Interleukin-1β and interleukin-1 receptor inhibitor gene cluster polymorphisms in patients with coronary artery disease after percutaneous angioplasty or coronary artery bypass grafting

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Abstract

Background: Pro-inflammatory cytokine interleukin-1β (IL-1β) plays a role in atherosclerosis. The results of several studies on the association between polymorphism of the IL-1β gene cluster and the course of coronary atherosclerosis have been inconclusive.

Aim: To investigate retrospectively whether the patients with the most common variants of polymorphism of the IL-1β gene cluster differ with respect to localisation and extent of coronary atherosclerosis to a degree which may influence the treatment strategy.

Methods: Ninety-two consecutive out-patients (age 39-83, male sex 74%) with coronary artery disease confirmed by angiography were included. In this group, 23 patients underwent coronary artery bypass grafting (CABG) and 69 percutaneous coronary interventions (PCI) of whom in 16 repeated treatment was performed. The polymorphisms of the IL-1β gene – transition C/T at -511 and -31 position – as well as of the IL-1 receptor antagonist gene (IL-1RN) – an 86-base pair variable-number tandem repeat in intron 2 – were determined by PCR. Out of the 54 theoretically possible combinations of polymorphisms, 17 were found in the studied group. The three most common combinations of polymorphisms were selected. The fraction of patients treated by means of primary or elective percutaneous coronary intervention (pPCI, ePCI) and by means of CABG were compared between the subgroups with one of the 3 most common combinations of polymorphisms.

Results: The most frequent combinations of polymorphisms were – Variant A: -31 C/T, -511C/T, RN 1/1 – 32.6%; Variant B: -31T/T, -511C/C, RN 1/1 – 27.1%; Variant C: -31C/T, -11C/T, RN 1/2 – 10.8%. The remaining patients (29.5%) represented 14 variants present in very small subgroups consisting only of 1, 2 or 3 persons. Statistical analysis showed that patients with the second most common variant of studied polymorphisms (variant B) were significantly more frequently treated with CABG in comparison to the two other variants. Also, repeated PCI was most frequent in this subgroup.

Conclusion: The data presented here suggest that carriers of the two relatively frequent variants of the IL-1β gene at -31 and -511 position, i.e. -31TT and -511CC, are at a higher risk of developing coronary artery disease requiring surgical treatment or two-stage percutaneous angioplasty.

Key words: gene polymorphism, interleukin-1β, coronary artery disease, percutaneous angioplasty, CABG

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Introduction

Studies dealing with development and progression of coronary artery atherosclerosis in adult identical twins brought up after adoption in different environments as well as an analysis of the disease prevalence among close relatives indicated the significance of hereditary factors in the aetiology of coronary artery disease (CAD) [1-3]. This has resulted in a growing interest in the genetic markers associated with the typical risk factors of atherosclerosis development. A number of studies published in the last decades documented the importance of inflammation in atherosclerosis promotion. Attention was also paid to the role of the cellular signalling pathways involving CD14 receptors and TLR (Toll-like receptors) [4, 5]. The importance of interleukin-1β (L-1β) as a stimulating mediator of interleukin-6 (IL-6), fibrinogen, C-reactive protein (CRP)

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or adhesive molecules expression by endothelial cells within cascade leading to development and destabilisation of the atherosclerotic plaque has been suggested [6-8]. It is thought that this locally produced cytokine stimulates the smooth muscle cells within atherosclerotic plaque and inhibits proliferation of the vascular endothelial cells [9]. Moreover, attention was also paid to the significance of IL-1 (IL-1RN) antagonist receptor tissue concentration, and thus various course of the series of chronic inflammatory diseases such as rheumatoid arthritis and inflammatory bowel disease [10, 11]. Many studies have shown that polymorphism of the genes associated with biosynthesis of the aforementioned factors, although not related to the presence of CAD, may modulate its development and dynamics. It is worth mentioning that IL-1β is a mediator, which, when released is associated with chronic Gram--negative bacterial infection – pathogens that are able to synthesise lipopolysaccharide (LPS). For many years, attempts have been made to find any causal relationship between LPS and atherosclerosis development [12, 13].

