

INTERLEUKIN-18 PROMOTER GENE POLYMORPHISMS ARE NOT ASSOCIATED WITH MYOCARDIAL INFARCTION IN TYPE 2 DIABETES IN SLOVENIA

Kariž S^{1*}, Petrovič D²

***Corresponding Author:** Stojan Kariž, Department of Internal Medicine, General Hospital Izola, Polje 35, Izola 6310, Slovenia; Tel.: +386-5-660-6480; Fax: +386-660-6305; E-mail: stojan.kariz@siol.net

ABSTRACT

Type 2 diabetes is a major risk factor for myocardial infarction (MI) and chronic inflammation may play a central role in both diseases. Interleukin (IL)-18 is a potent proinflammatory cytokine, which is considered important in acute coronary syndromes and type 2 diabetes. We investigated the association of the -137 (G>C), polymorphism (rs187238) and the -607 (C>A) polymorphism (rs1946518) of the IL-18 gene promoter region in 495 Caucasians with type 2 diabetes, of whom 169 had MI and 326 subjects had no clinically evident coronary artery disease (controls). We also investigated the impact of these polymorphisms on the serum IL-18 level in subsets of both groups and in a normal group. Genotype distributions of the polymorphisms showed no significant difference between cases and controls. However, IL-18 serum levels were significantly lower in diabetics with the 137 CC genotype than in those with other genotypes (241.5 ± 132.7 ng/L vs. 340.2 ± 167.4 ng/L; $p < 0.05$). High sensitivity C-reactive protein and IL-18 serum levels were higher in diabetics in the MI group than in the control group. We conclude that these IL-18 promoter gene polymor-

phisms are not risk factors for MI in Caucasians with type 2 diabetes.

Key words: Type 2 diabetes, Myocardial infarction (MI), Interleukin-18 (IL-18) gene polymorphisms.

INTRODUCTION

Type 2 diabetes is a major risk factor for development of coronary artery disease (CAD) and subsequent myocardial infarction (MI). Considerable data support the hypothesis that inflammation plays a central role in pathogenesis of both atherosclerosis [1] and type 2 diabetes [2]. In particular, diabetes is associated with enhanced inflammatory responses and accelerated atherosclerosis [3]. The risk of MI is increased 2- to 4-fold in diabetic patients [4]. The genetic variability of inflammatory genes may be involved in the pathogenesis of diabetes-accelerated atherosclerosis and its complications [1-3].

Interleukin-18 (IL-18) is a pro-inflammatory cytokine with an important role in the inflammatory process that contributes to atherosclerosis [5]. High levels of IL-18 have been detected in human atherosclerotic plaques and have been related to plaque instability [6]. Animal models support the pro-atherogenic role of IL-18 [7] and the favorable effect of inhibiting IL-18 on plaque composition and progression [8]. Increased levels of circulating IL-18 have been demonstrated

¹ Department of Internal Medicine, General Hospital Izola, Polje 35, Izola 6310, Slovenia

² Institute of Histology and Embryology, Medical Faculty, Slovenia

in patients with acute coronary syndromes [9] and in type 2 diabetic patients [10]. Diabetic patients with high IL-18 had a greater carotid intima-media thickness and a higher number of carotid plaques than those with normal IL-18 serum levels [11]. Moreover, acute hyperglycemia induces an increase in plasma IL-18 in normal subjects and in patients with impaired glucose tolerance [12]. Thus, elevated plasma IL-18 may be associated with acceleration of atherosclerosis and may play a role in acute coronary syndromes through plaque destabilization in type 2 diabetic patients.

The IL-18 gene locus is located at 11q22.2-q23.3 and several polymorphisms in its promoter region have been identified [13]. Substitution of G>C at position -137 changes a histone 4 transcription factor-1 (H4TF-1) nuclear factor-binding site, while a change of C>A at position -607 disrupts a cyclic adenosine monophosphate (cAMP) responsive element protein-binding site. These changes influence the transcriptional activity of the IL-18 gene [13]. Recently, genetic polymorphisms of the IL-18 gene have been associated with various immune and inflammatory diseases, including cardiovascular disease [14-16], type 1 diabetes mellitus [17], and Alzheimer's disease [18]. We have investigated the association of -137 (G>C) and -607 (C>A) polymorphisms of the IL-18 gene promoter region of MI patients of Caucasian origin with type 2 diabetes in Slovenia, and also the impact of the IL-18 gene polymorphisms on serum IL-18 levels.

