Identification of the $G^{994} \rightarrow T$ Missense Mutation in Exon 9 of the Plasma Platelet-Activating Factor Acetylhydrolase Gene as an Independent Risk Factor for Coronary Artery Disease in Japanese Men

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Platelet-activating factor (PAF) acetylhydrolase may play important roles in the pathophysiology of thrombosis and atherosclerosis related to its catalytic action in the degradation of PAF and oxidized phospholipids. A missense mutation (G → T transversion at nucleotide 994) in the plasma PAF acetylhydrolase gene results in a Val → Phe substitution at amino acid 279 of the mature protein and a consequent loss of catalytic activity. However, the role of a deficiency or low activity of this enzyme caused by the missense mutation in the etiology of coronary artery disease (CAD) has not been determined. The relation between this mutation and the incidence of CAD in the Japanese population is investigated herein. The genotype of plasma PAF acetylhydrolase (*MM,* normal; *Mm,* heterozygote; and *mm,* deficient homozygote) was determined with a polymerase chain reaction (PCR) assay for 454 patients with myocardial infarction (MI) and 602 control subjects. The frequency of the *m* allele was significantly higher in male patients with MI (odds ratio, 1.8) than in controls, an association that was more marked in a low-risk subgroup (odds ratio, 2.3). In contrast, the *m* allele was not associated with MI in women. These results indicate that the $G^{994} \rightarrow T$ missense mutation in exon 9 of the plasma PAF acetylhydrolase gene is an independent risk factor for CAD in Japanese men, especially low-risk individuals, but not in women.

CORONARY ARTERY DISEASE (CAD) is a major clinical problem because of its large contribution to mortality. Various CAD risk factors, including hypertension, hyperlipidemia, and diabetes mellitus, have been identified epidemiologically based on their relation to the incidence of CAD. However, in some individuals, CAD is not associated with such conventional risk factors, which suggests that other genetic factors may contribute to the predisposition to CAD.[1]

Platelet-activating factor (PAF) is a biologically active phospholipid that activates platelets, leukocytes, monocytes, and macrophages[2] as a result of its binding to a cell-surface receptor.[3] Recently, Mueller et al[4] showed that PAF is synthesized locally at the site of endothelial injury during thrombosis and accumulates in the atherosclerotic plaques of some patients with advanced CAD, suggesting that PAF actively participates in the pathophysiology of thrombosis and atherosclerosis. A strongly oxidizing environment induces fragmentation of the polyunsaturated fatty acids of membrane phospholipids.[5] The resulting oxidized phospholipids are structurally similar to PAF and mimic its biological actions.[6,7] The biological actions of both PAF and oxidized phospholipids are abolished by hydrolysis of the *sn*-2 residue, a reaction catalyzed by PAF acetylhydrolase.[8-11] In plasma, PAF acetylhydrolase associates tightly with both low-density lipoprotein (LDL) and high-density lipoprotein,[9,12] and it may serve not only to regulate the plasma concentration of PAF but also to protect LDL from oxidative modification,[13,14] which is thought to be an important step in atherosclerosis.[15,16] Given that PAF acetylhydrolase constitutes a key defense against the biological actions of PAF and oxidized phospholipids, a decrease in its activity may result in pathological conditions such as thrombosis and atherosclerosis.

Tjoelker et al[17] determined the corresponding cDNA sequence of human plasma PAF acetylhydrolase and demonstrated an antiinflammatory effect of the recombinant protein on PAF-induced inflammation in animal models. Recently, Stafforini et al[18] determined the genomic structure for human plasma PAF acetylhydrolase, showing that the gene is located in chromosome 6p12-21.1 and comprises 12 exons spanning at least 45 kilobases of DNA sequence. These researchers also detected a single point mutation (G → T transversion) at nucleotide position 994 in exon 9, which encodes the catalytic domain, in 14 Japanese families with a deficiency of plasma PAF acetylhydrolase activity. This nucleotide change results in a Val → Phe substitution at amino acid residue 279 of the mature protein and is responsible for the loss of catalytic activity. The activity of plasma PAF acetylhydrolase is decreased in children with severe bronchial asthma; the frequency of plasma PAF acetylhydrolase deficiency in children with this condition is threefold that in normal children.[19] However, the role of deficiency or low activity of this enzyme caused by the missense mutation in the etiology of CAD has not been determined.

