Endothelial nitric oxide synthase Asp298Glu (894G/T) gene polymorphism as a possible risk factor for the coronary slow flow phenomenon among Iranians

Yeganeh Karimi1, Fatemeh Sehati1, Ali Sarreshtedari1, Mina Mirzad1, Yasaman Khalili3, Reza Kiani2, Elham Taheri Bajgan4, Maryam Hosseini Moghadam3, Farzaneh Mehrvarz1, Hooman Bakhshandeh1, Maryam Parham2, Mahshid Malakootian3* and Parham Sadeghipour2,3*

Abstract

Background: Mounting evidence indicates an association between endothelial dysfunction and the coronary slow flow phenomenon (CSFP). In the present study, we aimed to evaluate the possible role of endothelial nitric oxide synthase (eNOS) 894G/T and interleukin-1β (IL-1β) 315C/T polymorphisms as possible risk factors for CSFP.

Methods: This prospective study enrolled patients with CSFP and individuals with normal coronary arteries. Genotypes were assessed using regular polymerase chain reaction and direct Sanger-sequencing techniques.

Results: The study population consisted of 267 individuals: 180 patients with CSFP (49 women [27.2%]) at a median age of 55 (48–62) years and 87 controls with normal coronary arteries (56 women [64.4%]) at a median age of 47 (41–58) years. The allelic distribution of eNOS 894G/T was significantly associated with CSFP (odds ratio [OR], 1.58; 95% confidence interval (CI), 1.04–2.42; \( P = 0.03 \)). This polymorphism increased the risk of CSFP under the dominant model (OR 1.73; 95% CI 1.02–2.95; \( P = 0.04 \)). However, the allelic frequencies (1.05; 95% CI 0.68–1.59; \( P = 0.83 \)) and genotypic frequencies (0.88; 95% CI 0.52–1.49; \( P = 0.63 \)) of the IL-1β 315C/T polymorphism were not associated with the incidence of CSFP in the Iranian population.

Conclusions: The CSFP and control groups were statistically different regarding the eNOS 894G/T polymorphism. Our findings also demonstrated that the IL-1β 315C/T polymorphism was not a risk factor for CSFP.

Keywords: Coronary artery disease, Endothelial dysfunction, Coronary slow flow phenomenon, Endothelial nitric oxide synthase, Interleukin-1β, rs1799983, rs1143634

Introduction

The coronary slow flow phenomenon (CSFP), an uncommon disease, is an angiographic finding characterized by the delayed opacification of the distal branch of the coronary arteries in the absence of obstructive coronary artery disease [1, 2]. It is only found in 7% of patients with coronary artery disease undergoing diagnostic angiography [3]. CSFP seems to be multifactorial, and its precise etiopathological mechanisms have yet to be elucidated.
Morphological abnormalities such as fibromuscular hyperplasia, medial hypertrophy, myointimal proliferation, and subclinical atherosclerosis, as well as anatomic factors, functional abnormalities, and inflammation, have been proposed as the pathogenic factors of the disease [1, 4, 5]. Mounting evidence indicates common single-nucleotide polymorphisms (SNPs) residing in different genes as genetic risk factors for CSFP [6–9].

Nitric oxide (NO) is a vasodilator synthesized from L-arginine by endothelial nitric oxide synthase (eNOS), which is encoded by a single eNOS (NOS3) gene located on chromosome 7q35-q36 [10]. One of the most studied SNPs of eNOS is 894G/T (rs1799983), which results in the decreased production of NO and is significantly associated with coronary artery disease in different populations [11–17]. In addition, numerous studies have indicated that the plasma level of NO is significantly lower in patients with CSFP than in healthy controls [9, 18–20]. The interleukin-1 (IL-1) family comprises a group of proinflammatory cytokines composed of α and β types. The family, the product of the IL-1 gene, modulates the chronic inflammatory response by increasing leukocyte adhesion to damaged endothelia, although several mediators are involved in the atherosclerosis process and cardiovascular disease [21].

The literature contains conflicting reports on the relationship between CSFP and eNOS 894G/T (rs1799983, Asp298Glu) and IL-1β 315C/T (rs1143634, Phe105=) from studies carried out on different populations across the world [7, 9, 22–25].

In the present study, we sought to investigate the association between CSFP and eNOS 894G/T (rs1799983, Asp298Glu) and IL-1β 315C/T (rs1143634, Phe105=) in a sample of the Iranian population, divided into patients with CSFP and normal individuals.

**Methods**

**Study population**

The study population was selected from candidates for coronary angiography in Rajaie Cardiovascular Medical and Research Center in Tehran, Iran. Patients with valvular heart disease, congenital heart disease, arrhythmia, connective tissue disease, collagen vascular disease, and more than 25% obstruction in the vessel diameter were excluded. The control group was chosen from individuals in whom diagnostic coronary angiography showed no coronary artery disease. Peripheral blood samples were taken from all the participants to determine genotypes, lipid profiles, cardiac enzyme levels, creatinine levels, cell blood counts, and erythrocyte sedimentation rates. The blood samples for genetic analysis were preserved at −70 °C.

The study protocol was approved by the Ethics Committee of Rajaie Cardiovascular Medical and Research Center (IR.RHC.REC.1399.075), and the study was conducted in accordance with the Helsinki Declaration.