PATIENTS AND METHODS

This cross-sectional analysis examined 495 subjects (263 males, 232 females; age range 46-78 years) with type 2 diabetes of more than 10 years duration: 169 subjects with MI (MI group) and 326 with no history of CAD, no signs of ischemic changes on the electrocardiogram and no ischemic changes during sub-maximal stress testing (control group). Subjects were classified as having type 2 diabetes according to the current criteria of the American Diabetes Association (ADA) [19]. The diagnosis of MI was made according to the criteria in [20], patients being studied 1 to 9 months after the acute event. All subjects were Slovenes of Slavic origin (Caucasians) and came from independent families. The body mass index (BMI) was calculated as weight in kilograms divided by the height in square meters. High sensitivity C-reactive protein (CRP), glycosylated hemoglobin (Hb A_{1c}), to-

tal cholesterol, low density lipoproteins (LDL), high density lipoproteins (HDL) and triglycerides were determined by standard biochemical methods. We also measured serum IL-18 levels in 70 subjects with type 2 diabetes (20 patients with MI and 50 patients without CAD) and 22 subjects without diabetes. Plasma IL-18 was determined with enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (Invitrogen, Carlsbad, CA, USA). The National Medical Ethics Committee approved the research protocol. All patients participating in the study gave written informed consent.

Genomic DNA was isolated from peripheral blood leukocytes by standard methods and stored at -20°C. Genotyping was carried out by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis. The -137 and -607 polymorphisms in the promoter of the IL-18 gene were determined as described in [13].

For the -137 G>C genotyping, a common reverse primer (5'-AGG AGG GCA AAA TGC ACT GG-3') and two sequence-specific forward primers [5'-CCC CAA CTT TTA CGG AAG AAA AG-3' (for allele G) and 5'-CCC CAA CTT TTA CGG AAG AAA AC-3' (for allele C)] were used to amplify a 261 bp product. A control forward primer was used to amplify a 446 bp fragment that contained the polymorphic site to serve as an amplification control. The PCR reaction was performed in a final volume of 5 µL containing 0.5 µL 10 mM dNTP, 1 µL 5 × PCR buffer, 0.2 µL 25 mM MgCl₂, 0.15 µL 10 µM of each primer, 0.5 µL genomic DNA, 2.55 µL H₂O and 0.1 µL (0.5 U) GoTaq DNA polymerase. Two PCR reactions were performed for each individual DNA sample (for both sequence-specific forward primers). The cycling conditions were: denaturation for 2 min. at 94°C, then five cycles each lasting 20 seconds at 94°C, 60 seconds at 68°C and 60 seconds at 68°C, respectively, followed by 25 cycles of 20 seconds at 94°C, 20 seconds at 62°C, 40 seconds at 72°C and a final elongation for 7 min. at 72°C. Polymerase chain reaction products were visualized by 2.0% agarose gel electrophoresis stained by »SYBR Green I« (Invitrogene, Carlsbad, CA, USA).

For the -607 C>A polymorphism, a common reverse primer (5'-TAA CCT CAT TCA GGA CTT CC-3') and two sequence-specific forward primers [5'-GTT GCA GAA AGT GTA AAA ATT ATT AC-3' (for allele C) and 5'-GTT GCA GAA AGT GTA AAA ATT ATT AA-3' (for allele A)] were used to amplify