These observations encouraged us to examine the hypothesis that coexistence of variants of two IL-1β gene polymorphisms (changing nucleotides containing cytosine and thymine (C and T) at positions -31 and -511) as well as a third polymorphism of the penta-allelic, 86-bp variable-number tandem repeat in the gene of IL-1 receptor antagonist (IL-1RN) may determine the extent and location of atherosclerosis within the coronary arteries as well as the strategy of either surgical or percutaneous management of CAD. Although several factors, such as patient preference, diabetes mellitus coexistence, the rate of restenosis following previous angioplasty procedures, and other concomitant disorders, have an impact on the choice between the two invasive therapeutic approaches (percutaneous or surgical) morphology and locations of the atherosclerotic lesions usually determine the patient management. Thus, it should be stressed that in the present study a choice of surgical option resulted from more advanced and extensive CAD. The need for repeat coronary intervention between 30 and 36 months after the initial procedure was also analysed. Additionally, the prevalence of other vascular pathologies such as stroke, aortic aneurysms and intracranial aneurysm was also evaluated with respect to their correlation with polymorphism of the IL-1 gene cluster.

Methods

Study groups

This study involved 92 consecutive patients with CAD treated in the cardiology outpatient clinic, in whom the disease was confirmed by coronary angiography. All these patients had previously undergone coronary intervention and gave an informed consent to participate in the genetic studies. The following groups of patients were excluded from the study: those with non-atherosclerotic aetiology of the coronary symptoms such as vasospastic angina, patients with muscular bridge causing haemodynamically significant systolic compression of the coronary artery, and patients with isolated stenosis of the left main stem.

A control group comprised 32 volunteers – individuals in good health without any disease diagnosed previously and who did not present any signs and symptoms, related particularly to the cardiovascular system. The study protocol was approved by the Local Ethical Committee. Data regarding CAD therapy as well as extracardiac vascular disorders treated in the past were verified on the basis of clinical reports issued by Cardiology, Cardiac Surgery, Vascular Surgery, Neurology and Neurosurgery Departments/Wards. Demographic data, prevalence of the typical risk factors of atherosclerosis development, concomitant disorders and treatment CAD options are outlined in Table I. Characteristics of lesions in the coronary arteries after division of patients into seven categories with respect to either localisation of significant atherosclerotic lesions or the number of coronary arteries involved are presented in Table II. This classification was adopted from Hannan et al. [14].

DNA isolation

DNA was isolated from the peripheral blood of the donors using the commercially available QIAamp DNA blood mini-kit (Qiagen, Hilden, Germany). Blood was taken to EDTA probes and then centrifuged for 10 minutes, 700 xg at room temperature. A fraction enriched with leucocytes (buffy coat) was collected to isolate DNA. Lysis of the leucocyte fraction was carried out using proteinase K. DNA was purified according to manufacturer's recommendations employing successive centrifugation cycles (6000 xg, 20 000 xg, 6000 xg), with the use of attached buffer kits. Isolated DNA probes of the consecutive patients were stored at a temperature of –20°C until amplification.

DNA amplification and electrophoresis

DNA amplification to evaluate IL-1-RN polymorphism was carried out by means of polymerase chain reaction (PCR) using the following primers:

5'-CTCAGCAACACTCCTAT-3' (upstream) and 5'-TCCTGGTCTGCAGGTAA-3' (downstream), in 40 cycles at a variable temperature (95, 60, 72, 10°C) in the UNO II thermocycler (Biometria, Gottingen, Germany) using the commercial kit BIOTOOLS DNA Polymerase Concentration – B&M Labs. SA, Madrid.

Polymorphism of the gene IL-1β promoter was assessed by means of PCR with restriction fragment length polymorphism (RFLP) analysis. The following primers for the locus in position 31 were used: (5'AGAAGCTTCCACCAATACTC-3) (upstream) and 5'AGCACCTAGTTGTAAGGAAG-3' (downstream). DNA amplification was conducted in 40 cycles at a variable temperature (94, 65, 72, 10°C). For the locus

Table I. Demographic and clinical characteristics of the study group

Parameter	
Age [years] range	38-81
Male gender [%]	73.9
Arterial hypertension [%]	59.7
Systolic/diastolic blood pressure range mean ± SD [mmHg]	 100-195/60-115 130 ± 21/79 ± 11
Diabetes mellitus type 1/2 [%]	0/22.8
Smoking [%]	40.2
Obesity [%]	13.0
BMI – range; mean ± SD	19.1-37.4; 27.2 ± 3.9
Gastric/duodenal ulcer disease [%]	6.4
Dyslipidemia [%]	59.7
Total cholesterol – range; mean ± SD [mg/dl]	86-347; 205 ± 49
LDL – range; mean ± SD [mg/dl]	38-243; 132 ± 48
HDL – range; mean ± SD [mg/dl]	19-80; 43 ± 11
Triglycerides – range; mean ± SD [mg/dl]	32-478; 148 ± 77
History of myocardial infarction [%]	85.8
Mean age at first myocardial infarction [years]	61
Primary coronary angioplasty [%]	68.5
Elective coronary angioplasty [%]	6.5
CABG [%]	25.0
Need for repeated procedure [%]	17.4
Mean age at bypass grafting [years]	61
History of stroke [%]	6.38
Peripheral artery disease in inferior limbs [%]	4.3
Aneurysm of abdominal aorta, peripheral or intracranial artery [%]	5.4

