Synergistic effects of apolipoprotein E gene epsilon polymorphism and some conventional risk factors on premature ischaemic heart disease development

Anna Balcerzyk[1], Iwona Żak[1], Jolanta Krauze[2]

[1] Department of Biochemistry and Medical Genetics, Medical University of Silesia, Katowice, Poland
[2] 1st Department and Clinic of Cardiology, Medical University of Silesia, Katowice, Poland

Abstract

Background: Ischaemic heart disease (IHD), which is a clinical manifestation of atherosclerotic changes in coronary arteries, results from the action of multiple genetic and environmental factors. Genetic susceptibility to IHD may be determined by specific polymorphic variants of genes encoding isoforms involved in processes important in the pathogenesis of atherosclerosis. Due to the multifactorial nature of IHD, participation of a single polymorphism in the determination of the disease risk is relatively small. However, it seems that its significance may increase in the presence of a specific genetic or environmental background.

Aim: To evaluate a possible association between the epsilon polymorphism of the apolipoprotein E gene (*apo E*) and premature IHD in the Polish population as well as to determine whether the genotype may modulate the influence of conventional risk factors on IHD.

Methods: We studied 247 caucasian subjects: 140 patients with angiographically confirmed IHD and 107 blood donors without a history of IHD. Polymorphism epsilon of the *apo E* gene was genotyped using the PCR-RFLP method.

Results: We observed a tendency to a higher prevalence of the ε4 allele and carriers of this allele in the IHD group compared to controls. However, these differences were not statistically significant. We also observed a synergistic effect between ε4 allele carrier state and smoking, elevated level of total cholesterol and, to a lower degree – LDL cholesterol, on IHD risk.

Conclusion: Presented data show the synergistic effects between ε4 allele carrier state and some traditional risk factors on determining the risk of premature coronary artery disease.

Key words: apolipoprotein E, ischaemic heart disease, risk factors, gene polymorphism

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Introduction

Ischaemic heart disease (IHD) is the most common clinical condition with phenotype manifestation being a result of progressive coronary artery atherosclerosis. In addition to the conventional risk factors for IHD development such as smoking, hypercholesterolaemia or obesity, genetic factors may also modulate susceptibility to the disease. Gene polymorphic variants coding isoforms, involved in the processes that are of importance for pathogenesis of atherosclerosis, result in genetic heterogeneity of the human population and also contribute to variability within species including susceptibility to a certain disease.

Undoubtedly, abnormalities of lipid transport and metabolism are among the most powerful risk factors for the development and progression of atherosclerotic plaques. Increased blood concentration of low density lipoproteins (LDL) may be a cause of endothelial damage and dysfunction. The LDL stimulates expression of adhesive molecules, leading to enhanced inflammatory cell migration and proliferation. Oxidised LDL, taken up by the macrophages, lead to their transformation into foam cells and subsequent

Address for correspondence:
Anna Balcerzyk MD, Zakład Biochemii i Genetyki Medycznej, Śląski Uniwersytet Medyczny, ul. Medyków 18, 40-752 Katowice,
tel.: +48 32 252 84 32, e-mail: abalcerzyk@slam.katowice.pl

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necrosis [1–3]. High density lipoproteins (HDL) play a significant role in the reduction of cholesterol content of atherosclerotic plaques [4].

One of the most important genes associated with lipid metabolism is the one coding apolipoprotein E (Apo E), a component of chylomicrons and very low density lipoproteins (VLDL) acting as a ligand for LDL and LRP receptors, thus regulating lipid transport and metabolism. Particles of Apo E also participate in removal of cholesterol from cells and have multiple pleiotropic effects. Apo E is involved in inflammatory response modulation, regulation of platelet function, apoptosis as well as oxidative stress. Such pronounced Apo E impact on the physiological processes may explain why polymorphism of the *apo E* gene seems to be associated not only with atherogenesis and IHD, but also with many other conditions such as Alzheimer disease, ischaemic stroke, some types of malignancies and cholelithiasis [5].

Due to the multifactorial nature of IHD, the contribution of single polymorphic variants to the risk of the disease is relatively low and so currently their analysis is irrelevant to clinical practice. However, it seems that the role of particular polymorphic variants may increase if they are accompanied by a defined genetic or environmental background. Thus, multivariate analysis involving both genetic and environmental risk factors is more than justified.

