ORIGINAL ARTICLE

The association between the insertion/deletion polymorphism of the angiotensin-converting enzyme gene and the plasma fibrinogen level in women and men with premature coronary artery atherosclerosis

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ABSTRACT

INTRODUCTION   The insertion/deletion (I/D) polymorphism of the angiotensin-converting enzyme (*ACE*) gene is associated with younger age at coronary artery disease (CAD) onset. Some data indicate the relationship between the DD genotype and the fibrinogen level. At the same time, the regulation of the renin–angiotensin system differs in women and men.

OBJECTIVES   The objective of the study was to evaluate the sex-dependent association of the *ACE* I/D polymorphism with the plasma fibrinogen level in patients with premature CAD.

PATIENTS AND METHODS   The study included 407 participants with premature CAD: 257 women not older than 55 years and 150 men not older than 45 years. Study participants had at least 1 stenosis ≥50% in a major epicardial coronary artery. The *ACE* I/D polymorphism (rs4343) was genotyped using polymerase chain reaction. Fibrinogen levels were measured with a modified Clauss method. We found a significant interaction indicating that sex modifies the influence of the I/D polymorphism of the *ACE* gene on fibrinogen levels ($P = 0.02$). The highest mean fibrinogen level, adjusted for age and smoking status, was observed in women with the DD genotype (575.7 mg/dl) and it was significantly higher than in men with the DD genotype (367.1 mg/dl; $P <0.001$) or in women with the ID genotype (491.7 mg/dl; $P = 0.04$). In men, there was no significant difference in mean adjusted fibrinogen levels across genotypes.

CONCLUSIONS   The DD genotype of the *ACE* gene was associated with higher plasma fibrinogen levels in women with premature CAD yet not in men.

INTRODUCTION   Coronary artery disease (CAD) is a multifactorial condition.[1-3] Genetic risk factors may play a significant role in premature CAD.[4]

One of the genetic factors that may be associated with the increased risk of atherosclerosis is the insertion/deletion (I/D) polymorphism located in the noncoding region of the angiotensin--converting enzyme (*ACE*) gene.[5] Interestingly, the DD genotype of the *ACE* gene was found

more frequently in younger patients with CAD.[6] The D allele is associated with a doubling of the mean plasma level of ACE in DD homozygotes relative to II homozygotes.[7] At the same time, the ID heterozygotes carry an intermediate plasma level of ACE.[7] No sex-related differences in these associations were reported.[7] A high level of ACE may increase atherogenesis by excessive conversion of angiotensin I into angiotensin II,

WHAT'S NEW?

This is the first study on the association of the insertion / deletion polymorphism of the angiotensin-converting enzyme (*ACE*) gene with the fibrinogen level in patients with premature coronary artery atherosclerosis. Moreover, we observed a strong interaction indicating that sex modifies this association. Our study shows that the DD genotype is significantly associated with higher plasma fibrinogen levels in women with premature coronary artery disease (CAD) yet not in men. These findings shed new light on the complexity of CAD as well as possible, though not investigated yet, regulatory mechanisms that may influence CAD progression differently in women and men. Our study opens a discussion on potential new differences in the pathophysiology of premature atherosclerosis in women and men.

which stimulates endothelial dysfunction and atherosclerotic plaque growth.[8] It also leads to increased synthesis of inflammatory agents, which may cause increased fibrinogen production.[9-11] It is known that high plasma fibrinogen levels enhance atherosclerotic plaque formation and thrombosis.[12-14] Fibrinogen has been found to have an additive effect on cardiovascular risk.[15,16]

Currently, synergistic interactions of the DD genotype with different cardiovascular risk factors (eg, environmental and genetic determinants or sex) are thought to hold the most specific risk.[5,17] The latest study showed an increased risk of ischemic stroke associated with an interaction of the DD genotype and the T148C polymorphism of the β-fibrinogen gene.[18] However, the differences between men and women were not analyzed.[18]

Although there are some differences in the frequency of classical risk factors between women and men with premature CAD, pathophysiological sex-related differences in premature atherosclerosis remain unclear.[19] However, several regulation patterns of the renin–angiotensin system differ between women and men.[20,21] Moreover, sex-dependent genetic architecture may play a significant role in the development of numerous sex-specific diseases.[22] Interestingly, a recent study reported on the association of fibrinogen levels with coronary artery atherosclerosis only in women, not in men.[23]

The objective of the present study was to evaluate the association of the *ACE* I/D polymorphism with the plasma fibrinogen level according to sex in patients with premature CAD.