a 196 bp product. A control forward primer (5'-CTT TGC TAT CAT TCC AGG AA-3') was used to amplify a 301 bp fragment covering the polymorphic site as an internal positive amplification control. The PCR reaction was performed in a final volume of 5 μ L consisting of 0.5 μ L 10 mM dNTP, 1 μ L 5 \times PCR buffer, 0.2 μ L 25 mM MgCl₂, 0.15 μ L 10 μ M of each primer, 0.5 μ L genomic DNA, 2.55 μ L H₂O and 0.1 μ L (0.5 U) GoTaq DNA polymerase. The cycling conditions were: denaturation for 2 min. at 94°C, then seven cycles each lasting 20 seconds at 94 °C, 40 seconds at 64 °C and 40 seconds at 72 °C, respectively, followed by 25 cycles of 20 seconds at 94 °C, 40 seconds at 57 °C, 40 seconds at 72 °C and a final elongation for 7 min. at 72 °C. PCR products were separated by electrophoresis on a 2% agarose gel and visualized by »SYBR Green I« (Invitrogen). Two investigators (SK, DP), blinded for the case or control status of the DNA sample, performed the assignment of genotype.

The χ^2 test was used to compare discrete variables and to compare genotype distributions. Continuous clinical data were compared by unpaired Student's *t*-test and presented as mean \pm standard deviation (SD). The Hardy-Weinberg equilibrium was confirmed using the χ^2 test. A *p* value of <0.05 was considered to be statistically significant. A statistical analysis was performed using the SPSS program for Windows 2000 version 17 (SPSS Inc., Chicago, IL, USA).

RESULTS

The clinical characteristics of the subjects and controls are listed in Table 1. The former were younger, predominantly male and had a higher incidence of cigarette smoking compared to the control group. They also had higher total cholesterol and LDL cholesterol levels, and longer duration of type 2 diabetes than the controls. The high sensitivity CRP serum levels were also higher in the diabetic patients with MI. There were no significant differences in the prevalence of hypertension, mean blood pressure, and mean BMI, and serum HDL cholesterol, triglyceride, and Hb A_{1c} levels between the two groups. Therapy for diabetes was similar in both groups.

The IL-18 genotype distributions in MI and control groups were compatible with Hardy-Weinberg expectations (-607: MI group $\chi^2 = 0.40$, *p* = 0.53; controls $\chi^2 = 1.65$, *p* = 0.2; -137: MI group $\chi^2 = 0.02$, *p* = 0.89; controls $\chi^2 = 1.07$, *p* = 0.3). The distribution of

genotypes in the promoter of the IL-18 gene in MI and in control groups are shown in Table 2. There were no differences between the two groups.

The serum IL-18 level in 70 diabetics did not differ significantly from those of 22 controls without diabetes (310.0 \pm 234.1 ng/L vs. 250.3 \pm 190.4 ng/L; *p* = 0.3). However, the level in 20 diabetics with MI was statistically significantly higher than that of 50 diabetics without CAD (534.0 \pm 397.5 ng/L vs. 286.2 \pm 202.4 ng/L; *p* <0.01). A significant difference in IL-18 serum levels was found in diabetics with the -137 CC genotype (10 subjects) compared to those with CG+GG genotypes (60 subjects) (241.5 \pm 132.7 ng/L vs. 340.2 \pm 167.4 ng/L; *p* <0.05). We found no significant difference in IL-18 level in diabetics with the -607 CC genotype (17 subjects) compared to those with CA+AA genotypes (53 subjects) (287.3 \pm 221.7 ng/L vs. 323.3 \pm 257.3 ng/L; *p* = 0.6).

DISCUSSION

In 495 patients with type 2 diabetes (169 patients with MI and 326 controls), we found no association between the -137 (G>C) and -607 (C>A) polymorphisms in the IL-18 gene promoter region and the occurrence of MI. The genotype distributions we found agreed with previously published results from healthy European Caucasians and from subjects with type 1 diabetes [21].

Type 2 diabetes is a chronic inflammatory disorder characterized by increased CRP serum levels [22], which independently increase the risk of cardiovascular events among these patients [23,24]. Accordingly, our patients had increased high sensitivity CRP levels, which was significantly higher in patients with MI. We also found significantly higher IL-18 levels in diabetic patients with MI than in those without clinical CAD. Our results agree with those of previous studies that elevated IL-18 levels may be associated with acceleration of atherosclerosis in type 2 diabetic patients [11,25]. It has been shown that IL-18 is an independent predictor of cardiovascular events in subjects with metabolic syndrome and especially in the presence of elevated fasting glucose, suggesting a synergistic effect of hyperglycemia and inflammation [26]. Unlike previous reports [10,27], we found no difference between IL-18 levels in diabetic and in normal patients.