**Definition of CSFP**

CSFP was diagnosed via the thrombolysis in myocardial infarction frame count (TFC) method. Participants with a corrected TFC greater than 2 standard deviations from the published normal range for the particular vessel were considered to have CSFP (the left anterior descending coronary artery (LADA) > 36.2 ± 2.6, the left circumflex artery (LCx) > 22.2 ± 4.1, and the right coronary artery (RCA) > 20.4 ± 3.0).

The standard method was drawn upon for left heart catheterization and coronary angiography. CSFP was defined based on the TFC method introduced by Gibson [26]. The number of cine frames required for the contrast to reach the standard landmark in the distal coronary artery is termed “TFC.” The first frame in TFC is obtained when the contrast material enters the coronary artery completely, with the entrance having 3 characteristics: (1) The contrast material should fill the full thickness of the vessel. (2) The contrast material should be in contact with both margins of the vessel. (3) The contrast agent should move forward. The last frame is obtained when the contrast material enters the distal landmark branch. The distal landmark branches are defined for each vessel separately: the last 2 branches for the left anterior descending, the last obtuse marginal branch for the left circumflex artery, and the first branch of the posterior left ventricular branch for the right coronary artery. The images were obtained at a rate of 15 frames per second, and the results were multiplied by 2. The frame counts of the left anterior descending were divided by 1.7 for correction because of its length. Patients who had a frame count above 27 for all vessels were considered to have CSFP.

**Genotyping of the eNOS and IL-1β gene polymorphisms**

**DNA extraction**

Genomic DNA was extracted from the peripheral blood samples, collected in EDTA tubes, using the salting-out method and the Exgene Blood SV Mini Kit (GeneAll, Seoul, South Korea). The NanoDrop Spectrophotometer (Thermo Fisher Scientific, US) was employed to determine the quantity of the extracted DNA.

**Polymerase chain reaction (PCR) and direct Sanger sequencing**

Appropriate PCR oligonucleotides were designed to amplify the desired part of the IL-1β and eNOS3 genes by utilizing the Gene Runner (Gene Runner 6.5.50).
and PerlPrimer (PerlPrimer 1.1.21) software tools. Further, 5′-AAGGCAGGACAGTGGATG-3′ (forward), 5′-CAATTTCCAGCAGCATGTTG-3′ (reverse), 5′-CGTATAGCCTAGGTGTCCTC-3′ (forward), and 5′-CATGGAGATTAGCAAAGCT-3′ (reverse) primers were used to amplify the part of eNOS (385 base pairs in length) and IL-1β (230 base pairs in length) that covered the desired variations with the following thermal program: 94 °C for 35 s, 63 °C (eNOS) or 55 °C (the IL-1β variant) for 30 s, and 72 °C for 45 s, with a final extension at 72 °C for 10 min. Amplicons were electrophoresed on a 1.5% agarose gel, stained with ethidium bromide, and visualized under ultraviolet light.

All the PCR products were subjected to direct Sanger sequencing with the ABI 3500 DNA Sequencer (Applied Biosystems, CA, US). The reverse primer of eNOS3 and the forward primer of IL-1β were used for direct sequencing.

**Statistical analysis**

HGMD [27], NCBI [28], UCSC [29], and VarSome [30] databases were utilized to evaluate the selected SNPs and the pathogenesis of the selected mutations.

The BioEdit software (BioEdit 7.2.1) was run to analyze the sequencing outcomes. The results were analyzed using the IBM SPSS statistics 26, the GraphPad Prism 9 software, and the SNPSTAT analyzer [31].

The 1-sample Kolmogorov–Smirnov test was first applied to test the normality of the data. Qualitative data were presented as numbers and percentages. The association between categorical variables was assessed using the χ² test; and if 20% of the cells had the expected count of lower than 5, the Fisher exact test was employed. Quantitative data were described as the medians (Q1–Q3) for nonparametric data. The independent samples t test was applied to compare the mean values, and the Mann–Whitney test was used to compare the median values between 2 groups. Additionally, ANOVA and Kruskal–Wallis tests were drawn upon to compare the mean and median values between more than 2 groups. Finally, the multivariante regression analysis was applied using STATA 13.

**Results**

**Clinical characteristics of the study population**

From 2016 through 2017, a total of 180 patients with CSFP (49 women [27.2%]) at a median age of 55 (48–62) years were enrolled in the CSFP group. From 2016 through 2018, a total of 87 individuals with normal coronary arteries (56 women [64.4%]) at a median age of 47 (41–58) years were enrolled in the control group. The baseline and clinical characteristics of both groups are summarized in Table 1. Table 2 shows the clinical and laboratory characteristics of the CSFP group. Both groups were similar regarding baseline characteristics and laboratory data except for age and sex. The patient and control groups were similar in terms of dyslipidemia, diabetes mellitus, hypertension, smoking, and a family history for coronary artery disease. Additionally, the levels of plasma creatinine, triglyceride, and hemoglobin were higher in the patients with CSFP than in the control group. The median value (Q1–Q3) TFC for the left anterior descending coronary artery, the left circumflex artery, and the right coronary artery was 36 (29–43), 40 (31–50), and 31.5 (24–40), respectively, in the CSF group.

**Allelic and Genotype Distributions of eNOS 894G/T and IL-1β 315C/T**

The results concerning the allelic and genotype distributions of the eNOS3 and IL-1β polymorphisms are depicted in Table 3. The genotype frequencies of eNOS 894G/T polymorphism was in accordance with the Hardy–Weinberg equilibrium in the CSFP group (χ² = 1.484, P = 0.48) and in control group (χ² = 0.1867, P = 0.91). Likewise, the genotype frequencies of IL-1β 315C/T polymorphism was in line with those predicted by the Hardy–Weinberg equilibrium in the CSFP group (χ² = 0.3557, P = 0.84) and in control group (χ² = 6.446, P = 0.04).