PATIENTS AND METHODS The study population included 407 participants with premature CAD. There were 257 consecutively sampled women with CAD onset before or at the age of 55 years. The group of men consisted of 150 consecutively sampled participants with CAD onset before or at the age of 45 years.

Women were sampled from the Premature Coronary Artery Disease in Women—Risk Factors and Prognosis (PRECADIW) study held at the National Institute of Cardiology, Warsaw, Poland, between January 2004 and April 2007. Men were sampled from a local registry of men with premature CAD (December 2005 to April 2007). These projects were designed to assess risk factors for premature CAD in women and men.[24] Premature CAD was defined as that occurring before or at the age of 55 years in women and before or at the age of 45 years in men.[25] Both women and men with premature CAD had at least 1 stenosis ≥50% in a major epicardial coronary artery, confirmed by coronary angiography. Patients included in the study group had their *ACE* I/D polymorphism (rs4343) genotyped. All participants were Caucasian.

The study participants were asked to complete structured questionnaires, and physical examinations were performed in the same manner for both women and men.[24] Fasting blood samples were drawn from every individual and collected for biochemical analyses. Lipid and glucose levels were measured in a single laboratory certified by the Center for Disease Control—Lipid Standardization Program in Atlanta, United States, and Randox International Quality Assessment Scheme. Plasma fibrinogen levels were measured with a modified Clauss method.[26] The list of reagents used for the laboratory tests is enclosed in the Supplementary material.

Genotyping of the I/D polymorphism of the *ACE* gene (rs4343) was performed by polymerase chain reaction (PCR), using the 5'CTGG AGACCACTCCCATCCTTTCT3' sense starter and then the 5'GATGTGGCCATCACATTCGTCAGT3' antisense starter. The fragments of DNA were then electrophoretically separated using 2% agarose gel.[27]

The institutional review board and local bioethics committee approved the study protocol (decision no., IK-NP-0021-87/851/04 and 1456). Each study participant provided 2 informed consents: one to take part in the study and another to have genetic tests performed.

We presented the definitions of cardiovascular risk factors elsewhere.[24] In brief, hypertension was defined as systolic blood pressure ≥140 mm Hg, diastolic blood pressure ≥90 mm Hg, or the use of antihypertensive drugs. Cigarette smoking was regarded as chronic if it started at least 12 months before study enrolment. The definition of hypercholesterolemia included a history of high cholesterol levels, statin and / or fibrate treatment before hospital admission, or a fasting cholesterol level ≥5.2 mmol/l. Diabetes was defined based on a previous medical diagnosis, a fasting glucose level ≥126 mg/dl, or the use of insulin or an oral hypoglycemic or an insulin-sensitizing agent. The conditions for a family history of cardiovascular disease included a history of myocardial infarction or stroke in a patient's parent.

The age of menopause was defined as the age of the last menstruation and lack of menstrual bleeding ≥12 months or the age of hysterectomy and oophorectomy.[28] Menopause was further subdivided into early postmenopause (up to 3 years) and late postmenopause (beyond 3 years).[29,30]

TABLE 1 Clinical characteristics of women and men with premature coronary artery disease

Parameter	Study group (n = 407)		P value
	Women (n = 257)	Men (n = 150)	
Age, y, mean (SD)	49.5 (4.6)	41.4 (4.7)	<0.001
BMI, kg/m², mean (SD)	27.8 (5.1)	28.2 (4.4)	0.48
Hypertension, n (%)	173 (67.3)	67 (44.7)	<0.001
Diabetes, n (%)	43 (16.7)	11 (7.3)	0.006
Smoking, n (%)	186 (72.4)	117 (78)	0.24
Hypercholesterolemia, n (%)	209 (81.3)	120 (80)	0.79
Family history of cardiovascular diseases, n (%)	54 (21)	24 (16)	0.24
Menopause ≤3 yrs, n (%)	78 (30.35)	–	–
Menopause >3 yrs, n (%)	97 (37.74)	–	–
Acute coronary syndrome, n (%)	164 (61)	105 (67.7)	0.18
STEMI, n (%)	96 (35.7)	61 (39.4)	0.47
Plasma fibrinogen level, mg/dl, mean (SD)	534.48 (180.9)	391.55 (161.7)	<0.001
White blood cells, × 10³/μl, median (IQR)	9.9 (7–12.2)	11.4 (8.6–14.4)	<0.001
High-sensitive CRP, mg/dl, median (IQR)	0.23 (0.11–0.55)	0.19 (0.1–0.38)	0.28