Interleukin-18 is a potent proinflammatory and proatherogenic cytokine directly associated with de-

Table 1. Clinical characteristics of type 2 diabetic patients with myocardial infarction and without coronary artery disease [values are presented as mean \pm standard deviation; percentage of cases (%) is shown in parentheses]

	MI Group	Control Group	p Value
Number	169	326	
Age (years)	61.0 \pm 11.9	66.2 \pm 9.8	<0.001
Males (% of males)	113 (66.9%)	150 (46.0%)	<0.001
Females (% of females)	56 (33.1%)	176 (54.0%)	<0.001
BMI (kg/m ²) ¹	28.7 \pm 3.9	29.0 \pm 4.6	0.4
Waist circumference (cm)	108.3 \pm 14.0	108.2 \pm 12.8	0.9
Arterial hypertension (%)	118 (69.8%)	222 (68.1%)	0.8
Duration of arterial hypertension (years)	12.9 \pm 9.7	9.7 \pm 8.8	0.001
Systolic BP (mmHg) ²	147.0 \pm 21.0	143.0 \pm 22.0	0.1
Diastolic BP (mmHg) ²	83.0 \pm 10.0	84.0 \pm 10.0	0.5
Smoking habit (%)	69 (40.8%)	39 (11.9%)	<0.001
Diabetes duration (years)	21.7 \pm 7.7	17.9 \pm 8.0	0.001
Insulin therapy (%)	99 (58.6%)	177 (54.3%)	0.4
Oral diabetic drug therapy (%)	71 (42.0%)	148 (45.4%)	0.8
Hb A _{1c} (%) ³	8.3 \pm 1.5	8.0 \pm 1.6	0.1
hs-CRP (mg/L) ⁴	7.4 \pm 16.1	3.9 \pm 4.5	0.01
Total cholesterol (mmol/L)	5.7 \pm 1.6	5.3 \pm 1.3	0.01
HDL cholesterol (mmol/L)	1.1 \pm 0.3	1.2 \pm 0.4	0.12
LDL cholesterol (mmol/L)	3.6 \pm 1.5	3.2 \pm 1.0	0.001
Triglycerides (mmol/L)	2.3 \pm 1.3	2.4 \pm 1.6	0.8

¹ Body mass index.

² Blood pressure.

³ Glycosylated hemoglobin.

⁴ High sensitivity C-reactive protein.

velopment of unstable atherosclerotic plaques [6]. Interleukin-18 induces the expression of interferon- γ and matrixmetalloproteinases, which may cause plaque destabilization and rupture [5]. In mouse models, exogenously-administered IL-18 accelerated the development of atherosclerotic lesion [28] and increased plaque size and inflammatory cell content [7]. On the other hand, the IL-18 binding protein, a natural antagonist of IL-18, decreased the inflammatory cell infiltrate and generated a stable plaque phenotype [8]. The IL-18 levels are higher among patients with unstable angina pectoris and previous MI [9,29,30] and

predict cardiovascular death in patients with CAD [31] and acute coronary events in healthy middle-aged men [32]. Likewise, serum IL-18 levels are increased in type 2 diabetic patients [10], and are a strong independent risk factor for the development of diabetes in middle-aged men and women [27]. They were also associated with nephropathy and atherosclerosis in Japanese patients with type 2 diabetes [25].