The aim of this study was to look for a relationship between the *apo E* gene polymorphism and premature development of IHD in the Polish population and to verify whether *apo E* genotype modulated the impact of traditional risk factors on the course of the disease.

Methods

Patients

The study involved 247 Caucasians aged between 31 and 55 years. Examined patients were split into two subgroups. The IHD group (n=140) consisted of 61 women and 79 men at the mean age of 43±5 years. These patients had atherosclerotic IHD confirmed by coronary angiography (>50% of stenosis in at least one major coronary artery) and were hospitalised in the First Department and Chair of Cardiology, Silesian Medical University, Katowice. Angiography was performed using the Judkins method. Myocardial infarction (MI) was diagnosed according to European Society of Cardiology recommendations [6]. The following exclusion criteria were adopted: non-vascular disease aetiology and clinical manifestation of cardiomyopathy, collagenosis and intoxication (with e.g. CO, amphetamine). The control group (n=107) involved 20 women and 87 men at the mean age of 42±7 years. This group comprised voluntary blood donors free from IHD symptoms, recruited from the Blood Donor Centre. Individuals with strong family history of IHD or history of ischaemic stroke were excluded from the study.

All patients were characterised based on medical history and special attention was paid to IHD risk factors such as hypertension, obesity, diabetes mellitus and smoking.

All patients expressed their informed consent to participate in the study. The study was approved by the Local Bioethics Committee of the Medical University of Silesia in Katowice (L. dz. NN-013-297/I/02).

Biochemical analysis

Serum concentration of triglycerides (TG), and total (TC) and HDL (HDL-C) cholesterol were measured by means of enzymatic methods using Analco diagnostic kits. The LDL cholesterol (LDL-C) concentration was calculated according to Friedewald's formula [7].

Analysis of polymorphism

Polymorphic variants were analysed by means of the RFLP-PCR technique (restriction fragment length polymorphism – polymerase chain reaction). DNA was isolated from peripheral blood lymphocytes using the MasterPure Genomic DNA Purification Kit (Epicentre). Epsilon polymorphism of the *apo E* gene was analysed using the method described by Hixson and colleagues [8] with own modifications. The following conditions of amplification were employed: preliminary denaturation – 5 min in 95°C, then 60 s in 95°C, 60 s in 64°C and 60 s in 72°C. Second, third and fourth stages were repeated twice, which resulted in a decrease of temperature of starters' attachment by 1 degree every two cycles till 61°C was reached, then 20 of the following cycles were performed: 60 s in 95°C, 60 s in 60°C, 120 s in 72°C, completed by final extension in 72°C for 30 min. The amplification product was digested with the use of HhaI (Promega) restrictive enzyme and ε3 allele fragments of 35, 38, 48, 91 bp in length as well as 35, 38, 48, 72 bp for ε4 allele were obtained.

Finally, restriction products were separated by means of electrophoresis in 8% polyacrylamide gel and visualised after staining with silver nitrate.

Statistical analysis

The results are presented as means ± SD or numbers and percentages. All data were analysed with Statistica 6.0 and EpiInfo-6 (WHO) computer software. In the

Table I. Clinical and biochemical characteristics of the examined group

Parameter	IHD n=140	Controls n=107
Gender		
females	61 (43.6%)	20 (18.7%)
males	79 (56.4%)	87 (81.3%)
Age [years]	42.9±5.5	41.8± 6.7
BMI	26.8±4.42*	25.4±3.7
Total cholesterol [mmol/l]	5.8±1.40*	5.3±1.3
LDL cholesterol [mmol/l]	3.9±1.20*	3.5±1.2
HDL cholesterol [mmol/l]	1.2±0.44	1.1±0.4
Triglyceride [mmol/l]	1.8±1.00*	1.5±0.7
Smokers	64 (45.7%)*	34 (31.8%)

** – p <0.05*

case of quantitative data, normal distribution was verified using Shapiro-Wilk's test. Mean values of variables following a normal distribution were compared by means of Student's t-test; otherwise Mann-Whitney U test was used. Based on genotype frequency, allele rate was estimated. Distribution compliance with Hardy-Weinberg equilibrium as well as comparison of genotype and allele rate between groups was performed using χ^2 test. Values of $p < 0.05$ were considered statistically significant. Association power of individual alleles and genotypes with the disease were evaluated based on odds ratios (OR) with a 95% confidence interval calculations. Odds ratio >1 means that the probability of disease in a group positive for the particular analysed risk factor is higher than in a group negative for this factor. Correlations between polymorphic variants and disease were assessed on the basis of OR values in either univariate analysis or a multivariate model of logistic regression that included conventional risk factors such as smoking, increased serum concentrations of total and LDL cholesterol, as well as triglycerides, overweight and obesity. Analysis of interactions between genetic and conventional risk factors was performed using synergy indices, according to the following formulae:

- in additive model (Rotman's synergy index) [9]:

$$SI = OR_{gtr} - 1/(OR_{tr} - 1) + (OR_g - 1)$$

- in multiplicative model (Khoury's synergy index) [10]:

$$SIM = OR_{gtr}/ORtr \times OR_g$$

where:

SI – synergy index in additive model,
SIM – synergy index in multiplicative model,
OR_{gtr} – odds ratio for the disease among individuals with genetic as well traditional risk factor,
OR_g – odds ratio for the disease among individuals with only genetic risk factors,
OR_{tr} – odds ratio for the disease among individuals with only traditional risk factors,

SIM >1 indicates the presence of synergy between genetic and traditional risk factors.

Results

Clinical and biochemical characteristics

Clinical and biochemical characteristics of the examined group are shown in the Table I. Patients with IHD had significantly higher serum level of total cholesterol, LDL cholesterol and triglycerides, body mass index (BMI) and proportion of smokers.

In the IHD group, individuals with a history of MI made up 80% of all patients, including 10% having had more than one MI. In more than half of examined patients, occlusion of an infarct-related artery (62.5%) or arterial hypertension (57.9%) was noted. A relatively low percentage of patients presented with comorbidities such as diabetes mellitus (6.4%), peripheral artery disease (9.3%) or ischaemic stroke (0.7%). Approximately half of studied patients (42%) received lipid-lowering drugs (statins) before the study.

Analysis of relation between *apo E* gene polymorphism and IHD

Distribution of *apo E* gene genotypes was consistent with the Hardy-Weinberg's principle. Subjects possessing at least one analysed allele, i.e. being heterozygotic or homozygotic with respect to a given allele, were defined as carriers.

In the IHD group, a trend towards a higher prevalence of ε4 allele was found, and more such allele carriers was identified. However, the differences did not reach statistical significance (Table II).

Analysis of interaction between genetic and conventional risk factors

According to the most recent recommendations on prevention of cardiovascular disease [11], the following conventional IHD risk factors were analysed: smoking, TC ≥5 mmol/l, LDL ≥3 mmol/l, TG >1.7 mmol/l and BMI ≥25. Decreased HDL concentration was not analysed because in the examined population this factor was not shown to be significantly associated with disease.

We found the synergistic effects between ε4 allele and smoking, increased total cholesterol and LDL cholesterol level in determining the IHD risk (Table III).

Table II. Comparison of genotype and *apo E* gene allele distribution between the IHD and control group

Gene	Genotype/allele	IHD (n=140)	Control (n=107)	p
apo E	ε3ε3	98 (70%)	82 (77%)	NS
	ε3ε4	29 (21%)	14 (13%)	NS
	ε3ε2	10 (7%)	10 (9%)	NS
	ε4ε4	2 (1%)	0 (0%)	NS
	ε2ε4	1 (1%)	1 (1%)	NS
	ε2ε2	0 (0%)	0 (0%)	NS
	ε3	235 (84%)	188 (88%)	NS
	ε4	34 (12%)	15 (7%)	NS
	ε2	11 (4%)	11 (5%)	NS

	Univariate analysis				Multivariate analysis			
	OR	95% CI	p	χ^2	OR	95% CI	p	χ^2
ε4+ε4ε4+ε2ε4 / ε3ε3+ε3ε2+ε2ε2	1.82	0.88-3.77	0.080	3.07	1.25	0.55-2.80	0.59	3.07
ε4 / ε3	1.83	0.93-3.64	0.060	3.58				

Table III. Synergistic effects between ε4 allele carriers and conventional IHD risk factors

