

Association of angiotensin converting enzyme and plasminogen activator inhibitor-1 promoter gene polymorphisms with features of the insulin resistance syndrome in patients with premature coronary heart disease

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Abstract

Polymorphisms of the angiotensin-converting enzyme (ACE) (insertion/deletion (I/D) in intron 16) and of the plasminogen activator inhibitor-1 (PAI-1) (promoter 4G/5G) genes have been linked with coronary heart disease (CHD) and/or myocardial infarction (MI). We studied the association of polymorphisms in these genes with CHD with linkage and association analyses in 118 families with premature and severe CHD and in 110 healthy controls. In linkage analysis there was no evidence for a linkage of the ACE or PAI-1 loci with CHD. However, in quantitative linkage analysis the ACE locus was linked with fasting glucose ($P = 0.047$) and fasting free fatty acid levels ($P = 0.029$). In association analysis the ACE genotype frequencies of probands with CHD did not differ from those of healthy controls. Normoglycemic probands with MI and with the ACE polymorphism DD genotype had characteristics of the insulin resistance syndrome. They had higher levels of 1-h glucose ($P = 0.008$) and 2-h free fatty acids ($P = 0.011$) in an oral glucose tolerance test and higher levels of total ($P = 0.005$) and very-low-density lipoprotein triglycerides ($P = 0.006$) than probands with the ID or the II genotypes. The PAI-1 gene polymorphism was not associated with any of the variables of glucose or lipid metabolism. In conclusion, the ACE and PAI-1 gene polymorphisms are not linked with early-onset CHD. However, the ACE gene polymorphism is associated with features of the insulin resistance syndrome. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Angiotensin-converting enzyme; Plasminogen activator inhibitor-1; Polymorphism; Coronary heart disease; Insulin resistance

1. Introduction

Genetic factors contribute to the development of early-onset atherosclerosis, and almost 50% of the incidence of premature coronary heart disease (CHD) can be explained by genetic factors [1,2]. Genes regulating cardiovascular risk factors for atherosclerosis and thrombosis (for example lipoprotein and glucose metabolism, blood pressure, blood coagulation and fibrinolysis) have been recently a focus of intensive

research. Among these genes the angiotensin-converting enzyme (ACE) [3–6] and plasminogen activator inhibitor-1 (PAI-1) [7,8] polymorphisms have been shown to be associated with CHD and myocardial infarction (MI).

ACE is a key enzyme in blood pressure regulation. It is responsible for the conversion of angiotensin I to angiotensin II, which is a potent vasoconstrictor of the renin–angiotensin system, and it can also inactivate bradykinin, a vasodilator of the kallikrein–kinin system [9]. In addition, the end product of the renin–angiotensin system, angiotensin II, has been suggested to have a proliferating effect on the vascular endothelium [10], a mechanism that could also promote atherosclerosis.

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rosis. The Etude Cas-Temoin de l'Infarctus du Myocarde (ECTIM) study was the first study to show an association of the deletion (D) allele of ACE polymorphism in intron 16 with MI [3]. Since then this association has been confirmed in other studies (for reviews see [4–6,11]) although a recent meta-analysis based on 46 studies casts doubt on the value of the ACE DD genotype as a marker for increased cardiovascular disease [12]. Another enzyme with a suggestive pathogenic role in CHD, especially in the thrombotic process of myocardial infarction, is PAI-1. The function of PAI-1 enzyme is to inhibit tissue plasminogen activator which catalyses the conversion of inactive plasminogen to active plasmin, a fibrin degrading agent. Thus, defects in the PAI-1 gene could lead to impaired fibrinolysis. The 4G allele of the PAI-1 (4G/5G) promoter polymorphism has been shown to be associated with increased levels of PAI-1 enzyme [13,14] and the risk of MI [7,8].

No studies are available on the linkage of the polymorphisms of the ACE and PAI-1 genes with early-onset CHD. Furthermore, it is not known whether or not these polymorphisms are related to features of the insulin resistance syndrome. Therefore, we investigated the association of these polymorphisms with CHD and MI in Finnish families with premature CHD, and with cardiovascular risk factors clustering with insulin resistance.