Polymorphisms in the promoter region of the IL-18 gene may influence transcriptional activity and thus the level of the cytokine expression [13]. We found the IL-18 levels to be significantly lower in diabetic patients with

Table 2. Distribution of interleukin-18 genotypes in type 2 diabetic patients with myocardial infarction and without coronary artery disease [percentage of cases (%) is shown in parentheses]

	MI Group (%)	Control Group (%)	OR (95% CI) ¹	p Value
IL-18 –607 (C>A)				
• genotype CC	55 (32.5%)	109 (33.4%)	1.0 (0.6-1.5) ²	0.9 ²
• genotype CA	86 (50.9%)	158 (48.5%)	1.2 (0.7-2.0) ³	0.6 ³
• genotype AA	28 (16.6%)	59 (18.1%)		
• Total	169	326		
IL-18 –137 (G>C)				
• genotype CC	8 (4.7%)	23 (7.1%)	0.7 (0.3-1.5) ²	0.3 ²
• genotype GC	71 (42.0%)	141 (43.3%)	0.9 (0.6-1.3) ³	0.5 ³
• genotype CC	90 (53.3%)	162 (49.7%)		
• Total	169	326		

¹ Odds ratio (95% confidence interval).

² p Value and OR for recessive model (–607: CC vs. CA plus AA; –137: CC vs. GC plus GG).

³ p Value and OR for dominant model (–607: CC plus CA vs. AA; –137: CC plus GC vs. GG).

the –137 CC genotype compared to those with CG+GG genotypes. Similarly, a greater capacity to produce IL-18 by monocytes has been reported in those with the –137 GG genotype [33], as has higher IL-18 mRNA levels in those with –607 CC and –137 GG genotypes compared to persons with other genotypes [13]. Despite the association between –137 (G>C) polymorphism and IL-18 serum levels, the polymorphism was not related to MI in our cohort of diabetic patients. However, in a recent Chinese study, the –137 GG genotype was linked to higher IL-18 serum levels and a greater likelihood of angiographically-proven CAD [16]. Likewise, C allele carriers of this polymorphism have decreased production of IL-18 and a lower risk for sudden cardiac death caused by CAD [15]. The same authors have also demonstrated that the –137 (G>C) polymorphism modulates the effect of hypertension on the development and complications of CAD [34].

The discrepancy between the results of our and other association studies may be due to differences in phenotype definition, the variation in the genetic or environmental background of the populations studied, or a sample not adequate to detect a modest association [35]. The negative result may also be due to survival bias, since we enrolled only patients who survived acute MI. Since the –137 (G>C) polymorphism has been associated with sudden cardiac death [15], this could also have influenced the results of our study. Only a prospective study could overcome this limitation.

In conclusion, the polymorphisms –137 (G>C) (rs187238) and –607 (C>A) (rs1946518) of the IL-18 gene are not risk factors for MI in patients with type 2 diabetes and cannot be used as genetic markers for MI in Caucasians with type 2 diabetes. Although the –137 (G>C) polymorphism was related to IL-18 serum levels, we assume the association is not strong enough to influence the risk of MI in diabetic patients.

REFERENCES

- Ross R. Atherosclerosis - an inflammatory disease. *N Engl J Med.* 1999; 340(2): 115-126.
- Pickup JC, Crook MA. Is type II diabetes mellitus a disease of the innate immune system? *Diabetologia.* 1998; 41(10): 1241-1248.
- Libby P. Inflammation in atherosclerosis. *Nature.* 2002; 420(6917) :868-874.
- Haffner SM. Coronary heart disease in patients with diabetes. *N Engl J Med.* 2000; 342(14): 1040-1042.
- Gerdes N, Sukhova GK, Libby P, Reynolds RS, Young JL, Schonbeck U. Expression of interleukin (IL)-18 and functional IL-18 receptor on human vascular endothelial cells, smooth muscle cells, and macrophages: implications for atherogenesis. *J Exp Med.* 2002; 195(2): 245-257.
- Mallat Z, Corbaz A, Scoazec A, Besnard S, Leseche G, Chvatchko Y, Tedgui A. Expression of interleukin-18 in human atherosclerotic plaques and relation to plaque instability. *Circulation.* 2001; 104(104): 1598-1603.