at position 511 respective upstream 5'-TGGCATTGATCTGGTTCATC-3' and downstream 5'-GTTTAGGAATCTTCCCACTT-3' primers were used. Amplification was carried out in 36 cycles at a variable temperature (95, 94, 54, 72, 10°C). The amplification products were then subjected to the restriction enzymes (Alu I, Ava I). The amplification product at position 31 contained 239 base pairs (bp) and after digestion allele C = 236 + 3bp, allele T = 139 + 97 3bp. At 511 locus the amplification product was 305bp and after digestion allele C = 185 + 116bp while allele T = 305bp.

In both studies, a partition of the amplified products and also a probe buffer itself and pattern mixture were done in 2% agarose gel enriched with ethidium bromide. The results of this division were then assessed under UV light. Representative findings and the method of their interpretation are presented in Figures 1, 2 and 3. Digital symbols used to describe the number of DNA sequence repeats with 86 bp, during assessment of the polymorphism of the IL-1 receptor (IL-1RN) antagonist, are outlined in Table III [15].

Table II. Characteristics of lesions found on coronary angiography

Sites of critical (> 70%) stenosis	Patients [%]
Three vessel disease with lesion in proximal segment of left anterior descending artery (LAD)	23.9
Three vessel disease with lesion in distal segment of LAD	2.2
Two vessel disease with lesion in proximal segment of LAD	20.6
Two vessel disease with lesion in distal segment of LAD	3.3
Two vessel disease without lesions in LAD	14.1
One vessel disease in LAD	17.4
One vessel disease without lesions in LAD	18.5

Table III. Explanation of nomenclature used for IL-RN polymorphism

Nomenclature of allels	Number of repeats of DNA sequence (86 bp)
Allel 1	4
Allel 2	2
Allel 3	5
Allel 4	3
Allel 5	6

Statistical analysis

The allele prevalence in the group of subjects with CAD and in the control group were compared. After the genotype for all three analysed loci was determined, the variants of three polymorphism combinations were calculated as well as their prevalence in the examined group. On the basis of allele prevalence the theoretical distribution of the genotypes was calculated according to the Hardy-Weinberg equation and conformity between actual and theoretical distribution was also assessed. A correlation between employed CAD treatment strategy and genotype regarding the examined genes was analysed for a combination of the three most prevalent variants of three polymorphisms. The χ^2 Pearson test was used to compare actual and predicted genotype distribution. In cases where the tests result suggested statistically significant differences, an attempt was made to identify responsible genotype. The difference between actual and predicted prevalence was calculated, then this difference was standardised to a normal distribution with mean 0 and standard deviation 1, respectively. A more pronounced absolute standardised difference (residuum) meant a more significant difference between predicted and actual prevalence.

The χ^2 test was used to evaluate any correlation between the selected variant combinations of three polymorphisms and the rate of surgical or percutaneous

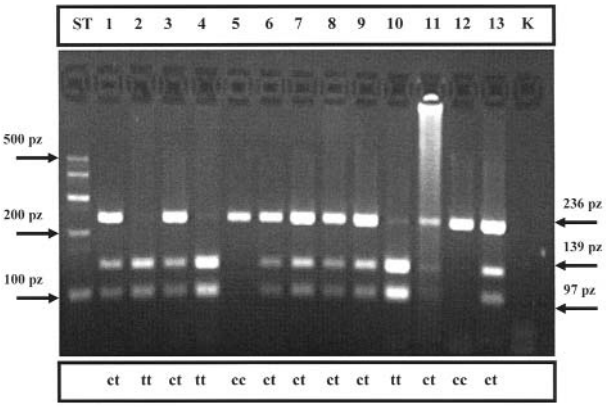

Figure 1. Results of DNA electrophoresis for assessment of polymorphism of IL-1β gene in locus -31. ST – DNA standard, K – negative control, allel C – 236 bp, allel T – 139 bp + 97 bp