ε4 allele carrier-state	Conventional risk factor	IHD Group	Control Group	OR (95% CI)	SIM	SI
	Smoking	(n=118)	(n=100)			
0	0	30	58	1		
0	1	60	27	4.30 (2.18-8.53)	1.7	2.17
1	0	6	10	1.16 (0.33-3.93)		
1	1	22	5	8.51 (2.68-28.72)		
	LDL ≥3 mmol/l	(n=133)	(n=97)			
0	0	28	33	1		
0	1	73	49	1.76 (0.90-3.42)	1.1	1.54
1	0	5	4	1.47 (0.30-7.39)		
1	1	27	11	2.89 (1.13-7.54)		
	TC ≥5 mmol/l	(n=135)	(n=97)			
0	0	30	37	1		
0	1	73	45	2.0 (1.04-3.85)	1.8	2.62
1	0	5	6	1.03 (0.24-4.33)		
1	1	27	9	3.70 (1.39-10.03)		
	TG >1.7 mmol/l	(n=134)	(n=97)			
0	0	47	58	1		
0	1	55	24	2.83 (1.46-5.49)	0.46	0.64
1	0	15	8	2.31 (0.83-6.59)		
1	1	17	7	3.0 (1.06-8.77)		
	BMI ≥25	(n=131)	(n=94)			
0	0	37	41	1		
0	1	64	38	1.87 (0.98-3.55)	0.92	1.23
1	0	14	9	1.72 (0.61-4.95)		
1	1	16	6	2.95 (0.95-9.54)		

Abbreviations: SIM – Khoury's synergy index, SI – Rotman's synergy index, TC – total cholesterol, TG – triglyceride, BMI – body mass index

Table IV. Serum lipid concentrations in relation to genotype

Genotype	Total cholesterol [mmol/l]	LDL-cholesterol [mmol/l]	HDL-cholesterol [mmol/l]	Triglyceride [mmol/l]
ε3ε3	5.77±1.41	3.90±1.18	1.17±0.50	1.79±0.92
ε3ε4+ ε4ε4	5.97±1.38	4.07±1.20	1.13±0.29	1.94±1.13
ε3ε2+ ε3ε2	5.63±1.43	3.54±1.50	1.10±0.26	2.29±1.47

Analysis of *apo E* gene polymorphism effects on serum lipid level

No significant differences in serum lipid levels were found between patients with various genotypes (Table IV). However, a trend towards elevated total cholesterol as well as LDL cholesterol was observed in the carriers of ε4 alleles and a reversed trend in the carriers of ε2 alleles (because of the reversed impact of ε2 and ε4 alleles, genotype ε2ε4 was not analysed). The carriers of either ε4 or ε2 alleles presented a slightly elevated level of triglycerides in comparison to individuals with ε3ε3 genotype.

Discussion

In this study, no significant relation between epsilon polymorphism of the *apo E* gene and IHD was noted. However, a trend towards a higher prevalence of ε4 allele and carriers of this allele was observed among patients with IHD than in a group of healthy blood donors. These differences were close to reaching a significant level (p=0.06 and p=0.08, respectively). The control group comprised voluntary blood donors with a negative history of IHD. For ethical and financial reasons, blood donors did not undergo angiography and so it was not possible to rule out that some of them had coronary atherosclerotic lesions not severe enough to be manifested clinically. To minimise the bias, an additional inclusion criterion for the control group was negative family history of premature IHD and no history of cerebral ischaemia.

None of the previous studies involving the Polish population documented any association between *apo E* gene polymorphism and IHD [12]. They included 170 randomly selected patients, of these 61 suffering from IHD. The IHD diagnosis was based only on clinical presentation and ECG recordings. Thus, the studies could have also included IHD patients with other than atherosclerotic aetiology. Moreover, the examined group of patients consisted of subjects aged between 41 and 69 years (mean 62 years), i.e. markedly older than individuals analysed in our study. Although these studies used completely different methodologies, no differences in allele distribution throughout the examined populations were seen.