Abbreviations: BMI, body mass index; CRP, C-reactive protein; IQR, interquartile range; STEMI, ST-segment elevation myocardial infarction

Statistical analysis Unless otherwise specified, normally distributed continuous data were presented as mean (SD), and variables without normal distribution as median (interquartile range). The *t* test, Mann–Whitney test, or Kruskal–Wallis test were used to search for significant differences in baseline characteristics expressed as the quantitative data. The χ^2 test or the Fisher exact test were applied to categorical data. Two-way and 3-way analysis of covariance (ANCOVA) were used to evaluate the relationship between the continuous dependent variable and 1 or more of the independent variables. The Bonferroni correction (adj=Bonferroni in the GLM procedure) was applied to all comparisons if there were more than 3 comparators.

The best subject-level gene model among 4 basic models (recessive, multiplicative, dominant, or overdominant) was calculated as described elsewhere.[31] To calculate odds ratios (ORs) for the original number of cases, we dichotomized fibrinogen as the continuous variable using the median value as the most appropriate one[32] and performed binary univariable and multivariable logistic regression. One-way analysis of variance (ANOVA) was used to calculate the statistical power of the sample size.

A *P* value less than or equal to 0.05 was considered significant. The statistical analysis was performed using the SAS 9.4 (SAS Inc., Cary, North Carolina, United States) and SPSS (SPSS Inc., Chicago, Illinois, United States) software.

RESULTS Clinical characteristics The mean (SD) age of women and men was 49.5 (4.6) years and 41.4 (4.7) years, respectively (*P* <0.001). Hypertension and diabetes were more common in women than in men (*P* <0.001 and *P* = 0.006, respectively). There were no statistical differences between women and men regarding the prevalence of other classical risk factors for CAD, acute coronary syndrome, and ST-segment elevation myocardial infarction. The baseline characteristics of the study group are presented in **TABLE 1**.

The I/D polymorphism of the *ACE* gene and classical cardiovascular risk factors The genotype distribution in the study group followed the Hardy–Weinberg equilibrium: II, 22.1% (90/407); ID, 47.7% (194/407); DD, 30.2% (123/407) (*P* = 0.41).

A significant difference was found in women, showing a higher frequency of a family history of cardiovascular disease in women with the DD genotype (32%) versus the ID genotype (16.1%) and the II genotype (16.4%) (*P* = 0.02).

In men, patients with the II genotype were younger (mean [SD] age, 39.1 [5.7] years) than men with the ID genotype (mean [SD] age, 41.6 [4.7] years) and the DD genotype (mean [SD] age, 42.6 [3.4] years) (*P* = 0.006). There were no other differences in risk factor distribution across genotypes in women and men (Supplementary material, *Tables S1* and *S2*).

Fibrinogen, the I/D polymorphism of the *ACE* gene, and sex differences in patients with premature coronary artery disease The mean (SD) plasma fibrinogen level was higher in women than in men (534.5 [180.9] mg/dl vs 391.6 [161.7] mg/dl; *P* <0.001) (**TABLE 1**). By contrast, leukocytosis, another parameter of inflammation, was more pronounced in men than in women (median [interquartile range] white blood cell count, 11.4 [8.6–14.4] × 10³/μl vs 9.2 [7–12.2] × 10³/μl; *P* <0.001). On the other hand, the median high-sensitivity C-reactive protein level was not elevated (**TABLE 1**).