The present study investigates whether the $G^{994} \rightarrow T$ missense mutation in exon 9 of the plasma PAF acetylhydrolase gene is an independent risk factor for CAD in the Japanese population.

SUBJECTS AND METHODS

Study Population

A total of 1,056 unrelated Japanese subjects (825 men and 231 women) who presented to 14 participating hospitals between July 1994 and July 1995 were entered onto the study. Informed consent was obtained from all subjects. Four hundred fifty-four patients with myocardial infarction ([MI] 373 men and 81 women) all underwent coronary angiography and left ventriculography. The diagnosis of MI was based on typical electrocardiographic changes and increased serum activities of enzymes such as creatinine kinase, aspartate aminotransferase, and lactate dehydrogenase, and was confirmed by the presence of wall-motion abnormality on left ventriculography. The 602 control subjects (452 men and 150 women) were randomly selected from

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individuals presenting to the participating hospitals and were matched with the MI group for age, sex, body mass index (BMI), and other risk factors for CAD, including a history of smoking, hypertension (systolic blood pressure ≥160 mm Hg and/or diastolic blood pressure ≥95 mm Hg), diabetes mellitus (fasting blood glucose ≥140 mg/dL), and hypercholesterolemia (total cholesterol ≥220 mg/dL). They had no history of CAD and had normal resting electrocardiograms and showed no signs of myocardial ischemia in exercise stress tests. We also selected low-risk men, defined as individuals with a BMI less than 27 kg/m^2 and no history of hypertension, diabetes mellitus, or hypercholesterolemia (94 MI patients and 155 control subjects).

Assay for Plasma PAF Acetylhydrolase Activity

Venous blood was collected into a tube containing EDTA (disodium salt, 50 mmol/L) and centrifuged at 1,600 × *g* for 15 minutes at 4°C. Plasma samples were stored at −30°C until assayed. The activity of PAF acetylhydrolase was measured as previously described.[8]

Genotyping of Plasma PAF Acetylhydrolase

For isolation of genomic DNA, 7 mL venous blood was collected into tubes containing EDTA (disodium salt, 50 mmol/L), and after separation of leukocytes, genomic DNA was isolated with a DNA extraction kit (Biologica, Nagoya, Japan). The genotype of plasma PAF acetylhydrolase was determined with an allele-specific polymerase chain reaction (PCR) as previously described.[18] The sense primer (5′-CTATAAATTTATATCATGCTT-3′) was the same for all reactions, and three antisense primers (1, 5′-TTTACTATTCTCTTGCTTTAC-3′; 2, 5′-TCACTAAGAGTCTGAATAAC-3′; and 3, 5′-TCACTAAGAGTC-TGAATAAA-3′) were used. Reactions were performed in a total volume of 50 μL containing 0.5 μg genomic DNA, 20 pmol of each primer, 0.2 mmol/L each of dATP, dGTP, dCTP, and dTTP, 1 U *Taq* DNA polymerase (Takara, Otsu, Japan), 50 mmol/L KCl, 1.5 mmol/L $MgCl_2$, and 10 mmol/L Tris hydrochloride (pH 8.3). The thermocycling procedure consisted of initial denaturation at 94°C for 5 minutes; five cycles of denaturation at 94°C for 1 minute, annealing at 56°C for 1 minute, and extension at 72°C for 1 minute; 30 cycles of 94°C for 30 seconds, 52°C for 30 seconds, and 72°C for 30 seconds; and a final extension at 72°C for 5 minutes. PCR products were analyzed by 2% agarose gel electrophoresis and visualized by ethidium bromide staining. The expected sizes of the PCR products were 160 base pairs (bp) with antisense primer 1 and 108 bp with antisense primers 2 or 3. Because the G → T transversion at nucleotide 994 produces a new restriction site for *Mae*II, genotypes were designated *MM* (normal), *Mm* (heterozygous), and *mm* (homozygous deficient). In some experiments, PCR products obtained with sense and antisense 1 primers were purified and digested with *Mae*II to confirm genotypes.

