-function mutation V279F in *PLA2G7* and its closely associated variant I198T are associated with carotid atherosclerosis in the similarly treated hypertensive Japanese. Our results indicated that a deficiency in pPAF-AH is a potential risk factor for atherosclerosis in hypertensive Japanese men, suggesting that a more definite risk management including blood pressure and lipid lowering is required for the hypertensive Japanese with 279F and 198T to prevent the progression of atherosclerosis.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

The authors are grateful to Yoko Tokunaga and Chiyako Imai for their excellent technical assistance. We also express our gratitude to Dr Soichiro Kitamura, President of the National Cardiovascular Center, for his support in our research. This study was supported by the Program for the Promotion of Fundamental Studies in Health Sciences of the Organization for Pharmaceutical and Medical Devices Agency (PMDA) of Japan and the Ministry of Health, Labor, and Welfare of Japan.

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