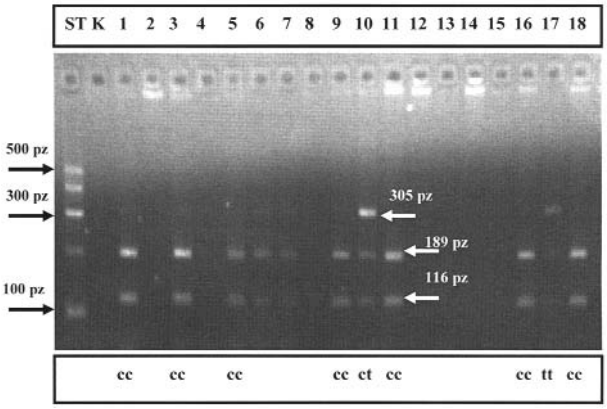

Figure 2. Results of DNA electrophoresis for assessment of polymorphism of IL-1β gene in locus -511. ST – DNA standard, allel C – 189 bp + 116 bp, allel T – 305 bp

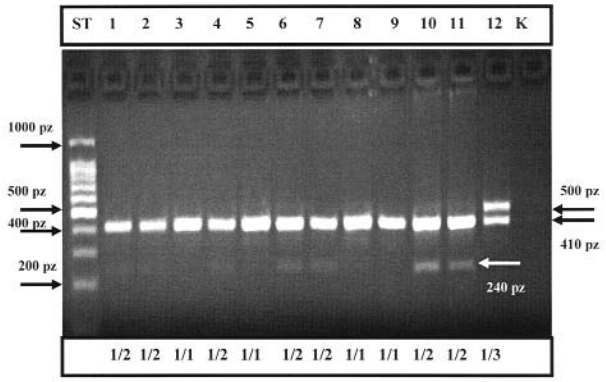

Figure 3. Results of DNA electrophoresis for assessment of polymorphism of IL-RN gene. ST – DNA standard, K – negative control, Allel 1 – 410 bp (4 repeats), allel 2 – 240 bp (2 repeats), allel 3 – 500 bp (5 repeats)

treatment option, and also prevalence of stroke and aneurysm, while in the case of statistically significant relations the power of correlation was calculated by means of the residuum, assuming that various combinations of the three polymorphisms did not influence the rate of the analysed event.

A p value < 0.05 was considered significant.

Results

The prevalence of individual alleles in the three loci did not differ significantly between the study and control groups and was as follows for the IL-1 antagonist gene – allele 1: 80% and 88% (p = 0.1223); allele 2: 17% and 12% (p = 0.3696); allele 3: 2% and 0% (p = 0.2266); for locus -31 of gene IL-1β allele T: 63% and 70% (p = 0.2432) and for locus -511 allele C: 62% and 69% (p = 0.1803), respectively.

Table IV presents data regarding prevalence of individual alleles, actual and theoretical genotype distributions, and statistical analysis of conformity between actual and theoretical distribution for loci -31 and -511 of the IL-1β gene and IL-1 receptor (IL-1RN) antagonist gene, respectively. No significant differences between actual and predicted genotype prevalence in both loci -31 and -511 were observed. However, conformity χ^2 test showed a significant difference with respect to the rate of IL-1 receptor antagonist gene. Homozygotes 1/1, 2/2 and 3/3 were more prevalent in the examined group than would be calculated by means of the Hardy-Weinberg equation, while 1/3 genotype was observed significantly less often.

Similar analysis (allele prevalence, actual and theoretical distribution of the genotypes and conformity of theoretical distribution to the actual one) was carried out for the combinations of two polymorphisms (at position -31 and -511) as well as the combination of three polymorphisms (-31, -511 and IL-1 receptor antagonist) – see Tables V and VI. Significant discrepancy between actual and predicted prevalence of the genotype was observed. Homozygotes CC/TT and TT/CC at loci -31/-511 and heterozygotes CT/CT were seen more often than the predicted rate calculated according to the Hardy-Weinberg equation, while genotypes CC/CC, TT/TT, CT/CC, CT/TT and TT/CT markedly less often presented. In prevalence assessment of variants of the three polymorphisms, triple homozygotes such as CC/TT 2/2, CC/TT 3/3, TT/CC 1/1, TT/CC 2/2 and variants CC/TT 1/2, CT/CT 1/1, CT/CT 1/2, CT/CT 2/2, CT/CT 3/3 and TT/CT 3/3 were also noted more frequently, but genotypes CT/CC 1/1, CT/CC 1/2, TT/CT 1/1 and TT/CT 1/2 at locus -31/-511 as well as for the IL-1 receptor antagonist gene were noted significantly less often than calculated by means of the Hardy-Weinberg equation. In patients with CAD three more prevalent genotype variants were detected: CT/CT 1/1 in 30 subjects (variant A), TT/CC 1/1