2. Materials and methods

2.1. Subjects

All subjects participating in this study were Finnish and living in eastern Finland. Finland is a genetic isolate [15] and the population of eastern Finland has been suggested to originate from one of the two groups of settlers who are founders of the present Finnish population [16].

Altogether 118 families with premature CHD were included in the study. Probands ($n = 118$; 70 men, 48 women) were identified from the coronary angiogram register of the Kuopio University Hospital. The inclusion criteria for the probands were: (1) age at the time of CHD diagnosis 55 years or less in men and 65 years or less in women; (2) stenosis exceeding 50% in coronary angiogram at least in two coronary arteries; and (3) family history of premature CHD. Affected siblings ($n = 140$; 111 men, 29 women) had the inclusion criteria (1)–(3) or a history of definite MI according to the World Health Organization (WHO) criteria based on chest pain, cardiac enzyme determinations and ECG changes [17]. From each family one unaffected sibling, if available, and matched for the age of proband, was included in the study. Unaf-

ected siblings ($n = 62$, 31 men, 31 women) did not have any signs or symptoms suggesting CHD based on medical history or Rose cardiovascular questionnaire [18] and ECG. Coronary angiogram was performed on 35 subjects of 62 unaffected siblings, and they were proven not to have a significant stenosis ($> 30\%$) in any of their coronary arteries. Control subjects were 110 healthy unrelated subjects (82 men, 28 women, age 51 ± 1 years) from our previous population studies. These subjects were selected from the same region from the control families in the myocardial infarction survivor study or from offspring of subjects who had a repeatedly normal glucose tolerance during 10-year follow-up [19–22]. They did not have any chronic disease or any signs or symptoms suggesting CHD according to the Rose cardiovascular questionnaire [18]. They were included as controls in the association study since they represent a normal allele distribution of the ACE and PAI-1 gene polymorphisms in a healthy population living in eastern Finland.

Informed consent was obtained from all subjects after the purpose and potential risks of the study were explained to them. The study protocol was approved by the Ethics Committee of the University of Kuopio and was in accordance with the Helsinki Declaration.

2.2. Evaluation of clinical and biochemical characteristics

Weight and height were measured with subjects wearing light clothing without shoes. The waist to hip ratio was used as an indicator of body fat distribution. Waist circumference was measured at the level of the umbilicus with the subjects standing and breathing normally. Hip circumference was measured at the level of the greatest hip girth. Blood pressure was measured on the right arm with subjects in the supine position with a mercury sphygmomanometer after a 5-min rest. Two readings were taken (1.5-min interval) and the latter reading was used in statistical analyses. In each measurement blood pressure was read to the nearest 2 mmHg. Subjects were defined to have hypertension if systolic blood pressure was ≥ 160 mmHg, or diastolic blood pressure was ≥ 95 mmHg, or if they were receiving drug treatment for hypertension. Diagnosis of diabetes was based on the WHO criteria [23]. Blood samples for laboratory analyses were drawn after a 12-h fast. An oral glucose tolerance test (OGTT) (75 g of glucose) was performed on all those individuals who had no previous diagnosis of diabetes. Blood samples for the determination of plasma glucose, plasma insulin and serum free fatty acids (FFAs) were drawn in the fasting state and after 1 and 2 h.