Epsilon polymorphism of the *apo E* gene is probably one of the most extensively studied polymorphisms with respect to its association with IHD. The results are not consistent; however, the most recent meta-analysis of 48 reports including 15 492 patients and 32 965 controls has shown that the ε4 allele has a modest but significant impact on the IHD risk [13]. The relationship between the ε4 allele and IHD may not only be a result of the effects of this polymorphism on serum lipid level [14], but also of extensive pleiotropic effects of the *apo E* gene [5]. The discrepant results of various studies on the contribution of the *apo E* gene polymorphism to IHD risk may, apart from methodological differences, be caused by ethnic and geographical variability as well as disease heterogeneity. This means that various groups of patients may possess different sets of genetic factors determining disease manifestation. Thus, some polymorphisms may be strongly associated with disease in one population while in another their contribution may be less pronounced due to the presence of different genetic as well as environmental risk factors. Thus, an analysis of a given phenotype in a specific patient population, as well as simultaneous evaluation of several genes and their interactions with factors other than genetic, seem justified. Earlier we reported [15] that coexistence of *apo E* gene ε4 and allele G of the ICAM1 gene (polymorphism K469E) was a powerful IHD risk factor, and that if analysed alone neither of these two was significantly associated with the disease.

In the present study we found that ε4 allele carrier state, if accompanied by smoking, had a more powerful impact on IHD risk than each of these factors alone. Similar synergistic effects were noted between ε4 allele carrier state and an increased level of total cholesterol and to a lesser degree of LDL cholesterol concentration. Interactions between *apo E* genotype and smoking were observed in previous studies. For example, a synergic impact of ε4 allele and smoking on the development of carotid artery atherosclerosis was observed [16]. Moreover, Humphries et al. [17] found an increased risk

of coronary artery disease in smoking men, especially among ε4 allele carriers. The interaction mechanism of *apo E* genotype and smoking is probably associated with increased oxidative stress and lipoprotein oxidation in smokers. Carriers of the ε4 allele have elevated LDL cholesterol concentration, and smoking may enhance oxidation of these lipoproteins, and in consequence increase the risk of atherosclerosis.

Synergic effects between ε4 allele carrier state and cholesterol concentration are probably related to the influence of epsilon polymorphism on serum lipid levels. Each particular Apo E isoform has a different affinity to the family of LDL receptors, resulting in different lipid levels related to a given genotype. Earlier studies documented that ε2 allele carriers had decreased total and LDL cholesterol concentrations in comparison to ε3ε3 homozygotes. Carriers of the ε4 allele express a trend towards higher TC and LDL concentrations than ε3ε3 individuals. Moreover, higher triglyceride level is associated with both ε2 and ε4 alleles compared to ε3ε3 homozygotes [14]. Although in our study no significant differences in serum lipid levels were seen with respect to genotype, the same trends as those mentioned earlier were observed. The lack of statistical significance may be a result of coexistence of many non-genetic environmental risk factors (e.g. diet), not analysed in this study, which have an impact on serum lipid levels. Moreover, approximately 50% of patients were on lipid-lowering drugs, which could significantly modify serum lipid levels, and this undoubtedly could have influenced our findings. An analysis of the interaction between lipid level and genotype of patients not receiving such therapy did not reveal any significant differences either. These data were not included in this report due to the small number of subjects in some classes of genotypes, limiting the reliability of the findings.

The additional main purpose of the studies on genetic background of IHD is to better understand disease pathogenesis and to assess susceptibility to IHD in early childhood, when phenotypic manifestation is still absent. However, methods to be useful for such evaluation must be superior to the tests assessing only conventional risk factors. In the case of atherosclerosis and IHD, measurement of lipid metabolism indices and assessment of risk factors such as smoking or overweight are currently much cheaper and easier to perform than individual genotyping. In the future, the costs of genetic analysis will certainly be reduced, but weak correlations between genetic polymorphisms and disease would remain problematic. Our previous and current studies indicate that the impact of suspected gene polymorphisms on IHD risk may be much stronger in certain subpopulations, such as subjects with specific genetic predispositions, and those exposed to certain environmental risk factors. We belive that future multivariate analyses of genetic as well as environmental factors may be important in risk stratification and IHD prevention.

Conclusions

The results of our study indicate the presence of a synergistic impact of ε4 allele carrier state and conventional IHD risk factors, such as smoking, increased total cholesterol concentration and, to a lesser degree, LDL cholesterol level, on the risk of premature IHD.

