

## Association of Interleukin-1 Gene cluster polymorphisms with coronary slow flow phenomenon

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### ABSTRACT

**Objective:** Coronary slow flow phenomenon (CSFP) is characterized by the decreased rate of contrast progression in epicardial coronary arteries in the absence of significant coronary stenosis. Mounting evidence has showed a significant association between inflammation and CSFP severity. This study aimed to evaluate possible associations between interleukin-1 receptor antagonist (IL-1ra) gene variable number tandem repeat (VNTR), IL-1 $\beta$ -511 single nucleotide (SNP), and IL-1 $\beta$ +3954 SNP mutations with CSFP.

**Methods:** Forty-eight patients with CSFP and 62 controls with angiographically normal coronary arteries were prospectively enrolled in the study. Genotypes were assessed using the polymerase chain reaction (PCR)-based restriction fragment length polymorphism (PCR-RFLP) technique.

**Results:** Homozygote genotype for allele 2 of +3954 C>T 2/2 genotype was significantly more frequent in patients with CSFP than in the control group, whereas 1/2 genotype was more frequent in the control group (35.4% versus 14.5% for 2/2 genotype and 25% versus 35.5% for 1/2 genotype in CSFP and control groups, respectively,  $X^2=6.6$ ;  $p=0.04$ ). The allelic frequency of allele 2 of this polymorphism was significantly higher in the CSFP group than in the control group (47.9% versus 28.6% in the control group,  $X^2=5.6$ ;  $p=0.02$ ). However, there was no significant difference with regard to genotype or allelic frequencies of IL-1ra VNTR or IL-1 $\beta$ -511 SNP polymorphisms between patients with CSFP and controls.

**Conclusion:** IL-1 $\beta$ +3954 SNP mutations are significantly more common in patients with CSFP. It may suggest that the tendency for inflammation may contribute to the presence of this phenomenon. (*Anatol J Cardiol* 2018; 19: 34-41)

**Keywords:** atherosclerosis, interleukins, oxidative stress, endothelium, vascular smooth muscle

### Introduction

Coronary slow flow phenomenon (CSFP) is a delay in the opacification of epicardial coronary arteries in coronary angiography in the absence of other factors, which might secondarily result in same appearance (1). The exact pathogenesis of CSFP seems to be multifactorial and includes morphological abnormalities in the vasculature, such as fibromuscular thickening, myofibrillar hypertrophy, occult atherosclerosis, and functional abnormalities, particularly endothelial dysfunction (2). A vast majority of evidence points out the crucial role of inflammation in the pathogenesis of this phenomenon (3, 4). Many inflammatory mediators and markers have been reported to be associated with this condition, indicating the presence of a proinflammatory process as the cause and/or result of the phenomenon (5-7).

Interleukin-1 (IL-1) alpha and beta are proinflammatory cytokines released from monocytes, lymphocytes, macrophages, platelets, and damaged endothelium (8). The interleukin-1 recep-

tor antagonist (IL-1Ra), which is also a member of the IL-1 cytokine family, shows structural similarity to IL-1, but antagonizes the effects of IL-1 $\beta$  and IL-1 $\alpha$  subunits at the receptor level (8).

The increased levels of preprocedural IL-1Ra indirectly reflecting atherosclerotic plaque activity were demonstrated in patients who had major adverse cardiac events following percutaneous coronary intervention (9,10). Evidences suggest that the balance between IL-1 $\beta$  and IL-1Ra determines the extent of inflammatory response elicited in coronary events (11).

Various polymorphisms are defined in the gene cluster coding for IL-2 and derivatives (12). IL-1Ra gene (RN2) polymorphism is associated with decreased expression of IL-1Ra and increased levels of IL-1 $\beta$ , shifting the IL-1/IL-1Ra balance toward IL-1 (13, 14). Some of these polymorphisms are associated with increased release of inflammatory mediators and severity and/or activation of inflammatory diseases (15). The associations between polymorphisms in this gene cluster and cardiovascular diseases such as coronary artery disease, stent restenosis after percutaneous

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**Accepted Date:** 02.10.2017 **Available Online Date:** 28.12.2017

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DOI:10.14744/AnatolJCardiol.2017.8071



**Table 1. Exclusion criteria**

Presentation with any kind of acute coronary syndrome (unstable angina pectoris, NSTEMI-ACS*, STEMI**)
Prior history of mechanical reperfusion (PTCA*** and/or stent)
Coronary ectasia in coronary angiography
Left ventricular systolic dysfunction (defined as LVEF**** <50%)
Coronary artery spasm (variant angina)
More than mild degree valvular disease in any of the valves
Known collagen tissue disorder
Known thyroid disease or thyroid dysfunction as shown by thyroid function tests at the time of coronary angiography

\* NSTEMI-ACS-non-ST segment elevation acute coronary syndrome; \*\* STEMI-ST-segment elevation myocardial infarction; \*\*\* PTCA- percutaneous transluminal coronary angioplasty; \*\*\*\* left ventricular ejection fraction

coronary interventions, carotid artery disease, and lone atrial fibrillation have already been demonstrated by studies (16-18). Also, polymorphisms of the IL-1Ra gene are shown to be associated with the development of acute coronary syndromes (19).

