Supplementary material

Kryczka KE, Płoski R, Księżycka E, et al. 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. Pol Arch Intern Med. 2020; 130: 748-756. doi:10.20452/pamw.15461

Table S1. Frequency of categorical traditional risk factors according to the insertion/deletion (I/D) polymorphism of the angiotensin-converting enzyme gene in women and men

		Genotypes				
		II	ID	DD	*P*	Heterogeneity of sex
		n=90 (22.1%)	n=194 (47.7%)	n=123 (30.2%)		
Sex	Women	61 (67.7%)	118 (60.8%)	78 (63.4%)		0,53
	Men	29 (32.2%)	76 (39.2%)	45 (36.6%)		
Hypertension		58 (64.4%)	108 (55.7%)	74 (60.2%)	0.36	
	Women	45 (73.8%)	76 (64.4%)	52 (66.7%)	0.44	0.48
	Men	13 (44.8%)	32 (42.1%)	22 (48.9%)	0.77	
Diabetes		9 (10.0%)	29 (14.9%)	16 (13.0%)	0.52	
	Women	7 (11.5%)	24 (20.3%)	12 (15.4%)	0.30	0.43
	Men	2 (6.9%)	5 (6.6%)	4 (8.9%)	0.92	
Hypercholesterolemia		73 (81.1%)	152 (78.3%)	104(84.5%)	0.39	
	Women	48 (78.7%)	93 (78.8%)	68 (87.2%)	0.28	0.40
	Men	25 (86.2%)	59 (77.6%)	36 (80.0%)	0.62	
Family history of cardiovascular diseases		15 (16.7%)	33 (17.0%)	30 (24.4%)	0.21	
	Women	10 (16.4%)	19 (16.1%)	25 (32.0%)	**0.016**	0.12
	Men	5 (17.2%)	14 (18.4%)	5 (11.1%)	0.56	
Smoking		61 (67.8%)	150 (77.3%)	92 (74.8%)	0.23	
	Women	37 (60.7%)	90 (76.3%)	59 (75.6%)	0.064	0.21

Men	24 (82.8%)	60 (78.9%)	33 (73.3%)	0.61	
Acute coronary syndrome	60 (66.7%)	122 (62.9%)	79 (123%)	0.83	
Women	41 (67.2%)	68 (57.3%)	50 (64.1%)	0.41	0.80
Men	19 (65.5%)	54 (71.0%)	29 (64.4%)	0.72	
ST-segment elevation myocardial infarction	33 (36.7%)	75 (38.7%)	46 (37.4%)	0.94	
Women	22 (36.1%)	44 (37.3%)	27 (34.6%)	0.93	0.96
Men	11 (37.9%)	31 (40.8%)	19 (42.2%)	0.93	

Table S2. Two-way ANOVA of continuous traditional risk factors according to the insertion/deletion (I/D) polymorphism of the angiotensin-converting enzyme gene in women and men

Risk factors mean [95% CI]	Genotypes				
	II n=90 (22.1%)	ID n=194 (47.7%)	DD n=123 (30.2%)	DD+ID+II	*P* *Two-way ANOVA, with interaction*
Age, years	44.0 [43.0-45.0]	45.7 [45.1 – 46.4]	46.0 [45.2-46.9]		Genotypes*Sex: *p*=0.085 **Sex: *p*<0.001** **Genotypes:*p*=0.006;** **DD vs II: 0.008** **ID vs II: 0.015**
Women	48.9 [47.7-50.0]	49.9 [49.1-50.7]	49.4 [48.4-50.5	49.4 [48.8-50.0]	
Men	39.1 [37.4-40.8]	41.6 [40.6-42.6]	42.6 [41.3-44.0]	41.1 [40.3-41.9]	
Body mass index, kg/m^2	28.3 [27.3-29.4]	28.1 [27.3-28.8]	27.7 [26.8-28.6]		Genotypes*Sex: *p*=0.35 Sex: *p*=0.43 Genotypes:*p*=0.71
Women	28.4 [27.1-29.6]	28.1 [27.2-29.0]	27.0 [25.9-28.1]	27.8 [27.2-28.5]	
Men	28.3 [26.5-30.1]	28.0 [26.9-29.1]	28.5 [27.0-29.9]	28.3 [27.4-29.1]	
High-sensitive C-reactive protein, mg/dl	0.21 [0.12-0.52]	0.21 [0.11-0.50]	0.23 [0.10-0.45]		Genotypes*Sex: *p*=0.085 Sex: *p*=0.91 Genotypes: *p*=0.27
Women	0.18 [0.11-0.51]	0.26 [0.12-0.62]	0.23 [0.09-0.55]	0.23 [0.11 – 0.55]	
Men	0.26 [0.15-0.85]	0.17 [0.09 -0.32]	0.21 [0.12 -0.62]	0.19 [0.10 -0.38]	
White blood	10.3	9.5	10.2		Genotypes*Sex:

cells, K/ul	[7.5-13.3]	[7.1-12.9]	[7.7-13.5]		*p*=0.22 Sex: ***p*<0.001**
Women	10.4 [7.0-12.9]	8.9 [6.9-11.4]	10.0 [7.4 – 12.4]	9.2 [7.0 – 12.25]	Genotype: *p*=0.29; **ID:**
Men	9.9 [8.8-13.4]	11.75 [8.6-14.4]	10.9 [8.6 – 14.5]	11.35 [8.6 – 14.4]	**Men vs women: *p*<0.001**

Table S3. Mean fibrinogen level according to presence of traditional cardiovascular risk factors

Fibrinogen, mg/dl Mean (standard deviation)			
Risk factor	Risk factor presents	Risk factor absents	*P*
Male sex	391.5 (161.2)	534.5 (180.9)	**<0.001**
Hypertension	488.3 (180.9)	472.4 (195.8)	0.40
Women	530.79 (173.68)	542.09 (195.84)	0.64
Men	378.63 (151.34)	401.97 (169.76)	0.38
Diabetes	482.0 (188.5)	481.8 (187.1)	0.99
Women	525.32 (181.20)	536.33 (181.23)	0.72
Men	312.72 (104.53)	397.79 (164.01)	0.93
Hypercholesterolemia	485.3 (182.8)	467.2 (204.6)	0.44
Women	532.98 (173.45)	541.03 (212.33)	0.78
Men	402.17 (169.12)	349.05 (120.90)	0.053
Family history of cardiovascular diseases	511.7 (184.3)	474.7 (187.3)	0.12
Women	557.29 (182.84)	528.42 (180.37)	0.30
Men	409.16 (144.26)	388.19 (165.12)	0.56
Smoking	492.3 (189.8)	451.1 (176.2)	**0.045**
Women	557.28 (183.88)	474.76 (159.18)	**0.001**
Men	389.11 (149.56)	400.20 (201.35)	0.73
Menopause Women	532.67 (188.69)	538.36 (164.13)	0.82
Menopause Women early ≤ 3years (vs late > 3 years)	507.67 (156.38)	552.77 (209.76)	0.12 Late vs lack of menopause p=0.61 Early vs lack of menopause p=0.23

Table S4. ANCOVA of different models tested. Dependent value: fibrinogen, mg/dl

Model's factors	Factors and interactions tested, *p*	Covariance: age, *p*
Two -way ANCOVA with interactions, *p*		

I/D genotypes, sex, I/D genotypes * sex	I/D genotypes: 0.61; **sex: <0.001**, **I/D genotypes * sex: 0.022**	0.11
I/D genotypes, hypertension, I/D genotypes * NT	I/D genotypes: 0.75; hypertension: 0.77, I/D genotypes * hypertension: 0.37	<0.001
I/D genotypes, diabetes, I/D genotypes * diabetes	I/D genotypes: 0.71; diabetes: 0.52, I/D genotypes * diabetes: 0.89	<0.001
I/D genotypes, hypercholesterolemia, I/D genotypes * hypercholesterolemia	I/D genotypes: 0.82; hypercholesterolemia: 0.70; I/D genotypes * hypercholesterolemia: 0.70	<0.001
I/D genotypes, family history of CVD, I/D genotypes * family history of CVD	I/D genotypes:0.20; family history of CVD: 0.16; I/D genotypes * family history of CVD: 0.06	<0.001
I/D genotypes, smoking, I/D genotypes * smoking	I/D genotypes: 0.23; **smoking:0.03**; I/D genotypes * smoking: 0.10	<0.001
	Three-way ANCOVA with interactions, p	
I/D genotypes, sex, smoking, I/D genotypes * sex, I/D genotypes * smoking	I/D genotypes: 0.30; **sex: <0.001, smoking: 0.03, I/D genotypes*** **sex: 0.019,** I/D genotypes * smoking: p=0.057	0.19