The mean fibrinogen level was found to be higher in women with the DD genotype than in those with the ID genotype (572.2

TABLE 2 Significant differences in mean fibrinogen levels across genotypes in women and men

Sex	Fibrinogen, mg/dl, mean (SD)			Difference between mean fibrinogen levels, mg/dl (95% CI)				
	II	ID	DD	ID vs DD	II vs ID	RE 'II' vs 'ID + DD'	DO 'II + ID' vs 'DD'	OV 'ID' vs 'II + DD'
Female	523.7 (152)	515.1 (185.7)	572.2 (190)	−57.1 (−111.1 to −3.1)	8.5 (−45 to 63)	−14.2 (−66.5 to 38.1)	−54.2 (−102 to −6.3]	−35.8 (−80.3 to 8.7)
	518 (174.6)		572.2 (190)					
	523.7 (152)	537.86 (189)						
	II + DD = 550.9 (175.7)							
	P value			0.038	0.76	0.59	0.027	0.11
	Adjusted for age and smoking status[a]			−57.6 (−111.1 to 4.1)	33.5 (−19.7 to 86.7)	−1.4 (53.7–50.8)	−50.5 (−97.7 to −3.2)[b]	−42.4 (−86.4 to 1.5)
	P value			0.035	0.21	0.96	0.036	0.06
Male	375.1 (162.5)	418.1 (163.9)	357.3 (152.8)	60.8 (1.3–120.4)	−43 (−113.8 to 27.8)	−20.4 (−86.6 to 45.8)	49 (−7.6 to 105.5)	53.8 (2.2–105.5)
	406.24 (163.87)		357.3 (152.8)					
	375.1 (162.5)	395.49 (162)						
	II + ID = 364.3 (155.8)							
	P value			0.045	0.23	0.54	0.09	0.041
	Adjusted for age and smoking status[a]			53.1 (−5.9 to 112.2)	−64.7 (−135.6 to 6.2)	−40.7 (−108.1 to 26.6)	40 (−17.1 to 97)	56.4[c] (5.4–107.4)
	P value			0.08	0.07	0.23	0.17	0.03

a 2-way analysis of covariance

b 3-way analysis of covariance for the dominant model in women; dominant model $P = 0.002$; factors: smoking ($P = 0.001$), DD genotype ($P = 0.036$); covariant: age ($P = 0.95$)

c 3-way analysis of covariance for the overdominant model in men; overdominant model $P = 0.018$; factors: ID genotype ($P = 0.03$); smoking status ($P = 0.69$); covariant: age ($P = 0.017$)

Abbreviations: RE, recessive; DD, dominant homozygote of the insertion / deletion polymorphism of the angiotensin-converting enzyme gene; DO, dominant; ID, heterozygote; II, recessive homozygote; OV, overdominant

[190.5] mg/dl vs 515.1 [185.7] mg/dl; $P = 0.04$) or in the combined group of those with the ID and II genotypes (vs 518.03 [174.6] mg/dl; $P = 0.03$). In men, the mean fibrinogen level was the highest in those with the ID genotype compared with men with the DD genotype (418.11 [163.89] mg/dl vs 357.3 [155.8] mg/dl; $P = 0.045$) and the combined group of men with the DD and II genotypes (vs 364.3 [155.8] mg/dl; $P = 0.04$) (TABLE 2).

A higher mean (SD) fibrinogen level was also observed in smoking women compared with those who did not smoke (557.28 [183.88] mg/dl vs 474.76 [159.18] mg/dl; $P = 0.001$) (Supplementary material, Table S3). There were no other significant differences in fibrinogen concentration regarding other cardiovascular risk factors (Supplementary material, Table S3).

Interaction of the I/D polymorphism of the *ACE* gene and sex modifying the fibrinogen level   We found a significant interaction indicating that sex modifies the relationship between the I/D polymorphism of the *ACE* gene and the plasma fibrinogen level. In the 2-way ANCOVA including 3 genotypes and sex, we found that sex is significantly associated with the fibrinogen level ($P < 0.001$). Furthermore, the significant interaction sex*genotypes of the I/D polymorphism, associated with the fibrinogen level, was also observed ($P = 0.02$) (Supplementary material, Table S4).

In different models, only smoking was found to be significantly associated with the fibrinogen level ($P = 0.03$); however, there was no interaction with the I/D genotypes (Supplementary material, Table S4).