Several studies investigated the associations between CSFP and genes coding nitric oxide synthesis, prothrombotic markers, and renin-angiotensin system (20, 21). However, only one study investigated the relation between inflammatory genes and CSFP and showed an association with IL-10 polymorphism in the Han Chinese population (22).

This study aimed to evaluate possible associations between CSFP and IL-1 gene cluster polymorphisms, i.e., IL-1Ra gene variable number tandem repeat (VNTR), IL-1 $\beta$  -511 single nucleotide (SNP), and IL-1 $\beta$ +3954 SNP mutations.

## Methods

### Study design

This cross-sectional, case-control study was conducted in our tertiary care center between December 2012 and June 2013. The patients were selected from the cardiac catheterization laboratory. Medical records of the patients who demonstrated CSFP and normal coronary arteries in their coronary angiography were examined. Among these patients, the patients in whom elective coronary angiography was performed for stable angina pectoris or equivalent symptoms, findings suggestive of myocardial ischemia in their resting ECG or noninvasive evaluation (consisting of positive exercise stress testing, nuclear perfusion scan, or dobutamin stress echocardiography), and preliminary diagnosis of coronary artery disease were further evaluated for the presence of possible exclusion criteria explained in the following section. Past medical history, blood assessments, and echocardiography reports were reviewed for conditions that might secondarily cause CSFP. Further assessments were performed as needed. The eligible patients were then asked to participate in the study. Blood for genetic analysis were drawn in patients who gave informed consent. The study was approved by the local Institutional Review Board.

### Study participants

Patients with documented coronary artery disease or other conditions, which may result in secondary CSFP (coronary ectasia, coronary spasm, acute coronary syndromes,  $\geq 50\%$  stenosis in at least one of the epicardial coronary arteries, coronary embolism, and left ventricular systolic dysfunction) were excluded. Exclusion criteria are shown in Table 1. Other exclusion criteria were variant angina, more than mild degree heart valve disease, known collagen tissue disorder, acute or chronic inflammatory disease, thyroid dysfunction, and known diabetes. Patients with normal coronary arteries who did not meet any exclusion criteria were enrolled to the control group.

All patients underwent basal demographic, clinical, laboratory, and transthoracic echocardiographic assessments prior to coronary angiography. Blood was drawn for biochemistry, thyroid function tests, blood count, erythrocyte sedimentation rate, and genetic analysis. Two-dimensional, pulsed Doppler, color Doppler, and tissue Doppler echocardiograms were acquired using phased array probes on a Vivid 7 Vingmed general electric ultrasound scanner (GE Vingmed Ultrasound, Horten, Norway) following a standardized protocol.

### Coronary angiography and diagnosis of coronary slow flow phenomenon

Coronary angiography was performed via a right femoral approach using the standard Judkins technique and 6F diagnostic catheters to cannulate coronary arteries. Nonionic contrast agent (Iopromide; Ultravist 370, Schering, Berlin, Germany) was used in all patients, and images of the coronary arteries were obtained in standardized projections at a film rate of 30 frames/s. For objective quantification of the coronary flow, an independent, experienced observer who was blinded to the study reviewed the coronary angiograms and coronary flow rates calculated using the TIMI frame counts method (23).

In patients who were visually suspected of having CSFP, the number of cine frames recorded at 30 frames/s required for the contrast to reach standard distal coronary landmarks in the left anterior descending, left circumflex, and right coronary arte-

ries were measured. TIMI frame counts  $>2$  standard deviations (SD) from the mean value previously defined for healthy subjects ( $36.2 \pm 2.6$  for LAD,  $22.2 \pm 4.1$  for CX,  $20.4 \pm 3.0$  for RCA) were accepted as "slow" (Table 2). The values for LAD were divided by the correction factor 1.7, and corrected TIMI frame counts (cTFC) were calculated for LAD, as previously described. Mean TIMI frame counts (mTFC) were calculated by summing up TIMI frame counts for each coronary artery and dividing the result by 3 (23).

### Genetic analysis

Blood samples of all individuals were taken from the antecubital vein following an overnight fasting state just after coronary angiography. Blood was collected into EDTA tubes for genetic analysis. Samples were refrigerated until all the samples were collected. DNA isolation from blood samples was performed with using the GenElute™ Blood Genomic DNA kit (St. Louis, MO, USA) from 20 mL of EDTA-anticoagulated blood. Isolated DNA was preserved at  $-20^{\circ}\text{C}$  until genetic assessments.