7. Whitman SC, Ravisankar P, Daugherty A. Interleukin-18 enhances atherosclerosis in apolipoprotein E(-/-) mice through release of interferon- γ . *Circ Res*. 2002; 90(2): e34-e38.
8. Mallat Z, Corbaz A, Scoazec A, Graber P, Alouani S, Esposito B, Humbert Y, Chvatchko Y, Tedgui A. Interleukin-18/interleukin-18 binding protein signaling modulates atherosclerotic lesion development and stability. *Circ Res*. 2001; 89(7): e41-e45.
9. Mallat Z, Henry P, Fressonnet R, Alouani S, Scoazec A, Beaufile P, Chvatchko Y, Tedgui A. Increased plasma concentrations of interleukin-18 in acute coronary syndromes. *Heart*. 2002; 88(5): 467-469.
10. Esposito K, Nappo F, Giugliano D, Di Palo C, Ciotola M, Barbieri M, Paolisso G, Giugliano D. Cytokine milieu tends toward inflammation in type 2 diabetes. *Diabetes Care*. 2003; 26(5): 1647.
11. Aso Y, Okumura KI, Takebayashi K, Wakabayashi S, Inukai T. Relationships of plasma interleukin-18 concentrations to hyperhomocysteinemia and carotid intima-media wall thickness in patients with type 2 diabetes. *Diabetes Care*. 2003; 26(9): 2622-2627.
12. Esposito K, Nappo F, Marfella R, Giugliano G, Giugliano F, Ciotola M, Quagliari L, Ceriello A, Giugliano D. Inflammatory cytokine concentrations are acutely increased by hyperglycemia in humans: role of oxidative stress. *Circulation*. 2002; 106(16): 2067-2072.
13. Giedraitis V, He B, Huang WX, Hillert J. Cloning and mutation analysis of the human IL-18 promoter: a possible role of polymorphisms in expression regulation. *J Neuroimmunol*. 2001; 112(1-2): 146-152.
14. Tiret L, Godefroy T, Lubos E, Nicaud V, Tregouet DA, Barbaux S, Schnabel R, Bickel C, Espinola-Klein C, Poirier O, Perret C, Munzel T, Rupprecht HJ, Lackner K, Cambien F, Blankenberg S, for the AtheroGene Investigators. Genetic analysis of the interleukin-18 aystem highlights the role of the interleukin-18 gene in cardiovascular disease. *Circulation*. 2005; 112(5): 643-650.
15. Hernesniemi JA, Karhunen PJ, Rontu R, Ilveskoski E, Kajander O, Goebeler S, Viiri LE, Pessi T, Hurme M, Lehtimäki T. Interleukin-18 promoter polymorphism associates with the occurrence of sudden cardiac death among Caucasian males: The Helsinki Sudden Death Study. *Atherosclerosis*. 2008; 196(2): 643-649.
16. Liu W, Tang Q, Jiang H, Ding X, Liu Y, Zhu R, Tang Y, Li B, Wei M. Promoter polymorphism of interleukin-18 in angiographically proven coronary artery disease. *Angiology*. 2009; 60(2): 180-185.
17. Kretowski A, Mironczuk K, Karpinska A, Bojaryn U, Kinalski M, Puchalski Z, Kinalska I. Interleukin-18 promoter polymorphisms in type 1 diabetes. *Diabetes*. 2002; 51(11): 3347-3349.
18. Bossu P, Ciaramella A, Moro ML, Bellincampi L, Bernardini S, Federici G, Trequatrini A, Macchiardi F, Spoletini I, Di Iulio F, Caltagirone C, Spalletta G. Interleukin 18 gene polymorphisms predict risk and outcome of Alzheimer's disease. *J Neurol Neurosurg Psychiatry* 2007; 78(8): 807-811.
19. Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Report of the expert committee on the diagnosis and classification of diabetes mellitus. *Diabetes Care*. 2003; 26(Suppl 1): S5-S20.
20. Alpert JS, Thygesen K, Antman E, Bassand JP. Myocardial infarction redefined--a consensus document of The Joint European Society of Cardiology/American College of Cardiology Committee for the redefinition of myocardial infarction. *J Am Coll Cardiol*. 2000; 36(3): 959-969.
21. Szeszko JS, Howson JM, Cooper JD, Walker NM, Twells RC, Stevens HE, Nutland SL, Todd JA. Analysis of polymorphisms of the interleukin-18 gene in type 1 diabetes and Hardy-Weinberg equilibrium testing. *Diabetes*. 2006; 55(2): 559-562.
22. Ford E. Body mass index, diabetes, and C-reactive protein among U.S. adults. *Diabetes Care*. 1999; 22(12): 1971-1977.
23. Schulze M, Rifai N, Rimm E, Stampfer M, Li T, Hu F. C-reactive protein and incident cardiovascular events among men with diabetes. *Diabetes Care*. 2004; 27(4): 889-894.
24. Soinio M, Marniemi J, Laakso M, Lehto S, Rönnemaa T. High-sensitivity C-reactive protein and coronary heart disease mortality in patients with type 2 diabetes: a 7-year follow-up study. *Diabetes Care*. 2006; 29(2): 329-333.
25. Nakamura A, Shikata K, Hiramatsu M, Nakatou T, Kitamura T, Wada J, Itoshima T, Makino H. Serum interleukin-18 levels are associated with nephropathy and atherosclerosis in Japanese patients with type 2 diabetes. *Diabetes Care*. 2005; 28(12): 2890-2895.
26. Trosheid M, Seljeflot I, Hjerkin EM, Arnesen H. Interleukin-18 is a strong predictor of cardiovascular events in elderly men with the metabolic syndrome: synergistic effect of inflammation and hyperglycemia. *Diabetes Care*. 2009; 32(3): 486-492.
27. Thorand B, Kolb H, Baumert J, Koenig W, Chambless L, Meisinger C, Illig T, Martin S, Herder C. Elevated levels of interleukin-18 predict the development of type 2 diabetes: results from the MONICA/KORA Augsburg Study, 1984-2002. *Diabetes*. 2005; 54(10): 2932-2938.
28. Tenger C, Sundborger A, Jawien J, Zhou X. IL-18 accelerates atherosclerosis accompanied by elevation of IFN-gamma and CXCL16 expression independently of T cells. *Arterioscler Thromb Vasc Biol*. 2005; 25(4): 791-796.