2.3. Analytical methods

Plasma glucose was measured by the glucose oxidase method (2300 Stat Plus, Yellow Springs Instrument Co. Inc., Yellow Springs, OH). For the determination of plasma insulin blood was collected in EDTA-containing tubes and after centrifugation the plasma was stored at -20°C until the analysis. Plasma insulin concentration was determined by a commercial double-antibody solid-phase radioimmunoassay (Phadeseph Insulin RIA 100; Pharmacia Diagnostics AB, Uppsala, Sweden). Serum FFAs were determined from fresh frozen samples by an enzymatic method (NEFA C test, Wako Chemicals GmbH, Neuss, Germany). Lipoprotein fractionation was performed with ultracentrifugation and selective precipitation [24] as previously described [25]. From fractionated serum samples cholesterol and triglyceride levels were assayed by automated enzymatic methods (Boehringer–Mannheim, Mannheim, Germany). Serum apolipoprotein (apo) AI and B concentrations were determined by a commercial immunoturbidometric method (Kone Instruments, Espoo, Finland). Plasma fibrinogen was measured by an automated analyzer (Thrombolyzer, Behnk Elektronik, Norderstedt, Germany). Plasma samples were assayed for PAI-1 activity with a commercial two-stage indirect enzymatic assay using microtest plate method (Spectrolyse, Biopool AB, Ventura, CA) [26].

2.4. Determination of the ACE and PAI-1 genotypes

DNA was prepared from peripheral blood leukocytes by proteinase K-phenol-chloroform extraction method. ACE and PAI-1 genotypes were determined in separate polymerase chain reactions (PCR) using primers and methods described previously [27,28]. PCR amplification of intron 16 of the ACE gene was conducted in a 10 μl volume containing 50 ng of genomic DNA, 5 pmol of each primer, 10 mmol/l Tris–HCl (pH 8.8), 50 mmol/l KCl, 1.5 mmol/l of MgCl_2 , 0.1% Triton X-100, 100 $\mu\text{mol/l}$ dNTPs, and 0.25 U of DNA polymerase (Dynazyme DNA polymerase, Finnzymes, Espoo, Finland). Conditions for amplification were denaturation at 94°C for 4 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 58°C for 30 s and extension at 72°C for 30 s with final extension at 72°C for 4 min. PCR amplification of the PAI-1 gene promoter region was conducted in a 10 μl volume containing 50 ng of genomic DNA, 10 pmol of insertion or deletion specific primer, 5 pmol of common downstream primer, 1.2 pmol common upstream primer, 10 mmol/l Tris–HCl (pH 8.8), 50 mmol/l KCl, 1.5 mmol/l of MgCl_2 , 0.1% Triton X-100, 100 $\mu\text{mol/l}$ dNTPs, and 0.25 U of DNA polymerase (Dynazyme DNA polymerase, Finnzymes, Espoo, Finland). Conditions for amplification were denaturation at 94°C for 5 min,

followed by 30 cycles of denaturation at 94°C for 60 s, annealing at 63°C for 45 s and extension at 72°C for 75 s with a final extension at 72°C for 5 min. Amplified PCR products of the ACE and PAI-1 genes were separated using 2% agarose gel electrophoresis and visualized by ethidium bromide staining.

2.5. Statistical analyses

2.5.1. Linkage analysis

Nonparametric linkage analysis between the ACE or PAI-1 polymorphisms and CHD in affected sib-pairs was based on the mean proportion of alleles shared identical by descent (IBD) at the ACE or PAI-1 loci as implemented in the SIBPAL program [29]. The same computer program was used to test linkage between quantitative traits and the ACE or PAI-1 loci in all siblings of the families by regression analysis where the squared difference in quantitative trait by siblings in a pair was the dependent variable and the estimated proportion of shared alleles IBD at the ACE or PAI-1 locus was the independent variable. Negative slope of the regression line that differed significantly from zero was interpreted as an evidence for linkage between the quantitative trait and the ACE or PAI-1 polymorphism [30].

2.5.2. Association analysis

All basic calculations were accomplished using the SPSS/Win 8.0 statistical package (SPSS Inc., Chicago, IL). Due to genetic dependence of siblings they were considered as paired samples, and in addition to probands only one affected and one healthy sibling were included in statistical analyses. The association analysis was performed on only those families where biochemical data were available for both affected and unaffected siblings. Differences in clinical characteristics and biochemical determinations between the study groups, and also in pair-wise comparisons, were assessed with Cochran's Q test for categorical variables and the analyses of variance (ANOVA) and covariance (ANCOVA) for repeated measures for continuous variables. In ANCOVA the adjustment was done for gender. Differences in genotype distributions between controls and other study groups were assessed with exact χ^2 -statistics using the StatXact 3.1 statistical program (Cytel Software Corporation, Cambridge, MA). ANOVA was used to examine the effect of polymorphisms on the variables of glucose and lipid metabolism in probands. If the difference between the genotype groups was statistically significant Helmert or Difference contrasts were used for pair-wise comparisons. Before statistical testing plasma glucose, and insulin, serum FFAs, serum VLDL cholesterol, serum triglycerides and plasma PAI-1 enzyme activity values were logarithmically transformed to achieve a normal distri-