Gene regions in exon 5 of the interleukin  $1\beta$  gene containing IL-1B+3954, promotor of the same gene containing IL1B -511 and intron 2 of IL-1RN gene containing VNTR polymorphisms were amplified using polymerase chain reaction (PCR). The oligonucleotide sequences 5'-CTCAGCAACTCCTAT-3' (forward) and 5'-TCCTGGTCTGCAGGTA-3' (reverse) were used for the amplification of IL-1RN gene region susceptible to polymorphisms. PCR cycles were as follows: (3 min at  $94^{\circ}\text{C}$ ) x 1; (30 s at  $94^{\circ}\text{C}$ ; 45 s at  $55^{\circ}\text{C}$  and  $72^{\circ}\text{C}$ ) x 35; (10 min at  $72^{\circ}\text{C}$ ) x 1 [8], and the size of PCR products was determined by 1.5 % agarose gel stained with ethidium bromide. Genotype was evaluated based on repeated unit size of PCR products.

For the detection of polymorphisms in -511 position, primers with 5'GTTTAGGAATCTTCCACTT-3' (forward) and 5'-TGGCATTGATCTGGTTCATC-3' (reverse) oligonucleotide sequences were used. PCR cycles were as follows: (3 min at  $94^{\circ}\text{C}$ ) x 1; (30 s  $94^{\circ}\text{C}$ , 30 s at  $54^{\circ}\text{C}$ , and 45 s at  $72^{\circ}\text{C}$ ) x 35; (10 min at  $72^{\circ}\text{C}$ ) x 1 [9]. For cleavage of the susceptible gene region, 5U Aval restriction endonuclease at  $37^{\circ}\text{C}$  overnight was used. Genotype determination was done based on the size of digested PCR product on 2.5% agarose gel. Digestion by Aval yielded two 190 and 114 base pair (bp) -long fragments when cytosine (C) was present and a single 304 bp-long fragment was detected when thymine (T) was present in the same position.

For IL-1 $\beta$ +3954 polymorphisms, the primer sequences were 5'GTTGTCATCAGACTTTGACC-3' (forward) and 5'TTCAGTTCATATGGACCAGA-3' (reverse). PCR cycles were as follows: (1 min at  $94^{\circ}\text{C}$ ) x 1; (30 s at  $94^{\circ}\text{C}$ , 30 s at  $54^{\circ}\text{C}$ , and 45 s at  $72^{\circ}\text{C}$ ) x 35; (10 min at  $72^{\circ}\text{C}$ ) x 1. Digestion was performed with 5U Taq1 restriction endonuclease at  $65^{\circ}\text{C}$  for overnight, followed by 3 % agarose gel electrophoresis analysis. 135 and 114 bp-long fragments were detected in the presence of allele C, whereas 249 bp fragment was seen in the presence of allele T. A "no template" control (water) and a positive control for the rare and

most common gene variant was used on each gel for electrophoresis of the gene products.

### Statistical analysis

Statistical analysis was performed using SPSS version 18 for Windows software. Parametric variables were summarized as mean  $\pm$  SD. Categorical variables were presented as percentages. Differences between groups with regard to ordinal and nominal variables were assessed using the Pearson's  $\chi^2$  test. Normal distribution of continuous variables was tested using the Kolmogorov-Smirnov test, and the equality of variances between groups was tested using the Levene's test. Differences of continuous variables between groups were assessed with the Student's t-test provided that the data was normally distributed. Statistical analysis was performed for both genotypic and allelic distributions. Pearson's  $\chi^2$  test was used for testing for Hardy-Weinberg equilibrium in the distribution of genetic polymorphisms. Rare alleles of IL-1RN were excluded from the statistical analysis. G\*Power (24) was used for determining the statistical power of our study. For detection of a moderate size effect of 0.30 moderate size effect for  $\alpha=0.05$ ,  $d(f)=2$ , the power of our study was 0.89. For all results,  $p<0.05$  was considered statistically significant.

### Results

In this study, 48 patients with CSFP (35 males and 13 females; mean age,  $50 \pm 9$  years) were enrolled in the CSFP group. The control group consisted of 62 patients (38 males and 24 females; mean age,  $50 \pm 7$  years) with normal coronary arteries. Baseline demographic, clinical, and laboratory characteristics including hs-CRP level were similar between the patients and control groups (0.95 vs. 1.07 mg/L, control group vs. CSFP group,  $p=0.50$ ), except for a higher level of LDL-C in the CSFP group (91.8 vs. 107.2 mg/dL,  $p=0.05$ , control group vs. CSFP group) (Table 2). Despite greater isovolumic relaxation time (IVRT) and smaller E/A ratio in the CSFP group (IVRT, 98.4 vs. 102 ms;  $p=0.05$ ; E/A ratio, 1.2 vs. 1.07;  $p<0.05$ , control group vs CSFP group), chamber dimensions and parameters of systolic and diastolic function were within normal range in both groups.