Statistical Analysis

Clinical laboratory data were compared between MI subjects and controls by unpaired Student's *t* test or the Mann-Whitney *U* test. Allele frequencies were estimated by the gene-counting method, and Hardy-Weinberg equilibrium was confirmed by the chi-square test. Data were compared among PAF acetylhydrolase genotypes by one-way ANOVA and Scheffe's multiple-range test. We performed multivariate logistic regression analysis to adjust risk factors, in which the dependent variable was MI and independent variables were age, BMI, smoking status (0, nonsmoker; 1, smoker), metabolic variables (0, no history of hypertension, diabetes mellitus, or hypercholesterolemia; 1, positive history), and PAF acetylhydrolase genotype (0, *MM;* 1, *Mm* and *mm*). The odds ratio and 95% confidence interval (CI) were also calculated. A *P* value less than .05 was considered statistically significant.

RESULTS

Characteristics of the study population are shown in Table 1. The age, BMI, and incidence of other conventional risk factors for CAD, including history of smoking, hypertension, diabetes mellitus, and hypercholesterolemia, did not differ between subjects with MI and controls for either men or women. The allele-specific PCR assay accurately detected the $G^{994} \rightarrow T$ mutation in the plasma PAF acetylhydrolase gene (Fig 1). There was a significant association between the plasma PAF acetylhydrolase activity and genotype for both men and women in both control and patient groups (Table 2). The plasma activity in individuals with the *MM* genotype significantly exceeded that in individuals with the *Mm* genotype. As expected, no activity was detected in *mm* homozygotes. Plasma activity in male patients with MI was significantly lower than in control subjects. Plasma activity did not differ significantly between men and women or between control subjects and MI patients of the same genotype.

Table 1. Characteristics of the Study Population

Characteristic	Men		Women	
	Control	MI	Control	MI
No. of subjects	452	373	150	81
Age (yr)	56.6 ± 7.7	57.3 ± 7.8	60.4 ± 8.3	62.2 ± 6.5
BMI (kg/m^2)	23.6 ± 2.5	23.5 ± 2.6	23.8 ± 3.2	23.3 ± 3.2
Positive history (%)				
Smoking	70.8	74.3	20.7	27.2
Hypertension	39.6	35.9	54.0	44.4
Diabetes mellitus	16.4	20.1	24.0	32.1
Hypercholesterolemia	40.9	44.8	67.3	66.7

NOTE. Age and BMI are expressed as the mean ± SD.

Among controls, the frequency of the *MM, Mm,* and *mm* genotype was 76.8%, 21.0%, and 2.2%, respectively, in men, and 68.7%, 30.0%, and 1.3%, respectively, in women (Table 3). The genotype distribution was in Hardy-Weinberg equilibrium and did not differ significantly between men and women. Logistic regression analysis revealed that the frequency of the *m* allele was significantly higher in MI subjects than in controls for men, but not for women (Table 3). Association of the *m* allele of the PAF acetylhydrolase gene with MI in men was further investigated in low-risk individuals. In the low-risk group, increased association of the *m* allele with MI was reflected in an odds ratio of 2.3 ($P = .007$; CI, 1.3 to 4.6).

DISCUSSION

The etiology of CAD is multifactorial, with complex interactions between genetic and environmental components. In general, the incidence of CAD shows an additive increase with an increase in the number of conventional risk factors.[20] However, some individuals with CAD exhibit no conventional risk factors. We have now demonstrated association of the *m* allele of the PAF acetylhydrolase gene with MI in Japanese men. Although the frequency of *mm* homozygotes was similar between male controls and patients with MI, the frequency of heterozygotes among male MI patients was significantly higher than among male control subjects. Furthermore, association of the *m* allele with MI in men was more marked in low-risk individuals. These results indicate that the *m* allele is an independent risk factor for CAD in Japanese men, especially those who lack other conventional risk factors.