In the univariate analysis of eNOS 894G/T, the frequencies of the T allele in the patient and control groups were 20% and 29%, respectively, and a significant difference was found in the allelic distribution of eNOS (odds ratio [OR], 1.58; 95% confidence interval [CI], 1.04–2.42; P = 0.03). Consequently, the higher presence of the T allele of eNOS in the control group hinted at a protective effect exerted by this allele on the study population. In the CSFP and control groups, respectively, the frequency of the G/G genotype was 65% versus 51.7%, the frequency of the G/T genotype was 29.4% versus 39.1%, and the frequency of the T/T genotype was 5.6% versus 9.2%. The analysis of the genotype distribution in the 2 groups demonstrated a significant association between the presence of the T allele of eNOS and CSFP (OR 1.73; 95% CI 1.02–2.95; P = 0.04) in a dominant model. Nonetheless, no significant differences were found between the recessive model and the codominant model.

In the univariate analysis of IL-1β 315C/T, the frequency of the T allele was estimated to be 24% and 25% in the patients and controls, respectively, and no significant difference was found in the allelic distribution of IL-1β (P = 0.83). The frequencies of the C/C, C/T, and T/T genotypes were 57.9% versus 60.9%, 35.4% versus 27.6%, and 6.7% versus 11.5% in the patients with CSFP and the participants with normal coronary arteries, respectively (P = 0.25). Additionally,
no significant differences in genotype distribution were found in the dominant ($P=0.63$), recessive ($P=0.19$), and codominant ($P=0.25$) models between the patients and the healthy controls concerning $IL-1\beta$.

Furthermore, the association between the CSFP phenotype and the combined genotypes of the $eNOS$ and $IL-1\beta$ polymorphisms was assessed, and the results were nonsignificant ($P=0.12$) (data not shown).

In the multivariable regression analysis, age (OR 1.08; 95% CI; 1.03 to 1.12; $P<0.01$) and the male sex (OR 0.22; 95% CI 0.08 to 0.62; $P<0.01$) were the only independent predictors of CSFP in the study population. In addition, no significant associations were found between the presence of the mutant allele and the wild type for the $eNOS$ and $IL-1\beta$ polymorphisms with the application of the multivariable analysis (OR 0.46; 95% CI 0.11 to 1.98; $P=0.29$ for $eNOS$, and OR 0.47; 95% CI 0.13 to 1.7; $P=0.24$ for $IL-1\beta$) (Table 4).

### Table 1 Comparison of baseline characteristics and lab data between the CSFP and control groups$^{ab}$

| Characteristics | Total (N = 267) | CSFP Group (n = 180) | Control Group (n = 87) | $P$ value |
|----------------|----------------|---------------------|-----------------------|-----------|
| Demographic | | | | |
| Age (years) | 52 (46–61) | 55 (48–62) | 47 (41–58) | <0.001 |
| Female/Male | 105 (39.3)/ 162 (60.7) | 49 (27.3)/ 131 (72.8) | 56 (64.4)/ 31 (35.6) | <0.001 |
| Dyslipidemia (%) | 87 (32.6) | 63 (35) | 24 (27.6) | 0.41 |
| Diabetes mellitus (%) | 78 (29.2) | 52 (28.9) | 26 (29.9) | 0.56 |
| Hypertension (%) | 118 (44.2) | 76 (42.2) | 42 (48.3) | 0.12 |
| Smoking status (%) | 56 (21.0) | 43 (23.9) | 13 (14.9) | 0.11 |
| Family history for CAD$^c$ (%) | 74 (27.7) | 55 (30.6) | 19 (21.8) | 0.26 |
| Laboratory Tests$^d$ | | | | |
| Fasting blood sugar (mg/dL) | 106 (95–134.5) | 109 (95–135) | 102.5 (94.75–132.75) | 0.37 |
| Plasma creatinine (mg/dL) | 1 (0.9–1.2) | 1 (0.9–1.2) | 0.9 (0.8–1) | <0.001 |
| Triglyceride (mg/dL) | 128 (88–181) | 140 (95.5–192) | 111 (78–156) | 0.01 |
| Total cholesterol (mg/dL) | 138.5 (116.25–163.75) | 141 (116.5–168) | 133 (115–155) | 0.08 |
| High-density lipoprotein-cholesterol (mg/dL) | 38 (34–42) | 38 (34.6–42) | 37 (32–42) | 0.33 |
| Low-density lipoprotein-cholesterol (mg/dL) | 76 (57–95) | 77 (57–98) | 72 (57–91) | 0.26 |
| Hemoglobin (g/dL) | 14.1 (12.8–15.2) | 14.2 (13.2–15.3) | 13.2 (12.12–14.77) | <0.001 |
| White blood cell (cells/mm$^3$) | 6800 (5675–8125) | 6800 (5700–7900) | 6900 (5600–8400) | 0.71 |
| Platelet ($\times$ 10$^9$/mm$^3$) | 219 (182–257.5) | 216.5 (180–251.25) | 225 (188.25–269.75) | 0.15 |
| Erythrocyte sedimentation rate (mm/h) | 10 (5–17) | 10 (5–15) | 12 (6–18.75) | 0.04 |

CSFP: coronary slow flow phenomenon; CAD: coronary artery disease

$^a$ Continuous variables are presented as the median (Q1–Q3)