The results of the genetic analysis are shown in Table 3. IL-1 $\beta$ +3954 SNP was found more frequently in patients with CSFP than in controls, and this difference was statistically significant (35.4% vs. 14.5% for 2/2 and 25% vs. 35.5% for 1/2 for patients and controls, respectively). The allelic frequency of allele 2 was also increased in the CSFP group ( $\chi^2=6.6$ ,  $p<0.05$  for both).

Genotypes with IL-1RN VNTR polymorphisms, namely genotypes 1/2 and 2/2 were not statistically different between the two groups. The 1/2 genotype was present in 27.1% of the patients with CSFP versus 22.6% of the controls, and the 2/2 genotype was present in 6.3% of the patients with CSFP and 6.5% of the controls ( $p=0.571$ ). The frequencies for allele 2 were also similar

**Table 2. Baseline clinical characteristics of the study participants**

Characteristics	Control (n=62)	CSFP (n=48)	P
Age, years	50±6.9	50.2±8.5	0.89
Male, %	38 (61.2)	35 (72.9)	0.24
Body mass index, kg/m <sup>2</sup>	22.9±2	23.1±2.2	0.65
Active smoker, %	15 (24.2)	12(25)	0.92
Systolic blood pressure, mm Hg	120.1±6.8	120.3±11	0.92
Diastolic blood pressure, mm Hg	68.6±14	69.0±15.1	0.89
Usage of medications, %			
Antiaggregants	27	25	0.37
Statins	15	13	0.68
ACE-I/ARBs	19	13	0.73
Beta-blockers	22	16	0.87
Calcium channel blockers	2	2	0.77
<b>Laboratory assessments</b>	Control	CSFP	P
Hemoglobin, g/dL	14.5±1.3	14.1±2	0.22
White blood cells, 10 <sup>3</sup> /μL	6.8±1.3	7±1.3	0.36
Fasting plasma glucose, mg/dL	92.8±15	100.3±23.4	0.56
Total cholesterol, mg/dL	178.7	181.5	0.65
Triglycerides, mg/dL	150.4	207.7	0.08
HDL-C, mg/dL	40.5	41.5	0.58
LDL-C, mg/dL	91.8	107.2	0.05
Creatinine, mg/dL	0.89±0.19	0.87±0.15	0.55
CRP, mg/L	0.95	1.07	0.50
<b>Echocardiographic parameters</b>	Control	CSFP	P
LVEDD, cm	4.6 (4.1-5.2)	4.4 (4.1-5.5)	0.56
LVESD, cm	2.6 (2-3.3)	2.5 (2.3-3.5)	0.78
LVEF, %	63.6 (±3.2)	60.1 (±3.1)	0.43
LA, cm	3.5 (3.2-4)	3.7 (3-4.1)	0.51
E wave, cm/s	60.5 (±5.4)	59.6 (±5.6)	0.26
IVRT, ms	98.4 (±9.7)	102 (±9.4)	0.05
E/A ratio	1.2 (±0.22)	1.07 (±0.19 )	0.005
<b>Coronary angiography</b>			
LAD TIMI frame count	31.4	42.8	0.000
RCA TIMI frame count	19.2	38.2	0.000
CX TIMI frame count	19.5	25.7	0.000
Mean TIMI frame count	19.0	29.7	0.000

(\* = mean±SD; minimum-maximum)

Differences between groups were assessed with Student's t test. The parameters demonstrating statistically significant difference are indicated with italic.

ACE-I-angiotensin-converting enzyme inhibitor; ARB-angiotensin receptor blocker; CRP-C Reactive protein; HDL-C-high-density lipoprotein cholesterol; IVRT-isovolumic relaxation time; LA-left atrial diameter; LAD-left anterior descending artery; LCX-left circumflex coronary artery; LDL-C-low-density lipoprotein cholesterol; LVEDD-left ventricular end-diastolic dimension; LVEF-left ventricular ejection fraction; LVESD-left ventricular end-systolic dimension; RCA-right coronary artery; TIMI-thrombolysis in myocardial infarction

in the patient and control groups (19.8% in patients with CSFP and 19.4% in controls; X<sup>2</sup>=1.16; p=0.56).

Likewise, genotypes with IL-1β -511 C/T SNP did not show a

statistically significant difference between the two groups. The 2/2 genotype was present in 14.6% of the patients with CSFP and in 17.7% of the controls. The 1/2 genotype was present in 29.2%

**Table 3. Genotype distribution and allelic frequencies of IL1RN VNTR, IL1 $\beta$  -511 (rs16944) and IL1 $\beta$ +3954 (rs1143634) polymorphisms in control group and CSFP patients**