bution. All data are presented as mean \pm S.E.M. *P*-value of <0.05 was considered to indicate a statistically significant difference.

3. Results

3.1. Clinical and biochemical characteristics

Clinical characteristics and biochemical determinations of unaffected siblings, affected siblings and probands, available on 62, 104 and 117 subjects, respectively, are shown in Table 1. The adjustment was done

for gender when the study groups were compared since there were more women among healthy siblings ($P = 0.043$) and more men among affected siblings ($P < 0.001$) than in probands. Probands were more often receiving cholesterol-lowering medication ($P < 0.001$), and had higher levels of 1-h ($P = 0.012$) and 2-h ($P = 0.013$) insulin and fasting FFAs ($P = 0.010$) in an OGTT than did healthy siblings. Additionally, probands had lower levels of HDL cholesterol ($P < 0.001$), higher levels of total ($P = 0.017$) and VLDL ($P = 0.011$) triglycerides and fibrinogen ($P = 0.004$) than healthy siblings. Affected siblings differed from probands only with respect to age ($P = 0.005$).

Table 1
Clinical characteristics of study groups^a

	Unaffected siblings (<i>n</i> = 62)	Affected siblings (<i>n</i> = 118)	Probands (<i>n</i> = 118)	ANOVA or Friedman test (<i>P</i> value)	ANCOVA ^b (<i>P</i> value)
Gender (M/F)	31/31	95/23	70/48	<0.001	–
Age (years)	54 \pm 1	57 \pm 1	56 \pm 1	NS	0.028
Body mass index (kg/m ²)	26.9 \pm 0.5	27.2 \pm 0.3	28.1 \pm 0.3	NS	NS
Waist-to-hip ratio	0.94 \pm 0.01	0.97 \pm 0.01	0.96 \pm 0.01	0.001	NS
Hypertension (%)	36	45	49	NS	–
Diabetes (%)	10	11	11	NS	–
Lipid lowering medication (%)	10	49	55	<0.001	–
<i>Oral glucose tolerance test</i>					
Plasma glucose (mmol/l)					
Fasting	5.7 \pm 0.1	6.3 \pm 0.2	6.2 \pm 0.2	0.017	NS
1-h	8.8 \pm 0.4	9.6 \pm 0.4	9.0 \pm 0.3	NS	NS
2-h	7.0 \pm 0.4	7.4 \pm 0.4	7.1 \pm 0.3	NS	NS
Plasma insulin (pmol/l)					
Fasting	68.4 \pm 3.6	84.0 \pm 7.2	87.0 \pm 6.6	0.008	0.018
1-h	434.4 \pm 31.8	564.0 \pm 45.0	606.0 \pm 53.4	0.006	0.024
2-h	327.6 \pm 31.2	393.6 \pm 36.0	463.2 \pm 60.0	0.001	0.001
FFAs (mmol/l)					
Fasting	0.68 \pm 0.03	0.64 \pm 0.03	0.58 \pm 0.02	0.007	0.043
1-h	0.19 \pm 0.02	0.18 \pm 0.01	0.17 \pm 0.01	NS	NS
2-h	0.12 \pm 0.01	0.12 \pm 0.01	0.11 \pm 0.01	NS	NS
Cholesterol (mmol/l)					
Total	6.58 \pm 0.16	6.08 \pm 0.11	5.95 \pm 0.09	0.046	NS
LDL	4.50 \pm 0.14	4.18 \pm 0.10	4.04 \pm 0.08	NS	NS
HDL	1.41 \pm 0.05	1.24 \pm 0.03	1.23 \pm 0.03	<0.001	0.001
VLDL	0.67 \pm 0.08	0.65 \pm 0.04	0.68 \pm 0.05	0.023	NS
Triglycerides (mmol/l)					
Total	1.68 \pm 0.13	1.89 \pm 0.12	1.91 \pm 0.11	0.016	0.032
LDL	0.40 \pm 0.02	0.41 \pm 0.01	0.42 \pm 0.01	NS	NS
HDL	0.21 \pm 0.01	0.21 \pm 0.01	0.23 \pm 0.01	NS	NS
VLDL	1.06 \pm 0.12	1.26 \pm 0.11	1.25 \pm 0.10	0.004	0.018
Apolipoprotein A1 (g/l)	1.55 \pm 0.03	1.46 \pm 0.03	1.43 \pm 0.02	0.016	NS
Apolipoprotein B (g/l)	1.25 \pm 0.04	1.22 \pm 0.03	1.20 \pm 0.03	NS	NS
Fibrinogen (g/l)	3.4 \pm 0.1	3.9 \pm 0.1	3.8 \pm 0.1	0.002	0.013
PAI-1 (U/ml)	16.6 \pm 1.7	18.6 \pm 1.4	22.5 \pm 1.7	0.030	NS