CVD: cardiovascular diseases, I/D: insertion/deletion polymorphism of the angiotensin-converting enzyme

Table S5. Significant differences in fibrinogen levels across genotypes- adjustment for multiple comparison: Bonferroni correction

Comparison		Difference [95% CI]	P
DD Women	**DD Men**	208.6 [102.2; 314.9]	**<0.001**
	ID Men	176.2 [70.7 ; 281.6]	<0.001
	II Men	217.1 [79.1 ; 355.0;]	<0.001
	ID Women	83 [2.7 ; 165.3]	**0.036**
II Women	**II Men**	160.4 [28.2; 292.6]	**0.01**
	ID Men	119.5 [16.0; 223.0]	0.01
	DD Men	151.9 [40.9; 262.9]	0.001

ID Women	DD Men	124.6 [17.0; 232.1]	0.01
Insignificant differences of fibrinogen levels across genotypes- adjustment for multiple comparison: Bonferroni			
ID Women	II Men	133.1 [-1.4 ; 267.5]	0.055
	ID Men	92.2 [-2.6 ; 186.8]	0.06
ID Men	DD Men	32.4 [-68.5; 133.3]	1.0
II Women	ID Women	27.3 [-56.5; 111.3]	1.0
	DD Women	-55.7 [-147.8 ; 34.6]	1.0
II Men	ID Men	-40.9 [-160.3 ; 78.5]	1.0
	DD Men	-8.5 [-138.0 ; -121.0]	1.0

DD: dominant homozygote, ID: heterozygote, II: recessive homozygote of the I/D polymorphism of the angiotensin-converting enzyme gene

The optimal subject-level gene model assessment based on Horita N, Kaneko T. Genetic model selection for a case-control study and a meta-analysis. Meta Gene 5 (2015) 1–8

Table S6. Four model strategy for women and men

	Fibrinogen, mg/dl	Genotype			**Odds Ratio (OR)** [95% CI]		**OR** [95% CI] Four models:				Optimal model
		II	ID	DD	ID vs DD	II vs ID	**RE** 'II' vs 'ID+DD'	**Multi** 'ID*II' vs 'DD'	**DO** 'II+ID' vs 'DD'	**OV** 'ID' vs 'DD+II'	
Women	>Median (526)	27 (44.3)	50 (42.4)	50 (64.1)	**0.41 [0.23-0.74]**	1.08 [0.58-2.01]	0.76 [0.43-1.36]	0.65 [0.46-0.92]	**0.42 [0.24-0.73]**	0.59 [0.36-0.97]	**Dominant**
	≤ Median (526)	34 (55.7)	68 (57.6)	28 (35.9)							
				P	**0.003**	0.809	0.356	**0.012**	**0.002**	**0.037**	
Men	> Median (357.5)	15 (51.7)	44 (57.9)	16 (35.6)	**2.49 [1.16-5.34]**	0.78 [0.33-1.84]	1.09 [0.48-2.45]	1.48 [0.92-2.37]	**2.32 [1.13-4.78]**	1.91 [1.00-3.65]	**Dominant**
	≤ Median (357.5)	14 (48.3)	32 (42.1)	29 (64.4)							
				P	**0.017**	0.569	0.836	0.099	**0.020**	**0.050**	

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

ANCOVA for dominant model. Dependent value: fibrinogen (mg/dl); **factors:** I/D genotype, sex, smoking; **covariance:** age. **Results:** ID+II: 0.25, **sex: <0.001,** smoking: 0.54**, sex * (ID+II): 0.013, sex* smoking: 0.038, (ID+II)*smoking: 0.028**