Genotype Frequencies	Controls (n=62) n, %	CSFP (n=48) n, %	Allelic Frequencies	Controls (n=62) n, %	CSFP (n=48) n, %
<b>IL1RN VNTR</b>					
1/1	41, 66.1	31, 64.1	Allele 1, %	96, 77.4	76, 79.2
1/2	14, 22.6	13, 27.1	Allele 2, %	24, 19.4	19, 19.8
2/2	4, 6.5	3, 6.3	Other, %	4, 3.2	1, 1
Other/Other	1, 1.6	–			
1/Other	–	1, 2.1			
2/Other	2, 3.2	–			
Control/CSFP	$X^2=3.849$ ; $P=0.571$		$X^2=1.16$ ; $P=0.56$		
<b>IL1<math>\beta</math>-511</b>					
1/1	26, 41.9	27, 56.3	Allele 1, %	77, 62.1	68, 70.8
1/2	25, 40.3	14, 29.2	Allele 2, %	47, 37.9	28, 29.2
2/2	11, 17.7	7, 14.6			
Control/CSFP	$X^2=2.26$ ; $P=0.32$		$X^2=1.83$ ; $P=0.17$		
<b>IL1<math>\beta</math>+3954</b>					
1/1	31, 50.0	19, 39.6	Allele 1, %	84, 71.4	50, 52.1
1/2	22, 35.5	12, 25	Allele 2, %	40, 28.6	46, 47.9
2/2	9, 14.5	17, 35.4			
Control/CSFP	$X^2= 6.6$ ; $P=0.04$		$X^2=5.6$ ; $P=0.02$		
Pearson's $X^2$ test was used for testing for Hardy-Weinberg equilibrium in distribution of genetic polymorphisms.					
CSFP-Coronary slow flow phenomenon; IL-1RN VNTR Interleukin-1 receptor antagonist gene variable number tandem repeat; IL1 $\beta$ -511 (rs16944), Interleukin-1 $\beta$ polymorphisms in-511 position; IL1 $\beta$ +3954 (rs1143634), Interleukin-1 $\beta$ polymorphisms in+3954 position.					

versus 40.3% of the patients with CSFP and controls, respectively ( $X^2=2.26$ ;  $p=0.17$ ).

## Discussion

As a pathological entity, CSFP is detected in 1%–7% of all coronary angiographies (25). Although there are many pathophysiological processes that might secondarily result in CSFP, there are a lot of unanswered questions regarding the pathophysiology of primary CSFP: Occlusive disease of small caliber coronary vessels; microvascular dysfunction; an increase in blood viscosity due to increase in hematocrit, hyperlipidemia, or hyperfibrinogenemia; disruption in flow dynamics due to diameter irregularities in coronary vasculature; and distortion of the balance between vasoconstrictor and vasodilator mediators were some of the suggested mechanisms (1, 25–29).

To find whether there is any relationship between systemic inflammation and CSFP, following inflammatory mediators and markers have been previously investigated by various groups and found to be increased: (1) red cell distribution width; (2) neutrophil lymphocyte ratio; (3) eosinophil count; (4) hs-CRP levels; (5) myeloperoxidase; (6) IL-6; (7) adhesion molecules; (8) E-selectin, and (9) soluble CD40 (6, 30–38). Despite all these positive results,

studying a causative relation between CSFP and circulating inflammatory markers is prone to some errors. As most of these studies had a cross-sectional methodology and case-control design and inflammation may be both the result and cause of CSFP, most of the current literature is lacking the most vital information, i.e., the direction of the causative relationship. Second, the inflammatory markers and mediators may fluctuate over time and show variations during and between acute attacks of CSFP. Thus, the timing of blood sample collection in relation to the CSFP episodes might confound the results. Furthermore, many patients with CSFP present with acute coronary syndromes associated with stress response and activated immune system. Unfortunately, many studies investigating the role of immune response in CSFP did not exclude these patients.

As it is in the present work, genetic polymorphism studies based on the restriction fragment length polymorphism (RFLP) technique have been developed as suitable tools for establishing a causative relationship between the development of a certain condition and its genetic predisposition. With this technique, Shi et al. (22) firstly investigated the impact of genetic tendency for inflammation and demonstrated an association between CSFP and IL-10 promoter-592 polymorphisms in patients with CSFP. There was also a decrease in the production of IL-10.



The major findings of our study were as follows: (i) To the best of our knowledge, the present study is the first to point out the potential relationship between IL-1 gene cluster polymorphisms and CSFP. (ii) We demonstrated that IL-1 $\beta$ +3954 SNP was more frequent in patients with CSFP than in controls, whereas IL-1Ra and IL-1 511 SNPs were similar between the groups. (iii) Activated immune system may be one of the causative factors for CSFP and this “chicken or egg” problem may be originated from a genetic predisposition.

IL-1 $\beta$ +3954 SNP was found to be more frequent in patients with CSFP than in controls. However, neither IL-1Ra nor IL-1 511 SNPs was associated with CSFP. Therefore, our findings are partially supportive of the hypothesis that CSFP is a consequence of activated immune system associated with a genetic predisposition.

In the present study, we hypothesized that genetic polymorphisms involving IL-1 gene cluster might be associated with CSFP by augmenting inflammatory system activity. These genetic polymorphisms were shown to be associated with changes in basal levels of inflammation in the body. So, the associations examined by us specifically reflected the association between life-long increased activity of inflammatory system and CSFP development. Second and most important, we excluded patients with acute coronary syndromes from the study. TIMI frame counts in each epicardial coronary artery and mean TIMI frame counts were lower in our CSFP group than those in previously published studies, which might again be associated with a patient group with a relatively lower level of inflammation.