$^b$ Categorical variables are presented as numbers (%)

$^c$ Family history of coronary artery diseases (CAD): the presence of CAD in a first-degree male or female relative before age 55 or 65 years, respectively

$^d$ Normal ranges of the measured lab tests were defined as follows: <100 mg/dL for fasting blood sugar, 0.6–1.4 mg/dL for plasma creatinine, <150 mg/dL for triglyceride, <200 mg/dL for total cholesterol, <110 mg/dL for low-density lipoprotein, 13.5–17.5 g/dL for men and 12–15.6 g/dL for women for the hemoglobin level, 4500–11,000 cells/mm$^3$ for the white blood cell count, 150–450 10$^3$/F/l for the platelet count, <22 mm/h for men and 29 mm/h for women for the erythrocyte sedimentation rate

### Relationships Between the eNOS3 864G/T and IL-1β 315C/T Genotypes and TFC and Electrocardiographic Findings in the CSFP Group

According to the univariate analysis, the median values of TFC for the left anterior descending ($P=0.80$), the left circumflex ($P=0.16$), and the right coronary artery ($P=0.80$) were not significantly different between the individuals with different genotypes of $eNOS$ 894G/T. Moreover, no significant differences were found in terms of the median values of TFC for the left anterior descending ($P=0.53$) and the left circumflex ($P=0.11$) between the genotypes of $IL-1\beta$ 315C/T. However, the median value of TFC for the right coronary artery was different between the $IL-1\beta$ genotypes ($P<0.01$; Table 5).

Sex (coefficient, $-3.48$; 95% CI $-7.06$ to 0.11; $P=0.05$) was the only predictor of TFC for the left anterior descending, and no statistically significant associations were found between TFC for the left
Table 2 Clinical, laboratory, and angiographic characteristics of the CSFP groupab

| Characteristics                          | Patients |
|-----------------------------------------|----------|
| **Clinical**                            |          |
| Chest pain                              |          |
| Typical                                 | 107 (59.4) |
| Atypical                                | 59 (32.8) |
| Dyspnea                                 | 58 (32.2) |
| Palpitation                             | 8 (4.4)  |
| Past medical history                    |          |
| Prior PCI                               | 14 (7.8) |
| Prior MI                                | 10 (5.6) |
| HF                                      | 1 (0.6)  |
| BMIa                                    | 28.3 (25.7–30.97) |
| **Baseline hemodynamics**               |          |
| Systolic blood pressure (mm Hg)         | 125 (119–135) |
| Diastolic blood pressure (mm Hg)        | 80 (70–80) |
| **Laboratory and Echocardiography**     |          |
| hs-CRP                                  | 1.7 (0.7–4) |
| Positive cardiac enzyme                 | 24 (13.3) |
| LVEF                                    | 50 (50–55) |
| **Angiographic characteristics**       |          |
| TFC                                      |          |
| Left anterior descending artery          | 36 (29–43) |
| Left circumflex artery                  | 40 (31–50) |
| Right coronary artery                   | 31.5 (24–40) |
| Single-vessel slow flow coronary artery | 15 (8.3) |
| Double-vessel slow flow coronary arteries | 57 (31.7) |
| Triple-vessel slow flow coronary arteries | 108 (60) |

a Continuous variables are presented as the median (Q1–Q3)  b Categorical variables are presented as numbers (%)  c Weight (in kilograms) divided by the square of the height (in meters)

anterior descending and the presence of the T allele of the eNOS 864 T/G polymorphism, the presence of the mutant allele of the IL-1β 315C/T polymorphism, body mass index, systolic blood pressure, and left ventricular ejection fraction in the multivariable regression analysis (P > 0.05) (Additional file 1: Table S1). Further, all 180 patients with CSFP underwent electrocardiography. Among them, ST-T changes were positive in 43 patients (23.9%), of whom 17 (39.5%) had the T allele in the eNOS3 locus. No significant differences were noted in electrocardiographic findings between the eNOS3 genotypes.

Similar to the eNOS results, no significant associations were found in electrocardiographic findings between the different genotypes of the studied IL-1β SNP. The distribution of the IL-1β genotypes was similar among those with a positive ST-T change finding (P = 0.26).

Discussion
In the present study, we examined the association between CSFP and eNOS3 (894G/T) and IL-1β (315C/T) polymorphisms in a sample of the Iranian population. Our results indicated that the distribution of the Asp298Glu variant of the eNOS gene was significantly different between patients with CSFP and controls with normal coronary arteries. Further, the mutant allele T of eNOS 894G/T polymorphism was lower in the CSFP suggesting that this polymorphism is protective. While there was no significant association between the IL-1β gene (315C/T) variant and CSFP in our studied population. We also assessed associations in diagnostic tests, clinical information, and lab data between the eNOS3 (894G/T) and IL-1β (315C/T) variants and found no significant associations.

