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Angiotensin-converting enzyme gene insertion/deletion polymorphism in Polish patients with myocardial infarction

Myocardial infarction occurs in more than 90,000 Polish people each year. Almost 50% of them do not survive even first few hours and another 30% one month after its onset.

Both environmental and genetic factors play an important role in the pathogenesis of coronary artery disease. Most of the environmental factors as cigarette smoking, overfeeding, lack of physical activity and social stress are well recognised. We are still far from fully understanding of genetic mechanisms contributing to susceptibility to myocardial infarction. Probably this predisposition is influenced by interaction of several genetic loci. Among many genes involved in its pathogenesis are those coding vasoactive agents, components of lipid metabolism pathways as well as factors affecting coagulation and fibrinolysis.

In view of angiotensin-converting enzyme (ACE) role in physiology and pathology and benefits of ACE-inhibitors therapy, the gene coding this protein meets criteria for candidate gene for the susceptibility to cardiovascular disease including myocardial infarction. ACE gene was cloned and mapped to the long arm of chromosome 17 (17q23). The insertion/deletion (I/D) polymorphism of a 287 base pair sequence, located in intron 16 has been extensively studied over the past decade (10). This polymorphism has a major effect on plasma and cellular enzyme activity. Genotype DD is associated with about 50% higher plasma ACE activity (11) . It was hypothesised that insertion variant (I allele) contains the so-called silencer sequence that causes a decrease in ACE gene expression. The associations of this polymorphism with left ventricle hypertrophy, diabetic nephropathy or male infertility were confirmed by many authors (2-4). Studies concerning insertion/deletion polymorphism in ACE gene locus and essential hypertension have brought inconsistent observations (8-9). Even more discrepant results were published on the subject of its association with myocardial infarction (1, 12).

The goal of our research was to assess the association of ACE gene I/D polymorphism with myocardial infarction in the Polish population regarding different variables related to the disease (smoking, concomitant hypertension, obesity, plasma cholesterol level and family history).

MATERIAL

The study population consisted of 314 individuals of Polish origin. 178 of them suffered from acute myocardial infarction or have survived it. Another 136 people without apparent signs and symptoms of cardiovascular pathology or diabetes served as control subjects. The diagnosis of myocardial infarction was made upon conventional clinical, electrocardiographic and enzymatic criteria or was well documented in patients clinical files. All subjects were hospitalised in the Department of Internal Diseases, Medical University of Lublin between 1995 and 2000 for different reasons.

Positive family history considered as the presence of coronary artery disease in at least one of first degree relatives was reported by 85 experimental subjects. No one from the control group reported positive family history. Detailed characteristics of the study population are shown in Table 1. Patient's informed consent was mandatory to enter the study. The study protocol was approved by local Bioethical Committee.

Table 1. Characteristics of the studied population

	Patients	Control
Number of subjects	178	136
Men	131 (74%)	50 (38%)
Women	47 (26%)	86 (62%)
Mean age	48.8	49.1
Number of subjects under 50 years	100 (56%)	74 (54%)
Number of subjects with hypertension	64 (36%)	0
Number of subjects with BMI $\leq$ 25kg/m^2	40 (22%)	107 (79%) *
Number of subjects with < 25kg/m^2 < BMI $\leq$ 30kg/m^2	87 (49%) *	23 (17%)
Number of subjects with BMI > 30kg/m^2	51 (29%) *	6 (4%)
Positive family history	85 (48%) *	0
Mean plasma total cholesterol concentration (mg/dl)	209 mg/dl *	171 mg/dl
Number of smokers	147 (83%) *	54 (40%)

*$p < 0.05$

METHODS

After obtaining an informed consent 15 ml of venous blood were drawn into EDTA tube. Standard procedure for genomic DNA preparation from peripheral blood leukocytes was used (7).