Studies regarding the associations between IL-1 gene cluster polymorphisms and coronary heart disease risk resulted with inconsistent findings. In a meta-analysis including 13 studies, no associations were found between IL-1 gene cluster polymorphisms and coronary artery disease (36). Likewise, studies on Turkish patients with coronary artery disease reported conflicting results regarding IL-1 genetic predispositions, showing no or only modest associations with the disease (37, 38). There might be various reasons for this discrepancy between findings. First, CSFP seems to be a distinct clinical entity not completely related to coronary artery disease. Second, most of the studies did not include the correlation with blood IL-1 $\beta$  and/or IL-1Ra levels. Genetic interactions in IL-1 gene cluster are very complex, and simple cause-effect relationships with these polymorphisms are difficult to explain.

Unlike the majority of the previous studies, genetic polymorphism studies like our study are not susceptible to errors related with the timing of the blood sample collection. Our patient and control groups did not show a significant difference with respect to hs-CRP, which might be attributed to the confounding effect of this timing of the sample collection. None the less, the clinical picture of CSFP is related to the response of the coronary circulation to the inflammation as well as the inflammatory system activation. As a result, since all patients enrolled in this study were symptomatic in a way to warrant coronary angiography, these individuals might have similarly increased levels of hs-CRP.

Another significant finding in our study was the decreased E/A ratio and the increased IVRT in the CSFP group. This finding most probably does not reflect a causative relationship, which would not be expected from a cross-sectional, case-control study. This finding might be because of subtle impairment in left ventricular diastolic filling profiles as a cause and/or consequence of CSFP. Although heart failure as well as other conditions resulting in increased left ventricular end-diastolic pressures is shown as a cause of secondary CSFP, isolated impaired diastolic filling has not previously been identified as a cause of CSFP. Impaired diastolic filling pattern might be because of significantly decreased resting coronary blood flow patterns in these patients, alternatively this could be a subclinical sign of the myocardial dysfunction in these patients (39).

Genetic investigations reached a turning point with the advent of next generation sequencing with linkage analysis and genome-wide association studies. Data on genetical basis of CAD is accumulating with new data from these studies. We believe that new studies using these contemporary modalities with population-based real-life data analyzed by the big-data analysis methods will be needed to better understand this relationships (40).

### Study limitations

First, our control group was selected from patients who underwent coronary angiography due to chest pain or other symptom suggestive of coronary artery disease either with or without resting ECG changes, exercise stress testing, or nuclear perfusion scan. Similar to the majority of the case-control studies in this patient group, we did not perform provocation tests to rule out vasospastic angina. Second, selecting healthy individuals who do not have any symptoms as the control group is expected to provide more accurate results. Another limitation of our study might be the small sample size. Likewise, a larger control group at least two times the size of our patient group would have yielded more robust results. Although our study population was not very small compared with other studies in the literature, larger studies with larger control groups would yield more valid and reliable results (7). Interobserver and intraobserver variability should be checked when possible if manual measurements are performed. Although not present in most of the studies in this area, the fact that it was not performed might be another limitation of our study. Also, we did not measure plasma levels of IL-1 $\beta$  and IL-1Ra. A larger scale study with plasma levels of IL-1 $\beta$  and IL-1Ra would demonstrate relationships more clearly.

### Conclusion

On the basis of the present study's results, systemic inflammation might contribute CSFP via increased immune system. The present study is the first clinical study that demonstrated that IL-1 $\beta$  -511 SNP is significantly more common in patients with CSFP than in patients with normal coronary arteries in coronary

angiography. Our preliminary report might be a start point for new prospective studies investigating the potential role of inflammation in CSFP using genetical analysis.

**Acknowledgments:** The paper was previously presented as an oral presentation in the 12<sup>th</sup> International Congress of Update in Cardiology and Cardiovascular Surgery, Antalya, Turkey in May, 2016, with the following title: "Coronary slow flow phenomenon and systemic inflammation: a "chicken or egg" problem."

**Funding:** None declared.

**Conflict of interest:** None declared.

**Peer-review:** Externally peer-reviewed.

**Authorship contributions:** Concept – F.O.M.; Design – F.O.M.; Supervision – D.U., B.G.; Funding – F.O.M.; Materials – F.O.M., B.G., O.B.; Data collection &/or processing – B.G., O.B., T.A.; Analysis &/or interpretation – F.O.M., B.G., O.B., T.A.; Literature search – F.O.M., B.G., D.U., O.B., T.A.; Writing – F.O.M., B.G., T.A.; Critical review – T.A., D.U.