We failed to detect an association of the *m* allele with MI in women. In Japan, the morbidity of CAD is low in women, especially those who are premenopausal, because they are likely

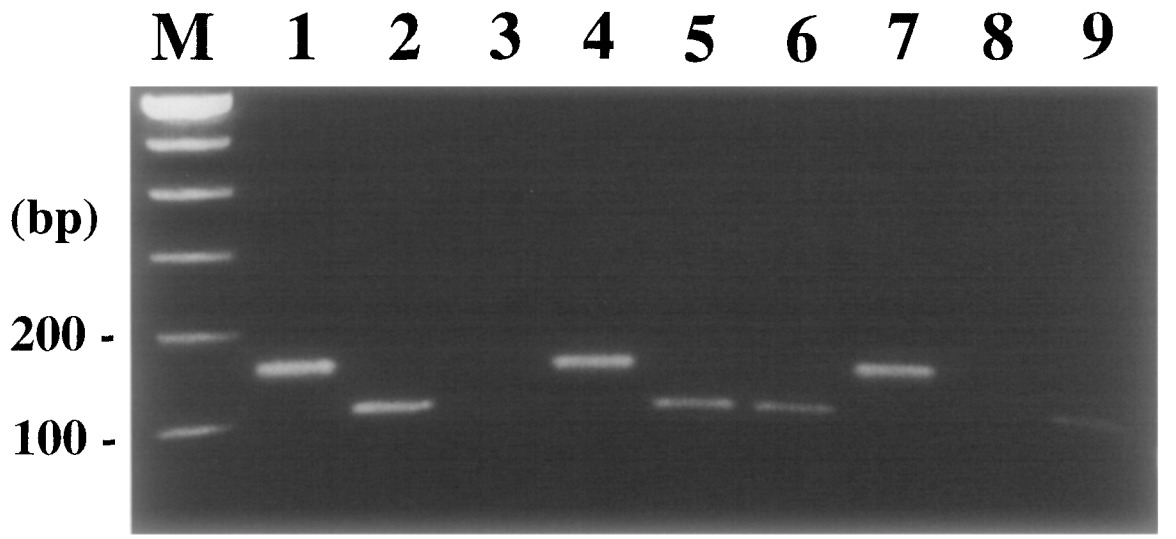

Fig 1. Amplification of the polymorphic region in exon 9 of the plasma PAF acetylhydrolase gene by allele-specific PCR. Lanes 1 to 3, normal (*MM* genotype); lanes 4 to 6, heterozygote (*Mm* genotype); lanes 7 to 9, deficient homozygote (*mm* genotype). PCR products in lanes 1, 4, and 7 correspond to the entire exon 9 (antisense primer 1); products in lanes 2, 5, and 8 correspond to a partial exon 9 product containing the normal sequence (antisense primer 2); and products in lanes 3, 6, and 9 correspond to a partial exon 9 product containing the mutation (antisense primer 3). Lane M, 100-bp ladder of molecular size markers.

protected by the secretion of large amounts of estrogen.[21] In the present study, MI mainly affected postmenopausal women. Our female population may be inappropriate for detection of an association, because the prevalence of MI in Japanese women does not approximate that of men until a much later age or the female sample size examined does not provide adequate power to detect an association. The activity of PAF acetylhydrolase is negatively correlated with the plasma concentration of estrogen.[22] Given that estrogen reduces plasma PAF acetylhydrolase activity in addition to its other antiatherosclerotic effects, its effect on CAD may be greater than that of the $G^{994} \rightarrow T$ mutation in the PAF acetylhydrolase gene.

Our study was performed in Nagoya and adjacent cities, a central area in Japan; the study population was thought to share the same ethnic ancestry and a homogeneous genetic background. Our control group consisted of individuals whose risk factors were matched with those of the MI group. Furthermore, the distribution of PAF acetylhydrolase genotypes in the controls was in Hardy-Weinberg equilibrium, confirming the genetic homogeneity of our study population. Miwa et al[19] detected plasma PAF acetylhydrolase deficiency in 3.9% of 816 healthy Japanese adults, and this condition was inherited in an autosomal recessive manner. Stafforini et al[18] showed that 4% of 127 randomly selected Japanese subjects were *mm* homozygotes. The frequency of *mm* homozygotes in our total study population was 2.0% (2.0% each in controls and MI patients). Our value may differ from those of the two previous studies[18,19] because of the difference in study populations: healthy adults[19] and the general population[18] versus MI patients and controls with conventional risk factors (our study). It is also possible that there are other missense mutations causing a loss of catalytic activity.