^a Clinical and biochemical data were available for 62 unaffected siblings, for 104 affected siblings and for 117 probands.

^b Adjusted for gender.

Table 2
Genotype frequencies (percentages in parenthesis) of the ACE and PAI-1 gene polymorphisms in the study groups

	Healthy controls (<i>n</i> = 110)	Unaffected siblings (<i>n</i> = 62)	Affected siblings (<i>n</i> = 118)	Probands (<i>n</i> = 118)
<i>ACE genotypes</i>				
DD	36 (32.7)	24 (38.7)	32 (27.1)	43 (36.4)
ID	57 (51.8)	28 (45.2)	64 (54.2)	48 (40.7)
II	17 (15.5)	10 (16.1)	22 (18.6)	27 (22.9)
<i>P</i> value (vs. controls)		0.698	0.594	0.189
<i>PAI-1 genotypes</i>				
4G/4G	31 (28.2)	17 (27.4)	24 (20.3)	30 (25.4)
4G/5G	51 (46.4)	28 (45.2)	65 (55.1)	63 (53.4)
5G/5G	28 (25.5)	17 (27.4)	29 (24.6)	25 (21.2)
<i>P</i> value (vs. controls)		0.958	0.319	0.569

3.2. Linkage analysis

In nonparametric linkage analysis the estimated proportion of alleles shared IBD was 0.50 ± 0.02 (mean \pm S.E.M.) at the ACE locus ($P = 0.429$) and 0.51 ± 0.01 at the PAI-1 locus ($P = 0.348$) in 163 affected sib-pairs of the families with premature CHD. In quantitative linkage analysis, with all family members included, a linkage of fasting plasma glucose ($P = 0.047$) and fasting serum FFA ($P = 0.029$) levels with the ACE locus was detected. However, the linkage with fasting plasma glucose disappeared after the exclusion of subjects with manifest diabetes ($P = 0.205$), although the linkage with fasting FFA level persisted ($P = 0.009$). No evidence for a linkage of PAI-1 enzyme activity or any other trait with the PAI-1 locus could be demonstrated.

3.3. Association analysis

Table 2 shows the genotype frequencies of the ACE and PAI-1 polymorphisms in healthy controls, unaffected siblings, affected siblings and probands. The ACE and PAI-1 genotype distributions were in the Hardy–Weinberg equilibrium. The distributions of the ACE or the PAI-1 polymorphism genotypes did not differ between the study groups (healthy controls vs. other groups). Because the ACE and PAI-1 gene polymorphisms have been associated with MI, we divided our probands into those who had ($n = 55$) and those who did not have a history of MI ($n = 63$). There were no differences in genotype frequencies of the ACE or PAI-1 polymorphisms in probands with a history of MI and without MI or healthy controls (data not shown). These results were essentially unchanged when we used a lower age criterion for probands (men ≤ 50 years and women ≤ 60 years at the time of CHD diagnosis, $n = 25$).