CSFP was first defined by Tambe et al. in 1972 as a delay in the progression of the contrast dye injected into the coronary arteries during coronary angiography without any obstructive disease [32]. The phenomenon is diagnosed mainly with an increased TFC. Although the etiology and pathogenesis of CSFP are not well-known, impaired balances between vasoconstrictor and vasodilator factors and increased inflammatory markers have been suggested [4, 9, 33]. Urotensin-II, as a potent vasoconstrictor, has been reported as a possible risk factor for CSFP (OR 1.01; 95% CI 1.00–1.014; P = 0.01) [34]. Furthermore, aortic pulse pressure and the pulsatility index in patients with CSFP tend to rise remarkably due to endothelial dysfunction. The role of inflammation in the pathophysiology of CSFP was expounded by Aksn G et al., who found that the serum levels of neutrophil gelatinase-associated lipocalin, as an inflammatory biomarker, were significantly higher in patients with CSF than in those with a normal coronary flow [35]. In addition, the hematocrit level, as well as erythrocyte, eosinophil, and basophil counts, was increased in patients with CSF compared with the group with a normal coronary flow, which may support the previous hypothesis [36]. Substantial evidence suggests that the eNOS Glu298Asp polymorphism is responsible for endothelial dysfunction [37–39].

NO plays a significant role as a vasorelaxation factor and has a protective effect on atherogenesis [40]. It has been shown that several polymorphisms of eNOS (NOS3) affect the serum level of NO [41]. Notably, the eNOS Asp298Glu polymorphism may be associated with CSFP in that it decreases the serum levels of
NO. Moreover, the \textit{IL-1\textbeta} gene, which releases \textit{IL-1\textbeta} as a proinflammatory agent, is associated with cardiovascular diseases, including coronary artery disease, stent restenosis after percutaneous coronary interventions, carotid artery disease, lone atrial fibrillation, and CSFP [42–45]. In addition, the 315C/T nucleotide transition of the \textit{IL-1\textbeta} gene probably modulates IL-1\textbeta protein synthesis and is associated with such cardiovascular diseases as CSFP, coronary artery disease, and myocardial infarction [25, 46–49].

Previous studies have also indicated the role of genetic predisposing factors in the occurrence of CSFP [22–24, 50]. There are dissimilarities in the frequencies of eNOS3 894G/T alleles in different races. Such differences have given rise to controversy as regards the application of the G allele as a mutant. The VarSome database recognizes

| Table 3 | Distributions of the eNOS3 864G/T and IL-1\textbeta 315C/T alleles and genotypes in the CSFP and control groups |
|---------|---------------------------------------------------------------|
|         | Patients With CSFP; N (%) | Controls; N (%) | OR (95% CI)<sup>b</sup> | \( P \) value<sup>a</sup> |
| \textit{eNOS} 894G/T | | | | |
| Allele Frequency | | | | |
| G | 287 (80) | 124 (71) | 1.00 | 0.03 |
| T | 73 (20) | 50 (29) | 1.58 (1.04–2.42) | |
| Total | 360 (100) | 174 (100) | | |
| Genotypes (codominant) | | | | |
| G/G | 117 (65) | 45 (51.7) | 1.00 | 0.11 |
| G/T | 53 (29.4) | 34 (39.1) | 0.60 (0.35–1.04) | |
| T/T | 10 (5.6) | 8 (9.2) | 0.48 (0.18–1.30) | |
| Total | 180 (100) | 87 (100) | | |
| Genotypes (dominant) | | | | |
| G/G | 117 (65) | 45 (51.7) | 1.00 | 0.04 |
| G/T-T/T | 63 (35) | 42 (48.3) | 1.73 (1.02–2.95) | |
| Genotypes (recessive) | | | | |
| G/G-G/T | 170 (94.4) | 79 (90.8) | 1.00 | 0.27 |
| T/T | 10 (5.6) | 8 (9.2) | 1.72 (0.66–4.68) | |
| \( \chi^2 \) | \( = 1.484, P = 0.48 \) | \( \chi^2 = 0.1867, P = 0.91 \) | | |
| \textit{IL-1\textbeta} 315C/T | | | | |
| Allele Frequency | | | | |
| C | 269 (76) | 130 (75) | 1.00 | 0.83 |
| T | 87 (24) | 44 (25) | 1.047 (0.68–1.59) | |
| Total | 356 (100) | 174 (100) | | |
| Genotypes (codominant) | | | | |
| C/C | 103 (57.9) | 53 (60.9) | 1.00 | 0.25 |
| C/T | 63 (35.4) | 24 (27.6) | 1.73 (0.84–3.56) | |
| T/T | 12 (6.7) | 10 (11.5) | 0.81 (0.26–2.52) | |
| Total | 178 (100) | 87 (100) | | |
| Genotypes (dominant) | | | | |
| C/C | 103 (57.9) | 53 (60.9) | 1.00 | 0.63 |
| C/T-T/T | 75 (42.1) | 34 (39.1) | 0.881 (0.52–1.49) | |
| Genotype (recessive) | | | | |
| C/C–C/T | 166 (93.3) | 77 (88.5) | 1.00 | 0.19 |
| T/T | 12 (6.7) | 10 (11.5) | 1.797 (0.73–4.10) | |
| \( \chi^2 \) | \( = 0.3557, P = 0.84 \) | \( \chi^2 = 6.446, P = 0.04 \) | | |

\textit{CSFP} coronary slow flow phenomenon

<sup>a</sup> Significant \( P \) values if \( P \leq 0.05 \)

<sup>b</sup> OR: odds ratio, 95% CI: 95%: confidence interval

<sup>c</sup> Hardy–Weinberg equilibrium
the T allele as the reference allele, and the Iranome database also cites the same allele for the Iranian population. However, the T allele has been reported as a possible risk factor for stroke and periventricular white matter hyperintensities. Marwa Ben et al. concluded that eNOS3 894G/T was significantly associated with coronary artery disease in additive and dominant models (but not in recessive models), concordant with our findings. In Pakistan, Nawaz et al. reported that the frequency of the T allele was higher than that of the G allele and introduced the TT genotype as a strong risk factor for coronary artery disease.