In the 3-way ANCOVA including all significant factors with age as a covariate, we confirmed that sex ($P < 0.001$), smoking status ($P = 0.03$), and sex interactions with the I/D genotypes ($P = 0.02$) were significantly associated with the fibrinogen level. No interaction between smoking and the I/D genotypes was observed ($P = 0.06$) (Supplementary material, Table S4).
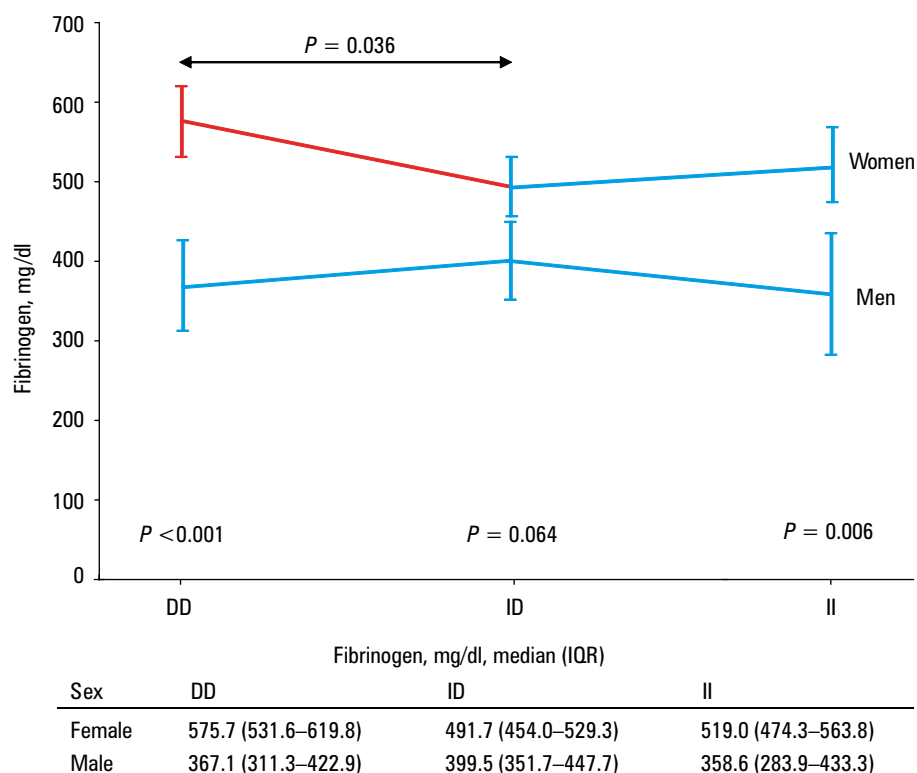
The adjusted fibrinogen level and its variations across the insertion/deletion genotypes of the *ACE* gene   According to the results of ANCOVA, the fibrinogen level in women and men was adjusted for age and smoking status. Adjusted mean fibrinogen levels are shown in FIGURE 1.

Fibrinogen, mg/dl, median (IQR)			
Sex	DD	ID	II
Female	575.7 (531.6–619.8)	491.7 (454.0–529.3)	519.0 (474.3–563.8)
Male	367.1 (311.3–422.9)	399.5 (351.7–447.7)	358.6 (283.9–433.3)

The observed interaction of sex with the I/D genotypes was primarily dependent on the DD genotype and secondarily on the ID genotype (**FIGURE 1**). The highest mean adjusted fibrinogen level was confirmed in women with the DD genotype (575.7 mg/dl), and it was significantly higher than in men with any of the analyzed genotypes: DD (367.1 mg/dl; $P < 0.001$), ID (399.5 mg/dl; $P < 0.001$), and II (358.6 mg/dl; $P < 0.001$), as well as in women with the ID genotype (491.7 mg/dl; $P = 0.036$).

The adjusted fibrinogen level in women with the II genotype (519 mg/dl) was also significantly higher than in men with the II (358.6 mg/dl; $P = 0.01$), ID (399.5 mg/dl; $P = 0.01$), and DD (367.1 mg/dl; $P = 0.001$) genotypes (Supplementary material, *Table S5*). The difference in adjusted mean fibrinogen levels between women and men with the ID genotypes was insignificant (491.7 mg/dl vs 399.5 mg/dl; $P = 0.06$).

In men, there was no significant difference in mean adjusted fibrinogen levels across various genotypes (Supplementary material, *Table S5*).

The optimal subject-level gene model The dominant model turned out to be the best gene model in both women and men (Supplementary material, *Table S6* and *Figure S1*). In women, the ID and II genotypes decreased the risk of presenting the fibrinogen level below the median value (OR, 0.42; 95% CI, 0.24–0.73), which is synonymous with a more than doubled risk of fibrinogen levels above the median value in women with the DD genotype (OR, 2.38; 95% CI, 1.37–4.17) (Supplementary material, *Table S6*). These results were consistent with a significant difference in mean fibrinogen levels between women with the DD

and non-DD genotypes (54.2 mg/dl; $P = 0.027$), as well as after adjustment for age and smoking status (50.5 mg/dl; $P = 0.036$) (**TABLE 2**).