A limitation of this study is its retrospective character. The study population actually consisted of survivors of MI, which does not appear representative of all MI cases. The prognosis of MI in Japan is better than in the United States and Europe: MI mortality in Japan is approximately one fifth of that in the United States and approximately one seventh of that in the United Kingdom. Therefore, we believe that a survivor bias is very small in our study population. However, it is possible that

Table 2. Plasma Activity of PAF Acetylhydrolase in Control Subjects and MI Patients According to Genotype

	Men		Women	
Parameter	Control	MI	Control	MI
No. of subjects	211	121	89	45
Age (yr)	57.8 ± 7.7	57.6 ± 7.6	58.8 ± 8.9	62.4 ± 6.4
PAF acetylhydrolase activity (nmol/mL/min)				
Genotype				
MM	33.2 ± 8.8*	31.8 ± 10.5*	32.0 ± 7.5*	32.6 ± 7.0*
Mm	17.7 ± 5.6†	18.5 ± 5.8†	18.9 ± 4.4†	20.3 ± 4.2†
mm	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Mean	29.2 ± 10.8	25.4 ± 12.3	28.1 ± 9.6	28.1 ± 10.3
P	.0035		.99	

NOTE. Age and activity are expressed as the mean ± SD.
*$P < .01$ *v Mm* and *mm*.
†$P < .01$ *v mm*.

Table 3. Distribution of Plasma PAF Acetylhydrolase Genotypes in Control Subjects and MI Patients

	Men				Women			
	Control		MI		Control		MI	
Parameter	No.	%	No.	%	No.	%	No.	%
Genotype								
MM	347	76.8	242	64.9	103	68.7	62	76.5
Mm	95	21.0	123	33.0	45	30.0	17	21.0
mm	10	2.2	8	2.1	2	1.3	2	2.5
P	.0002				.09			
Odds ratio	1.8							
95% CI	1.3-2.5							

the similar frequency of mutant homozygotes between control subjects and individuals with MI was attributable to a high mortality in MI subjects with a deficiency of plasma PAF acetylhydrolase activity.

The pathogenesis of atherosclerosis is complex, with many mediators, including growth factors and cytokines, playing a role.[23] One of the early key processes in the development of atherosclerosis is thought to be oxidative modification of LDL.[15,16,24] Oxidized LDL injures the endothelium directly and induces adherence and migration of monocytes.[15,16] Monocytes infiltrate the endothelium, differentiate into macrophages, and can then become loaded with additional oxidized LDL that is taken up by scavenger receptors.[25] The uncontrolled uptake of oxidized LDL by macrophages results in an increase in the number of foam cells followed by formation of fatty streaks, which are characterized by an accumulation of cells loaded with cholesterol esters.[15,16,23,25,26] PAF is produced by endothelial cells in response to oxidative injury or various physiological agonists, including thrombin, bradykinin, and histamine, and can induce macrophages to produce superoxide anions.[2] Therefore, local synthesis of PAF in segments of the vascular wall undergoing atherosclerotic changes may increase the oxidative modification of LDL, resulting in an amplification of the pathogenic process.[27] PAF acetylhydrolase degrades PAF to biologically inactive lyso-PAF, suggesting that this enzyme protects against PAF-mediated pathological events. This is supported by the observation that recombinant human PAF acetylhydrolase markedly inhibited PAF-induced inflammation in rats.[17] In addition, PAF acetylhydrolase protects LDL from oxidation by hydrolyzing oxidatively fragmented fatty acyl residues from the *sn*-2 position of phospholipids.[10,11,13,14] This action may help to prevent LDL from being recognized by the macrophage scavenger receptor. Thus, PAF acetylhydrolase may play a crucial role in the defense mechanism against atherosclerosis. PAF acetylhydrolase activity in plasma was shown to be increased in patients with atherosclerotic diseases such as MI[28] and ischemic stroke.[29] However, we demonstrated that the enzyme activity in plasma in male patients with MI was significantly lower than in control subjects. There is a difference between the previous studies[28,29] and ours: the other studies compared plasma activity between patients and healthy subjects, whereas we compared activity between MI patients and control subjects with conventional risk factors. It is also possible that the increase in plasma enzyme activity in patients in the previous studies[28,29] is an effect rather than a cause of the atherosclerotic process.