References

1. Stamler J, Daviglus ML, Garside DB, et al. Relationship of baseline serum cholesterol levels in 3 large cohorts of younger men to long-term coronary, cardiovascular, and all-cause mortality and to longevity. *JAMA* 2000; 284: 311-8.
2. Vasankari T, Ahotupa M, Toikka J, et al. Oxidized LDL and thickness of carotid intima-media are associated with coronary atherosclerosis in middle-aged men: lower levels of oxidized LDL with statin therapy. *Atherosclerosis* 2001; 155: 403-12.
3. Carpenter KL, Taylor SE, van der Veen C, et al. Lipids and oxidised lipids in human atherosclerotic lesions at different stages of development. *Biochim Biophys Acta* 1995; 1256: 141-50.
4. Despres JP, Lemieux I, Dagenais GR, et al. HDL-cholesterol as a marker of coronary heart disease risk: the Quebec cardiovascular study. *Atherosclerosis* 2000; 153: 263-72.
5. Balcerzyk A, Żak I. Apolipoprotein E – the role of polymorphism in pathogenesis of many diseases. *Postepy Biochem* 2004; 50: 344-52.
6. Myocardial infarction redefined – a consensus document of The Joint European Society of Cardiology/American College of Cardiology Committee for the redefinition of myocardial infarction. *Eur Heart J* 2000; 21: 1502-13.
7. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972; 18: 499-502.
8. Hixson JE, Vernier DT. Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with Hhal. *J Lipid Res* 1990; 31: 545-8.
9. Rothman KJ. Synergy and antagonism in cause-effect relationships. *Am J Epidemiol* 1974; 99: 385-8.
10. Khoury MJ, Flanders WD. Nontraditional epidemiologic approaches in the analysis of gene-environment interaction: case-control studies with no controls! *Am J Epidemiol* 1996; 144: 207-13.
11. De Backer G, Ambrosioni E, Borch-Johnsen K, et al. European guidelines on cardiovascular disease prevention in clinical practice. Third Joint Task Force Of European and other societies on cardiovascular disease prevention in clinical practice (constituted by representatives of eight societies and by invited experts). *Arch Mal Coeur Vaiss* 2004; 97: 1019-30.

12. Bednarska-Makaruk M, Broda G, Kurjata P, et al. Apolipoprotein E genotype, lipid levels and coronary heart disease in a Polish population group. *Eur J Epidemiol* 2001; 17: 789-92.
13. Song Y, Stampfer MJ, Liu S. Meta-analysis: apolipoprotein E genotypes and risk for coronary heart disease. *Ann Intern Med* 2004; 141: 137-47.
14. Eto M, Watanabe K, Ishii K. Reciprocal effects of apolipoprotein E alleles (epsilon 2 and epsilon 4) on plasma lipid levels in normolipidemic subjects. *Clin Genet* 1986; 29: 477-84.
15. Żak I, Balcerzyk A, Sarecka B, et al. Contemporaneous carrier-state of two or three 'proatherosclerotic' variants of APOE, ICAM1, PPARA and PAI-1 genes differentiate CAD patients from healthy individuals. *Clin Chim Acta* 2005; 362: 110-8.
16. Djousse L, Myers RH, Province MA, et al. Influence of apolipoprotein E, smoking, and alcohol intake on carotid atherosclerosis: National Heart, Lung, and Blood Institute Family Heart Study. *Stroke* 2002; 33: 1357-61.
17. Humphries SE, Talmud PJ, Hawe E, et al. Apolipoprotein E4 and coronary heart disease in middle-aged men who smoke: a prospective study. *Lancet* 2001; 358: 115-9.

Synergiczny wpływ polimorfizmu epsilon genu apolipoproteiny E oraz niektórych tradycyjnych czynników ryzyka na przedwczesną chorobę niedokrwienną serca

Anna Balcerzyk[1], Iwona Żak[1], Jolanta Krauze[2]

[1] Zakład Biochemii i Genetyki Medycznej, Śląski Uniwersytet Medyczny, Katowice
2 I Katedra i Klinika Kardiologii, Śląski Uniwersytet Medyczny, Katowice