29. Rosso R, Roth A, Herz I, Miller H, Keren G, George J. Serum levels of interleukin-18 in patients with stable and unstable angina pectoris. *Int J Cardiol.* 2005; 98(1): 45-48.
30. Hulthe J, McPheat W, Samnegard A, Tornvall P, Hamsten A, Eriksson P. Plasma interleukin (IL)-18 concentrations is elevated in patients with previous myocardial infarction and related to severity of coronary atherosclerosis independently of C-reactive protein and IL-6. *Atherosclerosis.* 2006; 188(2): 450-454.
31. Blankenberg S, Tiret L, Bickel C, Peetz D, Cambien F, Meyer J, Rupprecht Interleukin 18 is a strong predictor of cardiovascular death in stable and unstable angina. *Circulation.* 2002; HJ. 106(1): 24-30.
32. Blankenberg S, Luc G, Ducimetière P, Arveiler D, Ferrrières J, Amouyel P, Evans A, Cambien F, Tiret L, on behalf of the PRIME Study Group. Interleukin-18 and the risk of coronary heart disease in European men: The Prospective Epidemiological Study of Myocardial Infarction (PRIME). *Circulation.* 2003; 108(20): 2453-2459.
33. Arimitsu J, Hirano T, Higa S, Kawai M, Naka T, Ogata A, Shima Y, Fujimoto M, Yamadori T, Hagiwara K, Ohgawara T, Kuwabara Y, Kawase I, Tanaka T. IL-18 gene polymorphisms affect IL-18 production capability by monocytes. *Biochem Biophys Res Commun.* 2006; 342(4): 1413-1416.
34. Hernesniemi JA, Karhunen PJ, Oksala N, Kähönen M, Levula M, Rontu R, Ilveskoski E, Kajander O, Goebeler S, Viiri LE, Hurme M, Lehtimäki T. Interleukin 18 gene promoter polymorphism: a link between hypertension and pre-hospital sudden cardiac death: the Helsinki Sudden Death Study. *Eur Heart J.* 2009; 30(23): 2939-2946.
35. Kathiresan S, Newton-Cheh C, Gerszten RE. On the interpretation of genetic association studies. *Eur Heart J.* 2004; 25(16): 1378-1381.