In men, a slight inconsistency between the results of dichotomous and continuous levels of fibrinogen was observed. First, in men, the result was opposite to that seen in women: the ID and II genotypes more than doubled the risk of a fibrinogen level above the median value (OR, 2.32; 95% CI, 1.13–4.78; $P = 0.02$). However, when mean fibrinogen levels in study participants with the DD genotype and non-DD genotypes were compared, the difference was nonsignificant (49 mg/dl; $P = 0.09$) (**TABLE 2**).

On the other hand, we observed a significant difference in mean fibrinogen levels between men with the ID genotype and with the non-ID genotype (53.8 mg/dl; $P = 0.04$). The results were also significant after adjustment for age and smoking status (**TABLE 2**). Therefore, in the analysis assessing the best subject-level gene model, the overdominant model also showed significance (OR, 1.91; $P = 0.05$) (Supplementary material, *Table S6* and *Figure S1*).

The statistical power of the study groups For 1-way ANOVA with 3 levels of genotype, the statistical power of the female sample size was 0.65, and that of the male subgroup, 0.58.

DISCUSSION In this study, we found that the mean fibrinogen level in patients with premature CAD was higher in women than in men. The DD genotype was related to higher fibrinogen levels in women yet not in men. On the other hand, the mean fibrinogen level in men was found to be highest in patients with the ID genotype.

These relationships may suggest another difference in the pathogenetic mechanisms contributing to premature coronary atherosclerosis in women and men.

Sex differences and the association between the insertion/deletion polymorphism of the *ACE* gene, coronary artery disease, and fibrinogen levels The vast majority of studies on cardiovascular diseases have not analyzed sex differences among patients.[19,33] It is a consequence of the underrepresentation of women in most clinical trials.[19,33] In our study, however, the number of women with premature CAD was higher than that of men. Interestingly, our study revealed a significant interaction of the I/D polymorphism of the *ACE* gene with sex, which influenced the fibrinogen level in patients with premature CAD. Smoking and age were other risk factors that had an impact on the fibrinogen level.

The observed mean fibrinogen level was significantly higher in women than in men. This finding is consistent with previous studies, which indicated higher plasma fibrinogen levels in women compared with men.[34] However, the pathophysiological mechanism of this finding has not been elucidated yet. In our study group, women were older than men and showed a higher prevalence of hypertension and diabetes, which also may be associated with higher fibrinogen levels.[12,34] Additionally, the level of fibrinogen was significantly higher in the group of smoking women than in those nonsmoking, yet not in men.

A higher plasma fibrinogen level was most pronounced in women with the DD genotype. The DD genotype more than doubled the risk of presenting a fibrinogen level above the median value in women.

The fibrinogen level in men, adjusted for age and smoking status, was similar across genotypes (DD vs ID, DD vs II, ID vs II) (FIGURE 1, TABLE 2). A significant difference in the adjusted fibrinogen level in the study patients with the ID genotype was observed only when compared with the combined group of men with the DD and II genotypes (TABLE 2). Therefore, the overdominant model was also significant in the group of men (Supplementary material).

Nowadays, sex is widely recognized as a biological variable.[35] Although the sample size of the male subgroup was slightly underpowered in our study, our data may suggest that excluding sex from the analysis can lead to false conclusions about the nature of the association between the *ACE* I/D polymorphism and fibrinogen levels in the whole study group.

Novel findings in the group of women may be explained by sex-specific regulation and influence of the I/D polymorphism on physiological processes. Previous studies have shown that women, compared with men, are characterized by lower plasma renin activity.[20,21] Therefore, in women, other cardiovascular risk factors may synergistically increase the pathological influence of the DD

genotype on atherosclerosis development. Fibrinogen may be one of those factors and, by stimulating local inflammation in a vessel wall, it may increase renin activity and the angiotensinogen level in women.[14]

On the other hand, the DD genotype may elevate the fibrinogen level by increasing renin–angiotensin system activity, which stimulates inflammation and inhibits tissue plasminogen activator (tPA) and fibrin degradation (FIGURE 2).