Table IV. Comparison of real and theoretical (according to Hardy-Weinberg equilibrium) prevalence of alleles and genotypes in locus -31 and -511 of IL-1 and IL-1RN genes with statistical evaluation of differences in the group of patients with coronary artery disease

Locus	Allel/genotype	Real prevalence of allels/ /genotypes [%]	Theoretical prevalence according to Hardy-Weinberg equilibrium [%]	Residuum	*Significance of difference in genotype distribution/ /density of normal distribution N (0.1) for given value of residuum
-31	C/	36.9/	×		
	T/	63.1/	×		
	/CC	/10.9	13.6	×	*0.7561/
	/CT	/52.2	46.6		
	/TT	/36.9	39.8		
-511	T/	37.5/	×		
	C/	62.5/	×		
	/TT	/9.9	14.1	×	*0.3198/
	/CT	/55.4	46.8		
	/CC	/34.7	39.1		
-RN	1/	80.4/	×		
	2/	17.4/	×	×	*< 0.001/
	3/	2.2/	×		
	/1/1	/70.7	66.6	2.0	/0.0470
	/1/2	/17.4	27.9	-1.9	/0.0513
	/1/3	/2.1	3.8	-2.1	/0.0365
	/2/2	/8.7	3.0	3.3	/0.0008
	/2/3	/0.0	0.6	-1.3	/0.1920
	/3/3	/1.1	0.1	5.4	/< 0.0001

in 25 individuals (variant B) and CT/CT 1/2 in another 10 (variant C).

Table VII presents the frequency of surgical and percutaneous treatment as well as the rate of strokes and vascular anomalies (such as aortic, peripheral and intracranial artery aneurysms) in the subgroups with the three most prevalent variants of the examined polymorphisms – 2 regarding the IL-1β gene and one the IL-1 receptor antagonist gene. Significant correlations between the three selected genetic variants and the rate of elective coronary angioplasty, CABG and prevalence of aneurysm were found. In the group with the genetic variant TT/CC 1/1, for the IL-1β gene at locus -31/-511 and the IL-1 receptor antagonist gene, respectively, which was the second with respect to number in the examined group, CABG was carried out more often than would have resulted from random assignment. However, in the group, with the genetic variant CT/CT 1/1 (the most often represented variant), CABG was performed less frequently while in the group with the CT/CT 1/2 variant aneurysms were more prevalent and elective coronary angioplasty was carried out more often than would have resulted from random assignment.

The number of repeated therapeutic coronary procedures, their type and priority of treatment throughout follow-up lasting between 30 and 36 months after the first procedure are outlined in Table VIII.

Discussion

In the present study it was assumed that patients with critical stenosis in the proximal segments of two or three coronary arteries were selected for CABG. In recent years a tendency to employ the percutaneous method rather than surgery has been noted even in patients with three-vessel disease and concomitant diabetes mellitus [16]. This trend was also observed in the examined group of patients. Among 22 patients with three-vessel disease and simultaneous involvement of the proximal segment of the left anterior descending artery (LAD) almost one third of individuals, in spite of characteristics of lesions in the coronary arteries, were treated percutaneously, while in the group of 19 patients with two-vessel disease and proximal involvement of LAD the ratio was reversed: surgically treated to percutaneously treated rate was 5 : 14. Thus, a decision regarding one of the two therapeutic invasive methods was a result of the findings

Table V. Comparison of real and theoretical (according to Hardy-Weinberg equilibrium) prevalence of combination of 2 polymorphisms with evaluation of significance of differences in the CAD group

Genotype in 2 loci: -31 and -511	Real prevalence of genotype [%]	Theoretical prevalence of genotype (according to Hardy-Weinberg equilibrium) [%]	Residuum	*Significance of difference in genotype distribution/ density of normal distribution N (0.1) for given value of residuum *< 0.0001/
CT/CT	48.9	28.8	7.1	/< 0.0001
TT/CT	3.3	20.3	-3.3	/0.0011
CT/CC	1.1	18.0	-3.9	/0.0001
TT/CC	33.6	12.7	5.6	/< 0.0001
CC/CT	3.3	6.0	-1.3	/0.2030
CT/TT	2.2	5.1	-1.7	/0.0938
CC/CC	0.0	3.8	-2.3	/0.0235
TT/TT	0.0	3.5	-2.3	/0.0235
CC/TT	7.6	1.8	4.8	/< 0.0001