Streszczenie

Wstęp: Choroba niedokrwienna serca (IHD) jest najczęstszą jednostką kliniczną, której fenotyp ujawnia się w następstwie postępującej miażdżycy tętnic wieńcowych. Obok tradycyjnych czynników ryzyka IHD, takich jak palenie papierosów, hipercholesterolemia czy otyłość, podatność na chorobę mogą również modulować czynniki genetyczne. Warianty polimorficzne genów, kodujące izoformy zaangażowane w procesy istotne z punktu widzenia patogenezy miażdżycy, mogą różnicować genetycznie populację ludzką i warunkować zmienność wewnątrz gatunku, w tym również pod względem podatności na chorobę. Niewątpliwie jednym z najważniejszych czynników odpowiedzialnych za powstawanie i rozwój zmian miażdżycowych są zaburzenia w transporcie i metabolizmie lipidów. Jednym z ważniejszych genów związanych z metabolizmem lipidów jest gen kodujący apolipoproteinę E (Apo E), która jest składnikiem chylomikronów oraz lipoprotein VLDL i pełni funkcję liganda dla receptorów LDL i LRP, regulując transport i metabolizm lipidów. Apolipoproteina E bierze ponadto udział w usuwaniu cholesterolu z komórek i wykazuje szereg efektów plejotropowych. Ze względu na wieloczynnikowy charakter IHD udział pojedynczych wariantów polimorficznych w kształtowaniu ryzyka choroby jest stosunkowo niewielki, a w związku z tym ich analiza dotychczas nie ma większego znaczenia praktycznego. Wydaje się jednak, że rola określonych wariantów polimorficznych może wzrastać w obecności określonego tła genetycznego lub środowiskowego. Zasadna jest więc wieloczynnikowa analiza obejmująca zarówno czynniki genetyczne, jak i środowiskowe.

Cel: Wyjaśnienie, czy istnieje związek pomiędzy polimorfizmem genu *apo E* a wczesną IHD w populacji polskiej oraz czy genotyp *apo E* moduluje wpływ tradycyjnych czynników ryzyka na chorobę.

Metodyka: Badaniami objęto 247 osób rasy kaukaskiej, w tym: 140 pacjentów z angiograficznie potwierdzoną IHD oraz 107 dobrowolnych dawców krwi bez historii IHD. Warianty polimorficzne analizowano techniką RFLP-PCR (*Restriction Fragments Lenght Polymorphism – Polymerase Chain Reaction*). DNA izolowano z limfocytów krwi obwodowej. Polimorfizm epsilon genu *apo E* analizowano metodą opisaną przez Hixsona i wsp. z własnymi modyfikacjami. W badanych grupach analizowano ponadto następujące tradycyjne czynniki ryzyka IHD: nikotynizm, poziom cholesterolu całkowitego (TC) ≥5 mmol/l, poziom cholesterolu LDL ≥3 mmol/l, poziom trójglicerydów (TG) >1,7 mmol/l i indeks masy ciała (BMI) ≥25. Oznaczenia stężeń TG, TC i cholesterolu HDL w surowicy wykonano metodami enzymatycznymi. Uzyskane dane analizowano przy użyciu programu STATISTICA 6.0 oraz EpiInfo-6 (WHO).

Wyniki: W grupie osób z IHD obserwowano tendencję do częstszego w porównaniu z grupą krwiodawców występowania allela ε4 i nosicieli tego allela. Różnice te nie były jednak istotne statystycznie. Stwierdzono synergiczny wpływ nosicielstwa allela ε4 oraz palenia papierosów, podwyższonego poziomu TC, a także, w mniejszym stopniu, cholesterolu LDL na ryzyko IHD. Analizowano również wpływ polimorfizmu genu *apo E* na poziom lipidów osocza. Nie stwierdzono znamiennych statystycznie różnic w poziomie lipidów osocza w zależności od genotypu. Obserwowano jednak tendencję do podwyższonego u nosicieli allela ε4 i obniżonego u nosicieli allela ε2 stężenia TC i cholesterolu LDL. Nosiciele zarówno allela ε4, jak i ε2 charakteryzowali się nieco wyższym poziomem TG w porównaniu z osobnikami o genotypie ε3ε3.

Wnioski: Przedstawione wyniki wskazują na synergiczny wpływ nosicielstwa allela ε4 oraz tradycyjnych czynników ryzyka, takich jak palenie papierosów, podwyższony poziom TC oraz, w mniejszym stopniu, poziom cholesterolu LDL na kształtowanie ryzyka wczesnej IHD.

Słowa kluczowe: apolipoproteina E, choroba niedokrwienna serca, tradycyjne czynniki ryzyka

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Adres do korespondencji:

dr n. med. Anna Balcerzyk, Zakład Biochemii i Genetyki Medycznej, Śląski Uniwersytet Medyczny, ul. Medyków 18, 40-752 Katowice, tel.: +48 32 252 84 32, e-mail: abalcerzyk@slam.katowice.pl

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