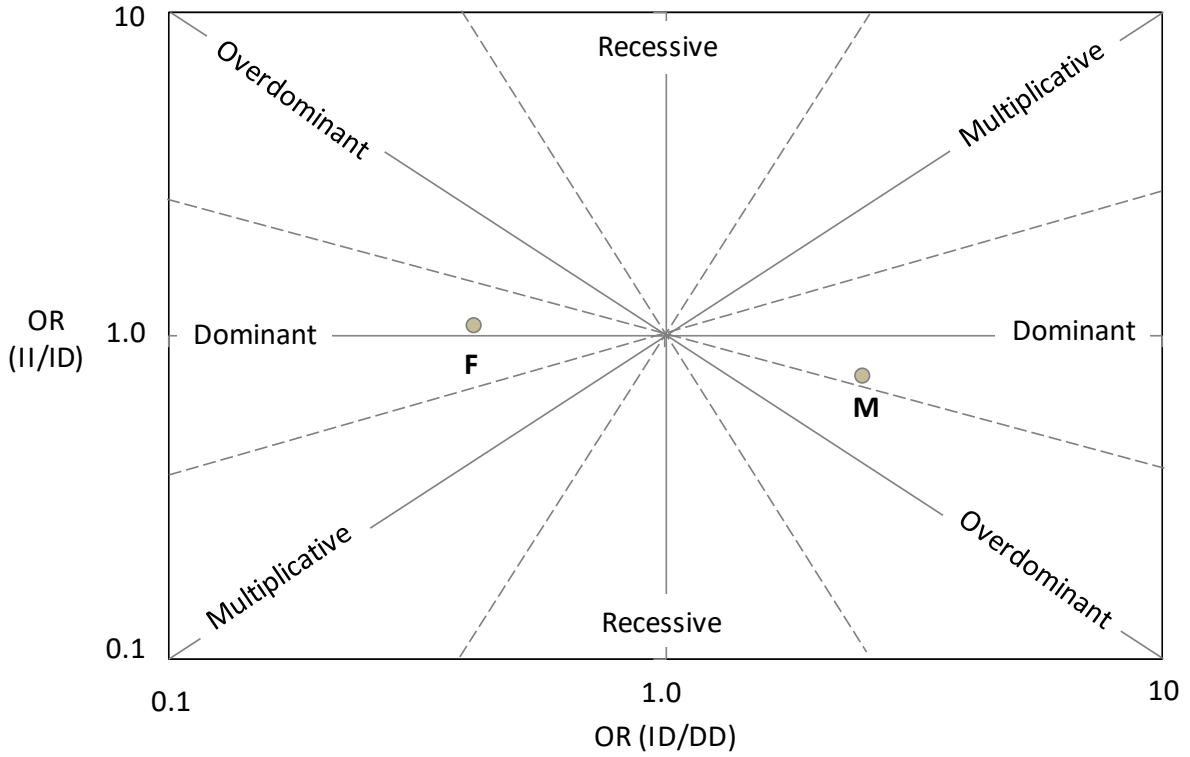

Figure S1. Four model strategy with optimal dominant model signed for women and men with premature coronary artery disease (logarithmic scale OR plane).

II: recessive homozygote, ID: heterozygote, DD: dominant homozygote of the insertion/deletion polymorphism of the angiotensin-converting enzyme gene

F: female; M: male

OR: odds ratio

The List of reagents for laboratory tests:

Lipids:

- Total cholesterol –Roche CHOL2 reagent, Cobas 6000 analyzer, enzymatic colorimetric method;
- High-density lipoprotein (HDL) cholesterol –Roche HDL4 reagent, Cobas 6000 analyzer, homogenous enzymatic colorimetric method;
- Low-density lipoprotein (LDL) cholesterol – Roche LDL3 reagent, Cobas 6000 analyzer, homogenous enzymatic colorimetric method;
- Triglycerides – Rosche TRIGL reagent, Cobas 6000 analyzer, homogenous enzymatic colorimetric method;

Glucose: Roche GLUC3 reagent, Cobas 6000 analyzer, hexokinase reference enzymatic method;

High-sensitive C-reactive protein (hs CRP): Roche CRPHS reagent, Cobas 6000 analyzer, latex-enhanced immunoturbidimetric method

Fibrinogen: SIEMENS „Multifibren® U" reagent, BCS analyzer, modified Clauss method;

Morphology:

- SYSMEX XN-1000 PURE Analyzer
- Reagents:
  - ✓ Lysercell WDF
  - ✓ Cellpack DCL
  - ✓ Sulfolyser
  - ✓ Fluorocell WDF

- ✓ Fluorocell WNR
- ✓ Lycercell WNR

- Methods:
  - ✓ Flow cytometry (White blood cells)