Tew et al[30] proposed that PAF acetylhydrolase exerts two opposite effects in vivo. On one hand, it degrades PAF and therefore would be expected to play an antiinflammatory role; on the other hand, given that it is responsible for the lysophosphatidylcholine content and the monocyte chemoattractant properties of oxidized LDL, its ability to hydrolyze oxidized phospholipids in LDL may confer a proinflammatory role. Among changes that occur during LDL modification, oxidized phosphatidylcholine molecules are generated and then hydrolyzed to lysophosphatidylcholine and oxidized fatty acids,[31] the latter of which derivatize apolipoprotein B-100 and thereby result in altered receptor recognition of the particle.[32] Stafforini et al[13] postulated that intact oxidized phospholipids may remain associated with LDL and react with amino acids on apolipoprotein B-100, given that they are more hydrophobic than the fatty acid products, which are water-soluble and would be readily bound by other serum components such as albumin. Thus, hydrolysis of oxidized phospholipids would actually be beneficial. These researchers showed that hydrolysis of oxidized phospholipids by PAF acetylhydrolase is not necessary for LDL modification and that the catalytic activity of the enzyme actually prevents oxidation of LDL.[13] These observations suggest that PAF acetylhydrolase is not proinflammatory, but acts as an antiinflammatory and antiatherosclerotic enzyme. Our results suggest that reduced plasma PAF acetylhydrolase activity is a risk factor for CAD—in other words, the enzyme has a protective effect against CAD, supporting the results of Stafforini et al.[13]

In conclusion, we demonstrated that the $G^{994} \rightarrow T$ missense mutation in exon 9 of the plasma PAF acetylhydrolase gene is associated with MI in Japanese men. The association of the mutation with MI was more prominent in low-risk individuals. Our results thus indicate that this mutation, which results in a loss of catalytic activity, is an independent risk factor for CAD in Japanese men and that the determination of PAF acetylhydrolase genotype or enzyme activity in plasma may contribute to the prevention and management of CAD, especially in men who lack conventional risk factors.

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REFERENCES

1. Marenberg ME, Risch N, Berkman LF, et al: Genetic susceptibility to death from coronary heart disease in a study of twins. N Engl J Med 330:1041-1046, 1994

2. Zimmerman GA, Prescott SM, McIntyre TM: Platelet-activating factor: A fluid-phase and cell-associated mediator of inflammation, in Gallin JI, Goldstein IM, Snyderman R (eds): Inflammation: Basic Principles and Clinical Correlates. New York, NY, Raven, 1992, pp 149-176

3. Honda Z, Nakamura M, Miki I, et al: Cloning by functional expression of platelet-activating factor receptor from guinea-pig lung. Nature 349:342-346, 1991

4. Mueller HW, Haught CA, McNatt JM, et al: Measurement of platelet-activating factor in a canine model of coronary thrombosis and in endarterectomy samples from patients with advanced coronary artery disease. Circ Res 77:54-63, 1995

5. Patel KD, Zimmerman GA, Prescott SM, et al: Novel leukocyte agonists are released by endothelial cells exposed to peroxide. J Biol Chem 267:15168-15175, 1992

6. Smiley PL, Stremler KE, Prescott SM, et al: Oxidatively fragmented phosphatidylcholines activate human neutrophils through the receptor for platelet-activating factor. J Biol Chem 266:11104-11110, 1991

7. Heery JM, Kozak M, Stafforini DM, et al: Oxidatively modified LDL contains phospholipids with platelet-activating factor–like activity and stimulates the growth of smooth muscle cells. J Clin Invest 96:2322-2330, 1995

8. Stafforini DM, Prescott SM, McIntyre TM: Human platelet-activating factor acetylhydrolase: Purification and properties. J Biol Chem 262:4223-4230, 1987

9. Stafforini DM, McIntyre TM, Carter ME, et al: Human plasma platelet-activating factor acetylhydrolase: Association with lipoprotein particles and role in the degradation of platelet-activating factor. J Biol Chem 262:4215-4222, 1987

10. Stremler KE, Stafforini DM, Prescott SM, et al: An oxidized derivative of phosphatidylcholine is a substrate for the platelet-activating factor acetylhydrolase from human plasma. J Biol Chem 264:5331-5334, 1989

11. Stremler KE, Stafforini DM, Prescott SM, et al: Human plasma platelet-activating factor acetylhydrolase: Oxidatively fragmented phospholipids as substrates. J Biol Chem 266:11095-11103, 1991