The effect of the ACE gene polymorphism on indicators of glucose and lipid metabolism was first studied in probands (data not shown). Statistically significant dif-

ferences between the ACE genotypes were detected only in levels of VLDL cholesterol ($P = 0.041$) and PAI-1 enzyme activity ($P = 0.044$). Since insulin resistance is associated with type 2 diabetes and the ACE polymorphism contributes to the risk of MI we studied the association of the ACE polymorphism with biochemical variables in only normoglycemic probands with a history of MI ($n = 45$) (Table 3). Probands with the DD genotype ($n = 19$) had higher levels of 1-h plasma glucose ($P = 0.008$) and 2-h FFAs ($P = 0.011$) in an OGTT than probands with the ID or the II genotypes ($n = 26$). Additionally, probands with the DD genotype had higher levels of total ($P = 0.005$) and VLDL triglycerides ($P = 0.006$) than probands with the ID or probands with the II genotypes. The results were essentially unchanged when we used a lower age criterion for probands (men ≤ 50 years and women ≤ 60 years at the time of CHD diagnosis, $n = 25$). The trends in glucose and lipid metabolism parameters according to the ACE polymorphism genotypes in healthy controls were essentially similar to those in probands (data not shown).

In probands the PAI-1 gene polymorphism modified 1-h FFA levels ($P = 0.019$) in an OGTT. Probands with the 5G/5G genotype ($n = 25$) had a higher level of 1-h FFAs (0.22 vs. 0.15 mmol/l, $P = 0.010$) than probands with the 4G/5G or the 4G/4G genotypes ($n = 93$). Analysis was repeated in normoglycemic probands with MI. No differences in the parameters of glucose and lipid metabolism or PAI-1 enzyme activity were found between the PAI-1 genotypes (data not shown).

4. Discussion

The novel finding of our study was that the ACE polymorphism was associated with features of the insulin resistance syndrome. However, our study failed to show any significant association or linkage of the ACE gene (deletion in intron 16) and the PAI-1 gene (pro-

moter 4G/5G) polymorphisms with CHD or MI in families with premature and severe CHD.

The lack of either association or linkage between the ACE or the PAI-1 gene polymorphisms and CHD or MI in families with premature and severe CHD could be explained by a low number of affected subjects with a history of MI [55 (46.6%) probands, 63 (53.4%) affected siblings]. Both genes are considered as candidate genes for MI and not for coronary stenosis, which was the original inclusion criterion for the study. In addition, other more important, but as yet unidentified, susceptibility genes may play a role in the pathogenesis of CHD in Eastern Finland. This explanation was previously suggested by Perola and coworkers [27] since in their study women from Eastern Finland with the ACE genotype II more frequently had parents with a history of MI than did those carrying the D allele. Additionally, the absence of linkage of the ACE or PAI-1 loci with CHD could be explained by the polymorphisms themselves because polymorphisms with only two alleles have a limited value as genetic markers. It should be emphasized, however, that the absence of an association or a linkage of the ACE or PAI-1 gene

polymorphisms with CHD or MI does not exclude the possibility that these polymorphisms have a modifying effect on the risk for CHD also in Eastern Finland.

In quantitative linkage analysis the ACE locus was associated with fasting plasma glucose and FFA levels. This finding was supported by the results of the association study where normoglycemic subjects with the DD genotype of the ACE gene polymorphism and who had suffered of MI had characteristics of the insulin resistance syndrome (impaired glucose tolerance, hyperinsulinemia, hypertriglyceridemia and hypofibrinolysis) when compared to subjects with the ID or the II genotypes. Our finding is in agreement with the results of Zingone and coworkers [31] because in their study population the subjects with the D allele of the ACE polymorphism had impaired glucose tolerance. However, in other studies nondiabetic or diabetic subjects with the I allele have been more insulin resistant [32,33]. One possible explanation for contradictory results could be that the ACE polymorphism is only a marker for another gene near the ACE gene contributing to abnormalities in glucose metabolism or insulin resistance. Therefore, in different ethnic populations either