Table VI. Comparison of real and theoretical (according to Hardy-Weinberg equilibrium) prevalence of combination of 3 polymorphisms with evaluation of significance of differences in the CAD group

Variants of 3 polymorphisms: IL-1 locus -31 and -511 and IL-RN	Real prevalence [%]	Theoretical prevalence of genotype (according to Hardy-Weinberg equilibrium) [%]	Residuum	*Significance of difference in genotype distribution/ density of normal distribution d N (0.1) for given value of residuum *< 0.0001/
CT/CT 1/1 Variant A	32.6	13.2	5.7	/0.0000
CT/CC 1/1	1.1	11.1	-2.9	/0.0033
TT/CT 1/1	3.2	11.1	-2.3	/0.0193
TT/CC 1/1 Variant B	27.1	9.4	6.8	/0.0000
CT/CT 1/2 Variant C	10.8	5.8	2.1	/0.0380
TT/CC 1/2	3.2	4.1	-0.4	/0.6574
CC/CT 1/1	2.2	3.9	-0.9	/0.3774
CT/TT 1/1	1.1	3.9	-1.4	/0.1608
CT/CT 1/3	1.1	1.8	-0.5	/0.5908
CC/CT 1/2	1.1	1.7	-0.5	/0.6287
CC/TT 1/1	3.2	1.2	1.9	/0.0629
CT/CT 2/2	3.2	0.6	3.2	/0.0015
CC/TT 1/2	2.2	0.5	2.2	/0.0260
CT/TT 1/3	1.1	0.5	0.7	/0.4814
TT/CC 2/2	3.2	0.4	4.0	/0.0001
CC/TT 2/2	2.2	0.1	8.6	/0.0000
CT/CT 3/3	1.1	0.1	3.9	/0.0001

Table VII. Relationship between strategy of CAD treatment, prevalence of coronary and extracoronary vascular events, and genetic variants of IL-1 β and IL-RN genes

Studied parameter	Three most common genetic variants Prevalence of studied parametr [%] *Residuum **Significance			Significance of difference in distribution in A, B, C variants (real vs. random)
	Variant A	Variant B	Variant C	
Primary coronary angioplasty	76.9	76.0	50.0	0.2294
Elective coronary angioplasty	3.3 *-1.2 **0.2363	0.0 *-1.9 **0.0622	40.0 *4.1 **< 0.0001	0.0002
CABG	13.3 *-2.3 **0.0244	44.0 *3.7 **0.0002	0.0 *-1.9 **0.0568	0.0006
Stroke in history	3.3	12.0	0.0	0.2914
Aneurysms of abdo- -minal aorta, peripheral or intracranial artery	3.3 *-1.2 **0.2363	0.0 *-1.9 **0.0622	40.0 *4.1 **< 0.0001	0.0002
History of myocardial infarction	93.3	76.0	70.0	0.1648

Table VIII. Comparison of repeated coronary artery procedures performed during 30-36 months follow-up after first treatment

Subgroups and its numbers/ succesity of procedures	Variant A n = 30	Variant B n = 25	Variant C n = 10	Remaining variants n = 27	Total n = 92
ePCI after pPCI	2	4	0	0	6
rePCI due to restenosis	1	2	1	1	5
CABG after pPCI	0	2	0	0	2
pPCI after pPCI	0	1	0	1	2
ePCI after ePCI	0	0	1	0	1
Total	3	9	2	2	16

Abbreviations: ePCI – elective PCI, pPCI – primary PCI, rePCI – repeated PCI

in the coronary angiography, the patient's clinical status, but also the patient's preference with respect to the method of treatment.

The analysis of the number of repeated procedures performed throughout the follow-up period lasting from 30 to 36 months after the initial invasive treatment indicated that patients with genotype variant B (-31TT -511CC RN1/1) most frequently needed repeated procedures (fraction 0.56). Among them almost one third of patients had to undergo repeated procedures. The most frequent consecutive procedure was elective PCI performed at the location of the new lesion carried out after primary PCI.