12. Yamada Y, Stafforini DM, Imaizumi T, et al: Characterization of the platelet-activating factor acetylhydrolase from human plasma by heterologous expression in *Xenopus laevis* oocytes. Proc Natl Acad Sci USA 91:10320-10324, 1994

13. Stafforini DM, Zimmerman GA, McIntyre TM, et al: The platelet-activating factor acetylhydrolase from human plasma prevents oxidative modification of low-density lipoprotein. Trans Assoc Am Physicians 105:44-63, 1992

14. Watson AD, Navab M, Hama SY, et al: Effect of platelet activating factor-acetylhydrolase on the formation and action of minimally oxidized low density lipoprotein. J Clin Invest 95:774-782, 1995

15. Steinberg D, Parthasarathy S, Carew TE, et al: Beyond cholesterol: Modifications of low-density lipoprotein that increase its atherogenicity. N Engl J Med 320:915-924, 1989

16. Witztum JL, Steinberg D: Role of oxidized low density lipoprotein in atherogenesis. J Clin Invest 88:1785-1792, 1991

17. Tjoelker LW, Wilder C, Eberhardt C, et al: Anti-inflammatory properties of a platelet-activating factor acetylhydrolase. Nature 374:549-553, 1995

18. Stafforini DM, Satoh K, Atkinson DL, et al: Platelet-activating factor acetylhydrolase deficiency: A missense mutation near the active site of an anti-inflammatory phospholipase. J Clin Invest 97:2784-2791, 1996

19. Miwa M, Miyake T, Yamanaka T, et al: Characterization of serum platelet-activating factor (PAF) acetylhydrolase: Correlation between deficiency of serum PAF acetylhydrolase and respiratory symptoms in asthmatic children. J Clin Invest 82:1983-1991, 1988

20. Nora JJ, Lortscher RH, Spangler RD, et al: Genetic-epidemiologic study of early-onset ischemic heart disease. Circulation 61:503-508, 1980

21. Guetta V, Cannon RO III: Cardiovascular effects of estrogen and lipid-lowering therapies in postmenopausal women. Circulation 93:1928-1937, 1996

22. Miyaura S, Maki N, Byrd W, et al: The hormonal regulation of platelet-activating factor acetylhydrolase activity in plasma. Lipids 26:1015-1020, 1991

23. Ross R: The pathogenesis of atherosclerosis: A perspective for the 1990s. Nature 362:801-809, 1993

24. Regnström J, Nilsson J, Tornvall P, et al: Susceptibility to low-density lipoprotein oxidation and coronary atherosclerosis in man. Lancet 339:1183-1186, 1992

25. Brown MS, Goldstein JL: Lipoprotein metabolism in the macrophage: Implications for cholesterol deposition in atherosclerosis. Annu Rev Biochem 52:223-261, 1983

26. Stary HC, Chandler AB, Dinsmore RE, et al: A definition of advanced types of atherosclerotic lesions and a histological classification of atherosclerosis: A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. Arterioscler Thromb Vasc Biol 15:1512-1531, 1995

27. Imaizumi T, Stafforini DM, Yamada Y, et al: Platelet-activating factor: A mediator for clinicians. J Intern Med 238:5-20, 1995

28. Osterman G, Lang A, Holtz H, et al: The degradation of platelet-activating factor in serum and its discriminative value in atherosclerotic patients. Thromb Res 52:529-540, 1988

29. Satoh K, Yoshida H, Imaizumi T, et al: Platelet-activating factor acetylhydrolase in plasma lipoproteins from patients with ischemic stroke. Stroke 23:1090-1092, 1992

30. Tew DG, Southan C, Rice SQJ, et al: Purification, properties, sequencing, and cloning of a lipoprotein-associated, serine-dependent phospholipase involved in the oxidative modification of low-density lipoproteins. Arterioscler Thromb Vasc Biol 16:591-599, 1996

31. Steinbrecher UP, Parthasarathy S, Leake DS, et al: Modification of low density lipoprotein by endothelial cells involves lipid peroxidation and degradation of low density lipoprotein phospholipids. Proc Natl Acad Sci USA 81:3883-3887, 1984

32. Steinbrecher UP, Lougheed M, Kwan W-C, et al: Recognition of oxidized low density lipoprotein by the scavenger receptor of macrophages results from derivatization of apolipoprotein B by production of fatty acid peroxidation. J Biol Chem 264:15216-15223, 1989