ACE GENOTYPING

To detect ACE I/D method of Rigat et al. (10) with some modification was used. Polymerase chain reaction (PCR) with specific primers (sense: 5'-GAT GTG GCC ATC ACA TTC GTC AGA-3' and antisense: 5'-CTG GAG ACC ACT CCC ATC CTT TCT-3') was performed in 50 μl volume. The reaction mixture contained 500 ng DNA, 1 unit of Taq polymerase in standard buffer with addition of 220pM of each primer, 2.5mM of each deoxynucleotide (dATP, dGTP, dCTP and dTTP) 1.5mM $MgCl_2$ (all reagents from MBI Fermentas) and 1.5% dimethylsulfoxide (DMSO). DNA amplification was performed in PTC-200 thermocycler MJ Research. After initial denaturation at 94° C for 6 min, 30 cycles followed consisting of denaturation at 94° C for 1 min, annealing at 60° C for 1 min. and chain elongation at 72° C for 1 min. A final extension was at 72°C for 7 min. PCR products were electrophoresed on 2% agarose gel (Prona) and visualised by ultraviolet transillumination after ethidium bromide staining. In the presence of insertion (allele I) 490bp band was seen, while 190bp band appeared in case of deletion (allele D). Polymorphic alleles and genotypes are shown in Figure 1.

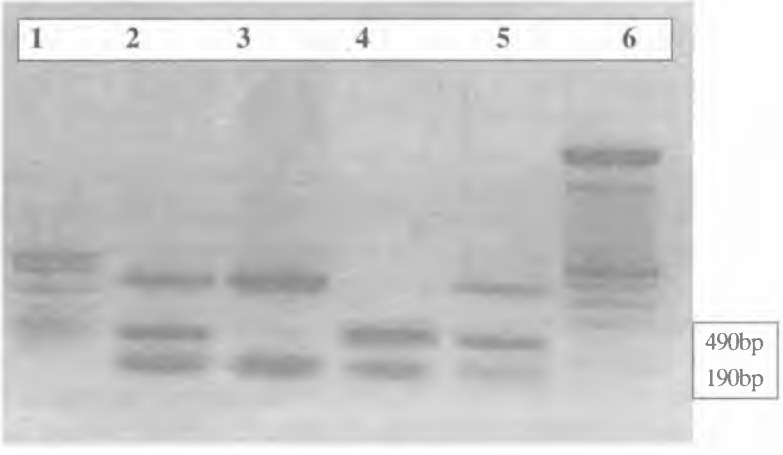

Fig. 1. Insertion/deletion polymorphism of the ACE gene. 490bp band reflecting I allele, 190bp band reflecting D allele; lanes 1 and 6 – DNA size markers; lanes 2 and 5 – ID genotype; lane 3 – genotype II; lane 4 – DD genotype (1.5% agarose gel under UV light)

To avoid mistyping of the DD genotype, second run PCR with insertion specific primers (sense: 5'-TGG GAC CAC AGC GCC CGC CAC TAC-3' and antisense: 5'-TCG CCA GCC CTC CCA TGC CCA TAA-3') was performed by the following method previously described by Odawara et al. (11).

STATISTICAL ANALYSIS

Differences in allele frequencies and genotype distribution between cases and controls were analysed by χ^2- statistic with different degrees of freedom. t–Student statistics was used for case and control population characteristics. (p) values less than 0.05 were considered significant.

RESULTS

Polymorphic allele frequencies and genotype distribution are shown in Table 2. They were similar to those reported in other Caucasian populations. Both allele frequencies and genotype distribution did not differ substantially between study groups.