Controversy, however, abounds regarding the association between CSFP and eNOS3 894G/T SNPs in different populations. In samples of the Turkish population, Caglayan et al. and subsequently Sezgin et al. reported no associations between 894G/T SNP and CSFP. Caglayan and colleagues assessed 85 individuals, consisting of 66 patients with CSFP and 19 subjects with normal coronary arteries, while they excluded patients with diabetes mellitus; hypertension; coronary artery disease history; coronary ectasia; atrial fibrillation; complete bundle branch block; serious conduction defects; mitral valve prolapse; hypertrophic, restrictive, and dilated cardiomyopathies; left ventricular hypertrophy; ejection fractions less than 50%; and pulmonary, renal, hepatic, and hematological disorders. In this study, the frequency of the variant allele was 0.41 and 0.38 in the control and patient groups, respectively. No statistically significant differences were found in allelic and genotype distributions between the CSFP and control groups. Sezgin and colleagues recruited 30 patients with CSFP and no other cardiac disease and 61 control subjects and reported no association between eNOS intron 4 VNTR and 894G/T SNPs in the Turkish population.

### Table 4: Multivariable logistic regression analyses of the possible predictors of CSFP in the study population

| Variables                                      | OR (95% CI)   | P value |
|------------------------------------------------|---------------|---------|
| Presence of allele ‘T’ of the eNOS 864G/T polymorphism | 0.46 (0.11–1.98) | 0.29    |
| Presence of allele ‘T’ of the IL-1β 315C/T polymorphism | 0.47 (0.13–1.7)  | 0.24    |
| Interaction of eNOS 864G/T and IL-1β 315C/T       | 1             |         |
| Age                                             | 1.08 (1.03–1.12) | <0.01   |
| Gender                                          | 0.22 (0.08–0.62) | <0.01   |
| Smoking                                         | 1.17 (0.48–2.83) | 0.73    |
| FH of CAD                                       | 2.15 (0.92–5.03) | 0.07    |
| DLP                                             | 1.16 (0.51–2.63) | 0.72    |
| DM                                              | 0.49 (0.19–1.25) | 0.13    |
| HTN                                             | 0.74 (0.33–1.64) | 0.45    |
| FBS                                             | 1.0 (0.99–1.01)  | 0.15    |
| Cr                                              | 2.51 (0.4–15.77) | 0.32    |
| LDL-cholesterol                                 | 1.01 (1–1.03)  | 0.05    |
| Hb                                              | 1.10 (0.81–1.50) | 0.52    |
| WBC                                             | 1 (1–1)        | 0.96    |
| Platelet ($\times10^3$)                         | 1.0 (0.99–1.01) | 0.79    |
| ESR                                             | 1.01 (0.96–1.05) | 0.77    |

CSFP: coronary slow flow phenomenon, FH of CAD: family history of coronary artery disease, DLP: dyslipidemia, DM: diabetes mellitus, HTN: hypertension, FBS: fasting blood sugar, Cr: creatinine, LDL: low-density lipoprotein, Hb: hemoglobin, WBC: white blood cell, ESR: erythrocyte sedimentation rate.

### Table 5: Relationships between the eNOS3 864G/T and IL-1β 315C/T genotypes and TFC findings of the CSFP group

| TFC, median (Q1–Q3) | eNOS 894G/T | IL-1β 315C/T |
|---------------------|-------------|--------------|
|                     | G/G         | G/T          | T/T          | C/C         | C/T          | T/T          |
| LAD                 | 35 (29–45)  | 37.5 (29–43.75) | 37.5 (29–51.75) | 0.80       | 38 (30–43.5) | 35 (29–41)  | 37.5 (29–51.75) | 0.53       |
| LCx                 | 40 (32–50)  | 37 (30–48)   | 46 (36–61.5) | 0.16        | 40 (32–50)  | 38 (30–44)  | 46 (36–61.5)  | 0.11        |
| RCA                 | 32 (24–40)  | 30 (23.5–40) | 25.5 (16.5–28) | 0.80       | 32 (25.5–41) | 30 (22–40)  | 25.5 (16.5–28) | <0.01       |

TFC: thrombolysis in myocardial infarction frame count, LAD: left anterior descending artery, LCx: left circumflex artery, RCA: right coronary artery.

* Categorical variables are presented as numbers (%).

* Significant P values if ≤0.05.
polymorphisms. Nevertheless, the plasma levels of NO were significantly lower in the CSFP group than in the control group ($P < 0.05$). In contrast, Gupta et al. reported a strong association between this nucleotide transition and CSFP in the North Indian population and suggested the T allele as an independent risk factor for CSFP [24]. This study assessed 27 patients with CSFP and 200 individuals as the control group. The exclusion criteria were the same as those in the study by Caglayan and colleagues. The results showed a significant association between the presence of the T allele and CSFP ($P = 0.014$; $\chi^2 = 6.1$). Our findings are different from those reported by the investigations in Turkey, but they chime in with those reported by Gupta and colleagues.