Table 3
Parameters of glucose and lipoprotein metabolism in normoglycemic probands with a history of MI according to the ACE polymorphism

	DD (n = 19)	ID (n = 16)	II (n = 10)	ANOVA
<i>Oral glucose tolerance test</i>				
Plasma glucose (mmol/l)				
Fasting	5.7 ± 0.1	5.7 ± 0.1	5.6 ± 0.1	NS
1-h	9.2 ± 0.5	7.5 ± 0.5 ^a	7.4 ± 0.7 ^a	0.028
2-h	6.1 ± 0.3	6.3 ± 0.3	5.4 ± 0.4	NS
Plasma insulin (pmol/l)				
Fasting	77.6 ± 5.8	71.6 ± 4.7	74.5 ± 7.2	NS
1-h	734.3 ± 111.7	462.8 ± 48.9	513.0 ± 102.4	NS
2-h	453.4 ± 80.7	349.2 ± 37.3	315.1 ± 92.4	NS
FFAs (mmol/l)				
Fasting	0.59 ± 0.05	0.63 ± 0.06	0.45 ± 0.07	NS
1-h	0.19 ± 0.02	0.14 ± 0.01	0.14 ± 0.03	NS
2-h	0.15 ± 0.03	0.07 ± 0.01 ^b	0.09 ± 0.02 ^b	0.012
Cholesterol (mmol/l)				
Total	6.33 ± 0.21	6.10 ± 0.20	6.02 ± 0.25	NS
LDL	4.36 ± 0.17	4.39 ± 0.20	4.26 ± 0.22	NS
HDL	1.22 ± 0.05	1.23 ± 0.05	1.21 ± 0.07	NS
VLDL	0.76 ± 0.08	0.49 ± 0.09	0.56 ± 0.11	NS
Triglycerides (mmol/l)				
Total	2.09 ± 0.15	1.48 ± 0.15 ^a	1.57 ± 0.17 ^a	0.013
LDL	0.48 ± 0.04	0.42 ± 0.04	0.37 ± 0.03	NS
HDL	0.25 ± 0.01	0.21 ± 0.02	0.19 ± 0.01	NS
VLDL	1.38 ± 0.14	0.85 ± 0.12 ^a	1.00 ± 0.16 ^a	0.019
Apolipoprotein AI (g/l)	1.47 ± 0.06	1.38 ± 0.04	1.38 ± 0.07	NS
Apolipoprotein B (g/l)	1.30 ± 0.06	1.18 ± 0.04	1.19 ± 0.07	NS
Fibrinogen (g/l)	3.9 ± 0.2	3.7 ± 0.1	3.6 ± 0.2	NS
PAI-1 enzyme activity (U/ml)	23.6 ± 4.8	18.5 ± 3.9	26.4 ± 5.6	NS

^a DD vs. ID and II, $P < 0.01$.

^b DD vs. ID and II, $P < 0.05$.

the D allele or the I allele could be in linkage disequilibrium with a functional polymorphism nearby.

We were not able to demonstrate that the promoter (4G/5G) polymorphism of the PAI-1 gene is associated with features of the insulin resistance syndrome either in linkage or association analyses. Neither were we able to show any trend in the PAI-1 enzyme activity levels in probands according to the PAI-1 promoter polymorphism, which has previously been demonstrated [13,14]. The explanation could be the relatively small sample size or that other yet unidentified genetic or environmental factors influence PAI-1 levels in different ethnic populations.

In summary, we demonstrated that normoglycemic subjects with the DD genotype of the ACE gene (intron 16 polymorphism) and a history of MI have characteristics of the insulin resistance syndrome. Furthermore, the ACE gene locus was linked with fasting glucose and FFAs. Both these findings imply that the ACE gene polymorphism could contribute to insulin resistance in Finnish subjects with early-onset CHD.

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