## References

- Tambe AA, Demany MA, Zimmerman HA, Mascarenhas E. Angina pectoris and slow flow velocity of dye in coronary arteries--a new angiographic finding. *Am Heart J* 1972; 84: 66-71.
- Wang X, Nie SP. The coronary slow flow phenomenon: characteristics, mechanisms and implications. *Cardiovasc Diagn Ther* 2011; 1: 37-43.
- Beltrame JF, Limaye SB, Wuttke RD, Horowitz JD. Coronary hemodynamic and metabolic studies of the coronary slow flow phenomenon. *Am Heart J* 2003; 146: 84-90.
- Barutcu I, Sezgin AT, Sezgin N, Güllü H, Esen AM, Topal E, et al. Elevated plasma homocysteine level in slow coronary flow. *Int J Cardiol* 2005; 101: 143-5.
- Sezgin N, Barutcu I, Sezgin AT, Güllü H, Türkmen M, Esen AM, et al. Plasma nitric oxide level and its role in slow coronary flow phenomenon. *Int Heart J* 2005; 46: 373-82.
- Turhan H, Saydam GS, Erbay AR, Ayaz S, Yaşar AS, Aksoy Y, et al. Increased plasma soluble adhesion molecules; ICAM-1, VCAM-1, and E-selectin levels in patients with slow coronary flow. *Int J Cardiol* 2006; 108: 224-30.
- Kopetz V, Kennedy J, Heresztyn T, Stafford I, Willoughby SR, Beltrame JF. Endothelial function, oxidative stress and inflammatory studies in chronic coronary slow flow phenomenon patients. *Cardiology* 2012; 121: 197-203.
- Sprague AH, Khalil RA. Inflammatory Cytokines in Vascular Dysfunction and Vascular Disease. *Biochem Pharmacol* 2009; 78: 539-52.
- Galea J, Armstrong J, Gadsdon P, Holden H, Francis SE, Holt CM. Interleukin-1 beta in coronary arteries of patients with ischemic heart disease. *Arterioscler Thromb Vasc Biol* 1996; 16: 1000-6.
- Patti G, Di Sciascio G, D'Ambrosio A, Dicuonzo G, Abbate A, Dobrina A. Prognostic value of interleukin-1 receptor antagonist in patients undergoing percutaneous coronary intervention. *Am J Cardiol* 2002; 89: 372-6.
- Arend WP. The balance between IL-1 and IL-1Ra in disease. *Cytokine Growth Factor Rev* 2002; 13: 323-40.
- Steinkasserer A, Spurr NK, Cox S, Jeggo P, Sim RB. The human IL-1 receptor antagonist gene (IL1RN) maps to chromosome 2q14-q21, in the region of the IL-1 alpha and IL-1 beta loci. *Genomics* 1992; 13: 654-7.
- Tountas NA, Casini-Raggi V, Yang H, Di Giovine FS, Vecchi M, Kam L, et al. Functional and ethnic association of allele 2 of the interleukin-1 receptor antagonist gene in ulcerative colitis. *Gastroenterology* 1999; 117: 806-13.
- Santtila S, Savinainen K, Hurme M. Presence of the IL-1RA allele 2 (IL1RN\*2) is associated with enhanced IL-1beta production in vitro. *Scand J Immunol* 1998; 47: 195-8.
- Bioque G, Crusius JB, Koutroubakis I, Bouma G, Kostense PJ, Meuwissen SG, et al. Allelic polymorphism in IL-1 beta and IL-1 receptor antagonist (IL-1Ra) genes in inflammatory bowel disease. *Clin Exp Immunol* 1995; 102: 379-83.
- Francis SE, Camp NJ, Burton AJ, Dewberry RM, Gunn J, Stephens-Lloyd A, et al. Interleukin 1 receptor antagonist gene polymorphism and restenosis after coronary angioplasty. *Heart* 2001; 86: 336-40.
- Manzoli A, Andreotti F, Varlotta C, Mollicelli N, Verde M, van de Greef W, et al. Allelic polymorphism of the interleukin-1 receptor antagonist gene in patients with acute or stable presentation of ischemic heart disease. *Cardiologia* 1999; 44: 825-30.
- Worrall BB, Azhar S, Nyquist PA, Ackerman RH, Hamm TL, DeGraba TJ. Interleukin-1 receptor antagonist gene polymorphisms in carotid atherosclerosis. *Stroke* 2003; 34: 790-3.
- Fragoso JM, Delgadillo H, Llorente L, Chuquiure E, Juarez-Cedillo T, Vallejo M, et al. Interleukin 1 receptor antagonist polymorphisms are associated with the risk of developing acute coronary syndrome in Mexicans. *Immunol Lett* 2010; 133: 106-11.
- Ekmekçi A, Güngör B, Özcan KS, Abacı N, İlhan E, Ekmekçi SS, et al. Evaluation of coronary microvascular function and nitric oxide synthase intron 4a/b polymorphism in patients with coronary slow flow. *Coron Artery Dis* 2013; 24: 461-7.
- Gazi E, Temiz A, Altun B, Barutcu A, Silan F, Çölkesen Y, et al. Endothelial function and germ-line ACE I/D, eNOS and PAI-1 gene profiles in patients with coronary slow flow in the Canakkale population: multiple thrombophilic gene profiles in coronary slow flow. *Cardiovasc J Afr* 2014; 25: 9-14.
- Shi GL, Cai XX, Su YM, Chen C, Deng XT, Pan HY, et al. Interleukin-10 promoter -592A/C polymorphism is associated with slow coronary flow in Han Chinese. *Int J Clin Exp Pathol* 2015; 8: 4091-8.
- Gibson CM, Cannon CP, Daley WL, Dodge JT Jr, Alexander B Jr, Marble SJ, et al. TIMI frame count: a quantitative method of assessing coronary artery flow. *Circulation* 1996; 93: 879-88.
- Faul F, Erdfelder E, Lang AG, Buchner A. G\* Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav Res Methods* 2007; 39: 175-91.
- Mangieri E, Macchiarelli G, Ciavolella M, Barilla F, Avella A, Martinotti A, et al. Slow coronary flow: clinical and histopathological features in patients with otherwise normal epicardial coronary arteries. *Cathet Cardiovasc Diagn* 1996; 37: 375-81.
- Mosseri M, Yarom R, Gotsman MS, Hasin Y. Histologic evidence for small-vessel coronary artery disease in patients with angina pectoris and patent large coronary arteries. *Circulation* 1986; 74: 964-72.
- Yazıcı M, Aksakal E, Demircan S, Şahin M, Sağkan O. Is slow coronary flow related with inflammation and procoagulant state? *Anadolu Kardiyol Derg* 2005; 5: 3-7.
- Madak N, Nazlı Y, Mergen H, Aysel S, Kandaz M, Yanık E, et al. Acute phase reactants in patients with coronary slow flow phenomenon. *Anadolu Kardiyol Derg* 2010; 10: 416-20.