Evaluation of the allele and genotype distribution prevalence

Clinicians' interest in the association between polymorphism of either the IL-1 gene cluster or IL-1RN receptor with CAD goes back to the end of the last decade. So far, data regarding the prevalence of those polymorphisms in Polish inhabitants have been neither studied nor published. In one of the first reports dealing with this issue Francis et al. noted the similar trends of more frequent (by approximately 10 per cent) prevalence of allele T at the position -511 of the IL-1 gene in CAD patients compared to the control group – patients enrolled in the study came from the British population [17]. The large size of the group examined by the aforementioned author guaranteed statistical significance of this difference, which was not noted in our markedly less numerous (difference of 7%). The second important value of Francis et al. observation was selection of the control group. After coronary angiography was performed, patients with normal arteries or including lesions not exceeding 30% of the vessel diameter were recruited.

Different results of genotype analysis were achieved by Vohnout et al., who examined gene IL-1b polymorphism at position -511 and IL-1 receptor (IL-1RN) among approximately 500 inhabitants of Italy. They did not find any difference with respect to prevalence of either allele or genotype distribution between CAD patients and a control group. However, comparing their results

with the findings of our study, their report showed lower prevalence of allele C by approximately 5% and around 10-fold higher rate of allele 3 of the IL-1 receptor antagonist gene among CAD patients – inhabitants of Central Poland [18]. In a recent publication regarding this issue, an analysis of the remote ethnic group of the Chinese revealed results consistent with our findings. An allele T carrier state at position -511 (i.e. individuals with CT and TT genotype analysed together) was noted more frequently in CAD patients than in controls [19].

IL-1β and IL-1RN polymorphism and course or severity of coronary artery disease

Among our patients we observed a correlation between one of the variants of two gene IL-1β polymorphisms and gene IL-1RN polymorphism with CAD severity that required surgical treatment and that has not been observed in previous publications. In the aforementioned study reported by Francis et al. after analysis of the four polymorphisms related to IL-1 distribution, a protective impact of gene IL-1RN allele 2 on the restenosis rate following balloon coronary angioplasty was found. It was probably linked to the possibility of different inflammatory response. However, these authors stressed that a statistically significant correlation of this polymorphism variant with lower restenosis risk was observed only in patients with critical stenosis in one coronary artery [20]. In our group of patients restenosis was seen in 5 patients and allele 2, considered as a protective one, was found in one of them who additionally was an IL-1RN 1/2 homozygote.

Gene IL-1β and IL-1RN polymorphisms and potential mechanisms accelerating atherosclerosis development

An attempt to explain the correlation between variety of IL-1β and IL-1RN gene variants and severity of atherosclerosis by inflammatory response difference was made. The studies dealing with intra- and extracellular expression of IL-1 receptor antagonist showed that allele 2 carrier subjects presented higher synthesis of this antagonist by monocytes and lowered by endothelial cells [21, 22].

Latkovskis et al. [23] observed that allele 2 carriers manifested lower CRP concentration and Momiyama et al. reported that a lack of allele T at position -511 and allele 2 presence in the IL-1 gene had the strongest association with myocardial infarction development if they were analysed in *Chlamydophila pneumoniae* seropositive subjects [24]. An interesting explanation with respect to the role of IL-1β gene polymorphism in intensity of inflammatory reaction was found in the *in vitro* studies by Wen et al. and Iacoviello et al. They noted that monocytes in subjects with TT/CC genotype at positions -31 and -511 of the described gene, after lipopolysaccharide (LPS) stimulation, produced two to three times more IL-1β than monocytes isolated from individuals with CC/TT genotype. [25, 26] In our study, individuals with TT/CC genotype at positions 31 and 511 constituted the second subgroup with the most frequent (44%) use of CABG, and more than half of the subjects (52%) had 3- or 2-vessel disease with proximal LAD involvement. Moreover, patients with this genotype and additionally being 1/1 homozygotes in the IL-1RN gene were the most numerous among subjects who required repeated therapeutic procedures. Higher intensity of the inflammatory processes influenced by bacterial LPS would explain the higher intensity and less stable coronary artery atherosclerotic lesions in these patients.