Mutluer et al. revealed an association between the rs1143634 of the IL-1β gene and CSFP in the Turkish population [48]. A study on the Han Chinese population reported an association between the IL-10 polymorphism and CSFP [45]. In contrast to the investigation in the Turkish population, our results showed no association between the 315C/T (rs1143634) of the IL-1β gene polymorphism and CSFP. It is worthy of note that had we recruited a larger population, our analysis might have yielded different results. To the best of our knowledge, this is the first report on the association between eNOS 894G/T and CSFP in the Iranian population. A previous investigation in Iran examined the predictive power of 2 common polymorphisms of the eNOS gene in relation to CSFP after primary percutaneous coronary interventions and reported no associations between CSFP and the 894G/T and −786T/C polymorphisms of the eNOS gene [58]. Heidari et al. found an association between the −813C/T (rs2070744) and 894G/T (rs1799983) polymorphisms of the eNOS gene and multiple sclerosis in Iranian patients [59]. In another study, no association was found between the 894G/T eNOS polymorphism and coronary artery disease in the northern Iranian population [60]. Accumulating evidence indicates that the Asp298Glu SNP of the eNOS gene is associated with coronary artery disease, ST-segment-elevation myocardial infarction, hypertension, coronary vasospasm, impaired coronary collateral development, impaired coronary blood flow, and obesity [14, 61–67].

**Limitations**

The observational nature of our investigation and its limited sample size precluded us from drawing a firm conclusion. Indeed, our results should be tested in a larger population to confirm the association between the studied eNOS gene polymorphisms and the IL-1β nucleotide transition. Additionally, the associations between the Asp298Glu transition of the eNOS gene and the plasma nitric oxide level and nitric oxide synthase activity were not assessed in this study due to technical and financial limitations. Finally, our results might have been influenced by dissimilarities between the patient and control groups.

**Conclusions**

The present preliminary study is the first to suggest an association between the 894G/T eNOS gene polymorphism and CSFP in the Iranian population. However, our results demonstrated no association between CSFP and the 315C/T IL-1β gene variant. Further, the allelic distribution and the presence of the variant allele of the 894G/T eNOS gene polymorphism were statistically associated with CSFP.

**Supplementary Information**

The online version contains supplementary material available at [https://doi.org/10.1186/s12872-022-02736-0](https://doi.org/10.1186/s12872-022-02736-0).

**Additional file 1:** Multivariable regression analysis between the TIMI frame count for the LAD and clinical and genetic parameters in the patients.

**Acknowledgements**

We would like to thank the patients and the healthy individuals for their kind participation in the study. We are also grateful to Maryam Zar Karimi for her technical assistance.

**Author Contributions**

YK: experiment design, data production, data interpretation, writing the first draft of the manuscript, and the final edit of the manuscript. FS: clinical evaluation of patients, and the final edit of the manuscript. AS: clinical evaluation of patients, and the final edit of the manuscript. MM: lab work, data production, and the final edit of the manuscript. YKh: WES analysis and the final edit of the manuscript. RK: clinical evaluation of patients, and the final edit of the manuscript. ETB: experiment design, data production, and the final edit of the manuscript. MHM: experiment design, data production, and the final edit of the manuscript. MK: original idea, clinical evaluation of patients, and the final edit of the manuscript. MM: lab work, data production, and the final edit of the manuscript. YK: experiment design, data production, data interpretation, writing the first draft of the manuscript, and the final edit of the manuscript. PS: clinical evaluation of patients, and the final edit of the manuscript. AS: clinical evaluation of patients, and the final edit of the manuscript. YKh: WES analysis and the final edit of the manuscript. RK: clinical evaluation of patients, and the final edit of the manuscript. ETB: experiment design, data production, and the final edit of the manuscript. MHM: experiment design, data production, and the final edit of the manuscript. YK: experiment design, data production, data interpretation, writing the first draft of the manuscript, and the final edit of the manuscript. FS: clinical evaluation of patients, and the final edit of the manuscript. AS: clinical evaluation of patients, and the final edit of the manuscript. MM: lab work, data production, and the final edit of the manuscript. YK: experiment design, data production, data interpretation, writing the first draft of the manuscript, and the final edit of the manuscript. PS: clinical evaluation of patients, data production, data interpretation, and the final edit of the manuscript. AS: clinical evaluation of patients, and the final edit of the manuscript. MM: lab work, data production, and the final edit of the manuscript. YK: experiment design, data production, data interpretation, writing the first draft of the manuscript, and the final edit of the manuscript. PS: clinical evaluation of patients, data production, data interpretation, and the final edit of the manuscript. AS: clinical evaluation of patients, and the final edit of the manuscript. MM: lab work, data production, and the final edit of the manuscript. YK: experiment design, data production, data interpretation, writing the first draft of the manuscript, and the final edit of the manuscript. PS: clinical evaluation of patients, data production, data interpretation, and the final edit of the manuscript. AS: clinical evaluation of patients, and the final edit of the manuscript. MM: lab work, data production, and the final edit of the manuscript. YK: experiment design, data production, data interpretation, writing the first draft of the manuscript, and the final edit of the manuscript. PS: clinical evaluation of patients, data production, data interpretation, and the final edit of the manuscript. AS: clinical evaluation of patients, and the final edit of the manuscript. MM: lab work, data production, and the final edit of the manuscript. YK: experiment design, data production, data interpretation, writing the first draft of the manuscript, and the final edit of the manuscript. PS: clinical evaluation of patients, data production, data interpretation, and the final edit of the manuscript. AS: clinical evaluation of patients, and the final edit of the manuscript. MM: lab work, data production, and the final edit of the manuscript.

**Funding**

This work was supported by a research grant to Dr Sadeghipour and Dr Mala-ki from the Research Deputyship of Rajaie Cardiovascular Medical and Research Center (9960).