29. Pekdemir H, Çiçek D, Çamsarı A, Akkuş MN, Cin VG, Doven O, et al. The relationship between plasma endothelin-1, nitric oxide levels, and heart rate variability in patients with coronary slow flow. *Ann Noninvasive Electrocardiol* 2004; 9: 24-33.
30. Özyurtlu F, Yavuz V, Çetin N, Acet H, Ayhan E, Işık T. The association between coronary slow flow and platelet distribution width among patients with stable angina pectoris. *Postepy Kardiologii Interwencyjnej* 2014; 10: 161-5.
31. Doğan M, Akyel A, Çimen T, Bilgin M, Sunman H, Kasapkara HA, et al. Relationship between neutrophil to lymphocyte ratio and slow coronary flow. *Clin Appl Thromb Hemost* 2015; 21: 251-4.
32. Altaş Y, Kurtoğlu E, Yaylak B, Baysal E, Ucaman B, Uğurlu HM, et al. The relationship between eosinophilia and slow coronary flow. *Ther Clin Risk Manag* 2015; 11: 1187-91.
33. Yurtdaş M, Yaylalı YT, Kaya Y, Özdemir M. Increased plasma high-sensitivity C-reactive protein and myeloperoxidase levels may predict ischemia during myocardial perfusion imaging in slow coronary flow. *Arch Med Res* 2014; 45: 63-9.
34. Li JJ, Qin XW, Li ZC, Zeng HS, Gao Z, Xu B, et al. Increased plasma C-reactive protein and interleukin-6 concentrations in patients with slow coronary flow. *Clin Chim Acta* 2007; 385: 43-7.
35. Durakoğlugil ME, Kocaman SA, Çetin M, Kırbaş A, Canga A, Erdoğan T, et al. Increased circulating soluble CD40 levels in patients with slow coronary flow phenomenon: an observational study. *Anadolu Kardiyol Derg* 2013; 13: 39-44.
36. Zhou L, Cai J, Liu G, Wei Y, Tang H. Associations between interleukin-1 gene polymorphisms and coronary heart disease risk: a meta-analysis. *PLoS One* 2012; 7: e45641.
37. Soylu O, Yıldırım A, Coker A, Tezel T, List EO, Arman A. Interleukin-1B (-511) gene polymorphism is associated with acute coronary syndrome in the Turkish population. *Eur Cytokine Netw* 2008; 19: 42-8.
38. Coker A, Arman A, Soylu O, Tezel T, Yıldırım A. Lack of association between IL-1 and IL-6 gene polymorphisms and myocardial infarction in Turkish population. *Int J Immunogenet* 2011; 38: 201-8.
40. Durda P, Sabourin J, Lange EM, Nalls MA, Mychaleckyj JC, Jenny NS, et al. Plasma Levels of Soluble Interleukin-2 Receptor alpha: Associations With Clinical Cardiovascular Events and Genome-Wide Association Scan. *Arterioscler Thromb Vasc Biol* 2015; 35: 2246-53.