Angiotensin II stimulates the synthesis of the plasminogen activator inhibitor that impedes tPA.[36,37] Additionally, ACE causes lysis of bradykinin, which also lowers the level of tPA.[38] These changes lead to the inhibition of fibrin degradation. As a result, the conversion of fibrinogen to fibrin is diminished (FIGURE 2). Therefore, the level of fibrinogen may be higher in persons with the DD genotype of the *ACE* gene.

Our findings regarding the different role of fibrinogen in the development of atherosclerosis in women are consistent with a recent study in which fibrinogen levels correlated with coronary atherosclerosis only in women, not in men.[24] Additionally, women with coronary plaque had lower fibrin clot lysability than women without coronary atherosclerosis.[23]

Different findings in men may be caused by the group's low statistical power. Additionally, as the I/D polymorphism of the *ACE* gene is suspected to play a regulatory role, its association with other traits or diseases may not be as regular as in the case of functional polymorphisms. However, this result may be interesting in the context of a previous study that reported a higher risk of CAD in patients with at least 1 copy of the D allele.[39] This study, however, did not examine sex-related differences.

Familial premature coronary artery disease and genetic risk factors Family history is a well-established risk factor for cardiovascular disease, especially in patients with premature CAD.[40] Some genetic risk factors, including the DD genotype of the *ACE* gene, were found more frequently in younger patients with CAD.[41] In a 1-family study assessing several genes associated with CAD, the DD genotype of the *ACE* gene was reported in premature familial CAD in men.[42] At the same time, there was no mutation of the β-fibrinogen gene in those young men with premature familial CAD.[42]

In our study, a higher frequency of the family history of cardiovascular disease was observed in women with the DD genotype. At the same time, women with this genotype had a higher mean fibrinogen level than that observed in men. This may be an additional argument supporting the hypothesis that the DD genotype of the *ACE* gene may have a more considerable impact on CAD development in women with a high fibrinogen level. According to our data, it is possible that observed higher levels of fibrinogen were caused by the polymorphisms of the fibrinogen gene (which was not assessed in our study). This hypothesis
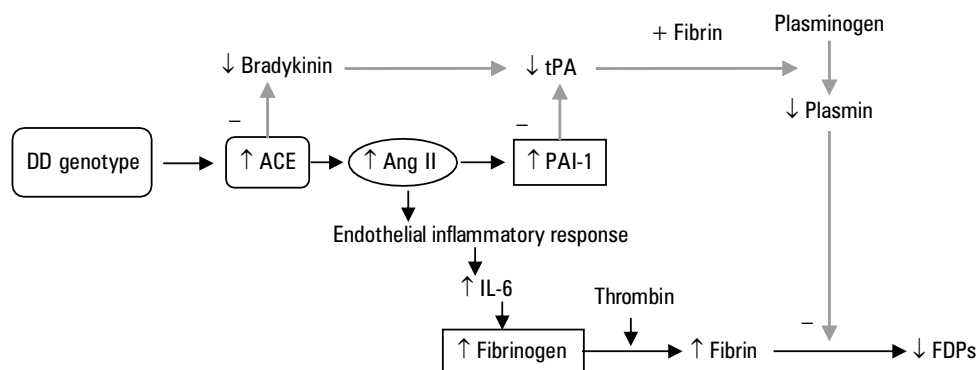
FIGURE 2 Influence of the DD genotype on hemostasis and fibrinogen levels

Abbreviations: ACE, angiotensin-converting enzyme; +, addition; Ang II, angiotensin II; ↓, decrease; FDPs, fibrinogen degradation products; IL-6, interleukin 6; ↑, increase; –, inhibition; PAI-1, plasminogen activator inhibitor 1; tPA, tissue plasminogen activator; others, see **FIGURE 1**

may be supported by the higher prevalence of comorbidities in hospitalized women compared with men.[35] Moreover, it has been shown that women carry more rare copy number variants, with a significantly greater number of affected genes, than men do.[36] In this context, the coexistence of high fibrinogen levels with the DD genotype may increase the risk of premature CAD by way of synergy, just as it has been reported for the DD genotype and β-fibrinogen 148CC or 148CT genotypes in the case of stroke.[18]

Fibrinogen levels and cardiovascular events: controversies regarding the causal role of fibrinogen in coronary artery disease Some controversy exists as to whether fibrinogen is only a marker of an ongoing inflammatory process stimulating atherosclerosis development and progression or is also a pathogenetic factor in atherosclerosis itself.[12] In our study, mean high-sensitivity C-reactive protein levels were not elevated and similar in women and men. Therefore, higher fibrinogen levels did not result from inflammation. Factors including diabetes, hypertension, smoking status, age, female sex, or menopause may be associated with increased fibrinogen levels.[12] In our study, hypertension and diabetes were observed more frequently in women than in men. This could be associated with women's older age. However, there was no difference in the prevalence of hypertension and diabetes across genotypes.