Table 2. Insertion/deletion allele and genotype occurrence in experimental and control subjects

Study group	Number		Alleles		Genotypes		
	subjects	alleles	I	D	DD	ID	II
Patients 50 years old	100	200	0.45	0.55	0.20	0.70	0.10
Patients > 50 years old	78	156	0.50	0.50	0.14	0.72	0.14
All patients	178	356	0.47	0.53	0.17	0.71	0.12
Control	136	272	0.45	0.55	0.23	0.65	0.12
			p>0.56		p>0.45		

Experimental subjects were then divided into two subgroups depending on the age of the onset of myocardial infarction. The occurrence of the disease before the age of 50 was considered as premature. Allele frequencies and genotype distribution in these subgroups are shown in Table 2. The observed differences between the two subgroups and control subjects were not statistically significant.

Further analysis did not detect any important differences in allele and genotype frequencies between experimental and control subjects regarding body mass index, total

plasma cholesterol level and concomitant arterial hypertension. In experimental subjects with negative family historyof coronary artery disease D allele and DD genotype occurred more often than in myocardial infarction patients reporting positive family history. However, the difference was not statistically significant ($p>0.5$) – see Table 3. Cigarette

Table 3. Polymorphic allele frequencies and genotype distribution in myocardial infarction subjects with positive and negative family history

Patients	Number subjects	Number alleles	Alleles		Genotypes		
			I	D	DD	ID	II
Positive family history	85	170	0.49	0.51	0.14	0.73	0.13
Negative family history	93	186	0.45	0.55	0.23	0.66	0.11
			p>0.42		p>0.61		

smoking status influenced allele frequencies and genotype distribution in the group of patients with premature myocardial infarction. DD genotype was observed more often among non-smoking patients who suffered from myocardial infarction before the age of 50 (DD genotype 0.40 vs. 0.16 respectively). These difference had reached the level of statistical significance ($p<0.05$).

DISCUSSION

Well known environmental factors such as cigarette smoking, lack of physical activity or social stress may lead to the onset of myocardial infarction. Undoubtedly genetic factors affect the susceptibility to this life threatening condition. Even after the completion of Human Genome project the nature of the genes involved in this process remain unrecognised, although tremendous progress was made in this field over the past few years. There is a strong need of elucidation of genetic mechanisms contributing to this disease.

The results published in the early 1990's strongly suggested the association of ACE gene I/D polymorphism with myocardial infarction. The presence of D variant had been even accepted as an independent risk factor of cardiovascular pathology. Several years later it was found that polymorphism detection method may cause mistyping in favour of D allele (8). After introducing some modifications to the method of I/D genotype detection studies had started bringing inconsistent results. Large meta-analysis performed by Keavney et al. in a group of about 5,000 cases and 6,000 controls did not confirm the existence of any important association of ACE gene I/D polymorphism with myocardial

infarction in general Caucasian population (6). The results presented above do not account for this association in the studied Polish population either.

Probably the susceptibility to myocardial infarction is influenced by several genetic loci. The effect of ACE gene could remain small or moderate in general population but may be stronger in a particular group of people. Therefore, the assessment of this association was performed depending on such variables as: the age of onset, family history, body mass index, plasma cholesterol level, concomitant hypertension and cigarette smoking habit. The only statistically important association found in our study was influenced by cigarette smoking status of the experimental subject with premature myocardial infarction. DD genotype occurred more often in non-smoking premature myocardial infarction patients when compared to the group of smokers. Cigarette smoking is a potent factor causing oxidative stress and decreasing nitric oxide synthesis that leads to endothelial dysfunction with its clinical consequences and myocardial infarction among them. DD variant of ACE gene is associated with higher plasma and cellular angiotensin-converting enzyme concentrations. This enzyme reveals also kininase activity. Extensive bradykinin break-down occurs in D allele bearers. Bradykinin is not only a strong vasodilator but also a stimulator of endothelial nitric oxide synthesis. Lower bradykinin concentrations may lead to endothelial dysfunction. This could be one of mechanisms that predispose DD genotype bearers to premature coronary artery disease even when endothelial cells are not jeopardised by cigarette smoking.