Although nowadays a genotypic examination is not employed to predict the course of CAD or to make decisions regarding management, studies dealing with the genetic markers of atherosclerosis development rate have a chance to gain such importance. In reports published in recent months, attempts have been made to assess the correlation between selected polymorphisms and CAD development in patients with diabetes mellitus. The control groups involved patients with diabetes mellitus but without CAD. Doria et al. studied the rs2383206 polymorphism at locus 9p21, but in reports by Drzewoski et al. two polymorphisms of the metalloproteinase 1 promoting gene were studied [27, 28]. Both studies showed that genetic examination may be useful in predicting rapid atherosclerosis development in patients with diabetes mellitus, which would suggest the necessity to intensify the medical therapy. However, similar to our study, both studies failed to compare CAD patients with subjects in whom atherosclerosis lesions in the coronary arteries were excluded in the coronary angiography. They enrolled subjects in the control groups on the basis of high probability of absence of atherosclerosis after history analysis, ECG study and negative stress test as a screening examination. These groups were used to disclose significant importance of the genetic examination in patients with CAD. It should also be stressed that a limitation of our study was the small size of studied populations, although our preliminary findings constitute a rationale for analyses of the significance of IL-1 gene cluster polymorphism in larger groups of patients with cardiovascular disorders.

Conclusions

The findings of this study suggest that in patients prone to development of atherosclerosis, polymorphism of the IL-1β gene cluster may be associated with the extent and dynamics of lesions in the coronary arteries. Detection of polymorphism of the IL-1β gene cluster may be of importance when selecting the type of CAD treatment.

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Polimorfizmy klastra genów interleukiny 1β i inhibitora receptora interleukiny 1 wśród osób z chorobą niedokrwienną serca leczonych metodą przezskórnej angioplastyki wieńcowej lub chirurgicznie

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Streszczenie

Wstęp: Prozapalna interleukina 1β (IL-1β) odgrywa niekorzystną rolę w procesie miażdżycy. Mediator ten w kaskadzie zdarzeń prowadzących do powstania i destabilizacji blaszki miażdżycowej stymuluje syntezę interleukiny 6 (IL-6), fibrynogenu, białka C-reaktywnego (CRP) i ekspresję cząsteczek adhezyjnych przez komórki śródbłonka. Tej produkowanej miejscowo cytokinie przypisuje się zdolność pobudzania komórek mięśni gładkich w blaszce miażdżycowej i hamowania proliferacji komórek śródbłonka naczyniowego.

Cel: Retrospektywna ocena, czy chorzy z najczęstszymi w badanej grupie wariantami polimorfizmu klastra genów IL-1β różnią się zaawansowaniem i lokalizacją zmian miażdżycowych w tętnicach wieńcowych w stopniu wpływającym na zastosowaną strategię leczenia choroby niedokrwiennej serca.

Metody: Grupa badana składała się z 92 kolejnych chorych leczonych ambulatoryjnie (wiek 39–83 lat, mężczyźni 74%) z potwierdzoną w badaniu angiograficznym chorobą wieńcową. W grupie tej 23 chorych było leczonych metodą pomostowania aortalno-wieńcowego (CABG), 69 – przezskórnej interwencji wieńcowej (PCI), 16 chorych wymagało ponownego zabiegu. Polimorfizmy genu IL-1β – zamiana C na T w pozycji -511 i -31, oraz genu antagonisty receptora interleukiny 1 (IL-RN) – zmienna ilość powtórzeń odcinków DNA, określono metodą PCR. Z teoretycznie możliwych 54 kombinacji polimorfizmów w badanej grupie znaleziono 17, z których wyodrębniono 3 najczęstsze. Pozostałe warianty były reprezentowane w podgrupach 3-, 2- lub 1-osobowych. Frakcje chorych leczonych metodą pierwotnej PCI lub planowej PCI oraz CABG zostały porównane między wyłonionymi trzema podgrupami.

Wyniki: Najczęstsze kombinacje to: wariant A: -31C/T, -511C/T, RN 1/1 – 32,6% chorych, wariant B: -31T/T, -511C/C, RN 1/1 – 27,1%, wariant C: -31C/T, -11C/T, RN 1/2 – 10,8%. Analiza statystyczna wykazała, że nosiciele drugiego co do częstości wariantu badanych polimorfizmów (wariant B) byli znamiennie częściej leczeni kardiochirurgicznie z powodu choroby wieńcowej niż nosiciele dwóch pozostałych wariantów. Także osoby z tym wariantem najczęściej wymagały kolejnej PCI jako leczenia dwuetapowego.

Wniosek: Wyniki powyższych badań sugerują, że polimorfizm klastra genów IL-1β może mieć związek ze zróżnicowanym nasileniem miażdżycy tętnic wieńcowych w stopniu determinującym wybór chirurgicznej bądź sekwencyjnej przezskórnej metody leczenia choroby niedokrwiennej serca.

Słowa kluczowe: polimorfizm genu, interleukina 1β, choroba wieńcowa, przezskórna angioplastyka, pomostowanie aortalno-wieńcowe

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