Congenital fibrinogen disorders are a heterogeneous group of abnormalities, mainly causing bleeding complications due to genetically conditioned low fibrinogen levels or defects in the fibrinogen structure.[43] However, paradoxical thrombosis, including stroke, was observed.[43] Potentially, this mechanism may also contribute to premature myocardial infarction. On the other hand, there are polymorphisms of the β-fibrinogen gene that cause higher plasma fibrinogen levels.[44]

Higher fibrinogen levels were found in patients with atherosclerosis.[45] Interestingly, a meta-analysis that evaluated 52 prospective studies revealed that assessing the fibrinogen level in people at intermediate risk for cardiovascular events would prevent 1 additional event over 10 years for every 400 to 500 people screened.[15]

Fibrinogen may stimulate acute cardiovascular events by increasing blood viscosity and platelet aggregation. Also, it was found to cumulate in vessel walls and facilitate the accumulation of LDL cholesterol and the development of atherosclerosis.[12]

Other factors may enhance the role of fibrinogen in atherosclerosis, such as a low level of serum albumins, which also increases blood viscosity and induces endothelial dysfunction.[46] In patients with ST-segment elevation myocardial infarction, a high fibrinogen-to-albumin ratio has been associated with more severe coronary artery atherosclerosis.[46] Leukocytosis has also been related to increased fibrin deposition in blood vessels.[47] In our study, median leukocytosis was higher in men than in women. Therefore, the potential interference of leukocytosis with fibrinogen levels was of no concern in the group of women.

Our study was the first to evaluate the association between the I/D polymorphism of the *ACE* gene and plasma fibrinogen levels in premature CAD. Previously, only 1 study reported on higher plasma fibrinogen levels in patients with untreated hypertension and the DD genotype compared with those with the ID or II genotypes.[48]

Gene expression and its influence on a trait or disease depends on numerous factors, including other genes, genetic architecture, and regulation, as well as internal and external environmental factors.[35] Luo et al[18] reported that the interaction between the DD genotype and the T148C polymorphism of the β-fibrinogen gene resulted in a higher risk of stroke. Our analyses may additionally indicate a greater role of this interaction of the DD genotype and the T148C polymorphism of the β-fibrinogen gene in women with premature CAD.

Practical implications The results of our study indicate a potential opportunity to use drugs that interfere with the renin–angiotensin system more

widely for prophylaxis or at higher doses, especially in women with high plasma fibrinogen levels, the DD genotype, and premature CAD. Moreover, pharmacoeconomic analyses show that genotyping is cost-effective and more efficient than standard methods of choosing and monitoring pharmacological treatment.[49,50]

However, considering the current state of knowledge, further evaluation of the association of the *ACE* I/D polymorphism with plasma fibrinogen levels in premature CAD in the general population is needed.

Study limitations Our study had several limitations. First, the study group was heterogeneous, showing significant differences in baseline characteristics of women and men with regard to the frequency of hypertension, diabetes, and mean plasma fibrinogen levels. This was unavoidable because of different age of premature CAD onset in women and men. Second, collecting a representative sample was difficult, as premature CAD is relatively rare, especially in patients under 45 years of age. Therefore, sample size calculation for male subgroups revealed slight underpowering. Another limitation of our study was lack of a control group without CAD. As our findings are novel, further research is needed to validate the differences between women and men regarding the association of the I/D polymorphism of the *ACE* gene with fibrinogen levels.

Conclusions Women with premature CAD had higher fibrinogen levels than men. Sex was a factor modifying the influence of the I/D polymorphism of the *ACE* gene on the plasma fibrinogen levels. The DD genotype of the *ACE* gene was associated with higher plasma fibrinogen levels in women with premature coronary atherosclerosis, yet not in men.

SUPPLEMENTARY MATERIAL

Supplementary material is available at www.mp.pl/paim.

ARTICLE INFORMATION

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