Since about half of deaths from acute myocardial infarction occur in the first few hours, before admission to hospital, the experimental group lacks subjects of the highest risk. The presented results need to be confirmed on a larger group of subjects in prospective studies including patients who lose their lives in the first hours of myocardial infarction.

CONCLUSIONS

1. Insertion/deletion polymorphism in ACE gene does not seem to be associated with myocardial infarction in the studied Polish population.
2. The obtained results suggest that DD genotype may be associated with smoking-dependent risk of myocardial infarction. Further studies are needed to prove this hypothesis.

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SUMMARY

Both environmental and genetic factors play an important role in the pathogenesis of coronary artery disease. In view of angiotensin-converting enzyme (ACE) role in physiology and pathology and benefits of ACE-inhibitors therapy, the gene coding this protein meets criteria for candidate gene for the susceptibility to cardiovascular disease including myocardial infarction. The goal of our research was to assess the association of ACE gene I/D polymorphism with myocardial infarction in Polish population regarding differ-

ent variables related to the disease (smoking, concomitant hypertension, obesity, plasma cholesterol level and family history). To detect ACE I/D polymorphism method of Rigat with some modification was used. The study population consisted of 314 individuals of Polish origin. 178 of them suffered from acute myocardial infarction or have survived it. Another 136 people without apparent signs and symptoms of cardiovascular pathology or diabetes served as control subjects. No one from the control group reported positive family history. Both allele frequencies and genotype distribution did not differ substantially between study groups. They were similar to those reported in other Caucasian populations. Further analysis did not detect any important differences in allele and genotype frequencies between experimental and control subjects regarding body mass index, total plasma cholesterol level and concomitant arterial hypertension. Cigarette smoking status influenced allele frequencies and genotype distribution in the group of patients with premature myocardial infarction. DD genotype was observed more often among non-smoking patients who suffered from myocardial infarction before the age of 50. These differences had reached the level of statistical significance ($p<0.05$). Conclusions: 1. Insertion/deletion polymorphism in ACE gene does not seem to be associated with myocardial infarction in the studied Polish population. 2. The obtained results suggest that DD genotype may be associated with smoking-dependent risk of myocardial infarction. Further studies are needed to prove this hypothesis.

Polimorfizm insercyjno-delecyjny genu enzymu konwertującego w polskiej populacji pacjentów z zawałem serca

Do wystąpienia zawału serca predysponują dobrze znane czynniki środowiskowe jak również pewne uwarunkowania genetyczne. Gen kodujący enzym konwertujący angiotensynę II jest genem kandydatem w patogenezie chorób układu sercowo-naczyniowego. Celem pracy była ocena związku polimorfizmu insercyjno-delecyjnego genu ACE z zawałem serca w populacji polskiej z uwzględnieniem wpływu środowiskowych czynników ryzyka. Polimorfizm wykrywano stosując reakcję łańcuchową polimerazy. Grupę badaną stanowiło 178 osób ze świeżym bądź przebytym zawałem serca, a grupę kontrolną 136 osób bez uchwytnych objawów schorzeń układu sercowo-naczyniowego. Częstość występowania polimorficznych alleli i genotypów nie różniła się istotnie między grupą osób z zawałem a grupą kontrolną i była zbliżona do obserwowanych w innych populacjach rasy kaukaskiej. Nie obserwowano również związku polimorfizmu I/D z zawałem serca po uwzględnieniu takich czynników, jak wiek wystąpienia zawału, wywiad rodzinny, hipercholesterolemia, wskaźnik masy ciała oraz współistniejące nadciśnienie tętnicze. Stwierdzono, że u niepalących osób z zawałem serca genotyp DD występował znacznie częściej niż u palących zawałowców ($p<0.05$). Wnioski: 1. Polimorfizm insercyjno-delecyjny genu ACE nie jest związany z zawałem serca w badanej populacji polskiej. 2. Uzyskane wyniki sugerują możliwość związku genotypu DD z ryzykiem zawału serca związanym z paleniem tytoniu.