**Availability of data and materials**

All the oligos and the information regarding the study are provided in the paper. The accession numbers of the nucleotide transitions are 894G/T (rs1799983) for the eNOS change and 315C/T (rs1143634) for the IL-1β change ([https://www.ncbi.nlm.nih.gov/search/all/?term=dbsnp](https://www.ncbi.nlm.nih.gov/search/all/?term=dbsnp)). HGMD ([http://www.hgmd.cf.ac.uk/ac/index.php](http://www.hgmd.cf.ac.uk/ac/index.php)), NCBI ([www.ncbi.nlm.nih.gov/clinvar](https://www.ncbi.nlm.nih.gov/clinvar)), UCSC ([https://genome.ucsc.edu](https://genome.ucsc.edu)), and VarSome ([https://varsome.com/](https://varsome.com/)) databases were utilized to investigate the selected SNPs and the pathogenesis of the selected nucleotide variations. The accession numbers and all the repositories
used for the study are both mentioned in the article and declarations part as well.

**Declarations**

**Ethical approval and consent to participate**
The study protocol was approved by the Ethics Committee of Rajaie Cardiovascular Medical and Research Center (IR.RHCR. REC.1399.075). The study was conducted in accordance with the Helsinki Declaration. All the individuals who joined the study signed the written informed consent.

**Consent for publication**
Not applicable.

**Competing interests**
All the authors have read and approved the data presented in the manuscript and declare that there are no conflicts of interest.

**Author details**
1. Rajaie Cardiovascular Medical and Research Center, Iran University of Medical Sciences, Tehran, Iran.
2. Cardiovascular Interventional Research Center, Rajaie Cardiovascular Medical and Research Center, Iran University of Medical Sciences, Vali-Asr Ave, Neyyayesh Blvd, Tehran 1996911101, Iran.
3. Cardiovascular Research Center, Rajaie Cardiovascular Medical and Research Center, Iran University of Medical Sciences, Vali-Asr Ave, Neyyayesh Blvd, Tehran 1996911101, Iran.
4. Molecular Genetics Department, Faculty of Biological Sciences, Tarbiat Modarres University, Tehran, Iran.

**Received:** 20 February 2022  **Accepted:** 27 June 2022  **Published online:** 30 June 2022

**References**

1. Beltrame JF, Limaye SB, Wustke RD, Horovitz JD. Coronary hemodynamic and metabolic studies of the coronary slow flow phenomenon. Am Heart J. 2003;146(1):84–90.
2. Cutri N, Zeitz C, Kucia AM, Beltrame JF. ST/T wave changes during acute coronary syndrome presentation in patients with the coronary slow flow phenomenon. Int J Cardiol. 2011;146(3):457–8.
3. Mangieni E, Macchiarella G, Giavola M, Barilla F, Avella A, Martinotti A, Dell’Italia LJ, Scibilla G, Motta P,ampa PP. Slow coronary flow: clinical and histopathological features in patients with otherwise normal epicardial coronary arteries. Cathet Cardiovasc Diagn. 1996;37(4):375–81.
4. Wang X, Nie S-P. The coronary slow flow phenomenon: characteristics, mechanisms and implications. Cardiovasc Diagn Ther. 2011;1(1):37.
5. Barutcu I, Sezgin AT, Sezgin N, Gullu H, Esen AM, Topal E, Ozdemir R. Elevated plasma homocysteine level in slow coronary flow. Int J Cardiol. 2005;101(1):143–5.
6. Mütltuer FO, Ural D, Gungör B, Bolca O, Aksu T. Interleukin-1 gene cluster polymorphisms associated with coronary slow flow phenomenon. Cardiovasc J Afr. 2013;24(9):355–9.
7. Gupta MD, Akkarappaty C, Girish MP, Kumar R, Raini M, Tyagi S, Pasha MAQ. Association between the Glu298Asp and 4b/4a polymorphisms at exon 7 of the endothelial nitric oxide synthase gene and coronary slow flow. Coron Artery Dis. 2013;24(6):457–8.
8. Gazi E, Temiz A, Altun B, Barutcu A, Silan F, Colkesen Y, Ozdemir O. Endothelial function and germ-line ACE I/D, eNOS and PAI-1 gene profiles in patients with coronary slow flow. Scand J Clin Lab Invest. 2012;72(6):495–500.
9. Moyer CF, Sajuthi D, Tulli H, Williams J. Synthesis of IL-1 alpha and IL-1 beta by arterial cells in atherosclerosis. Am J Pathol. 1991;138(4):951.
10. Emekli K, Gungör B, Özcan KS, Abaci N, İhan E, Emekli SS, Kemaloglu T, Osmanov D, Ustek D, Eren M. Evaluation of coronary microvascular function and nitric oxide synthase intron 4a/b polymorphism in patients with coronary slow flow. Coron Artery Dis. 2013;24(6):461–7.
11. Cáza E, Temisz A, Altun B, Barutcu A, Silan F, Colkesen Y, Ozdemir O. 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 cardiovascular. Cardiovasc J Afr. 2014;25(1):9–19.
12. Gupta MD, Akkarappaty C, Girish MP, Kumar R, Raini M, Tyagi S, Pasha MAQ. Association between the Glu298Asp and 4b/4a polymorphisms of endothelial nitric oxide synthase and coronary slow flow in the North Indian population. Coron Artery Dis. 2014;25(3):192–7.
13. Tsimikas S, Duff GW, Berger PB, Rogers J, Hutten K, Clapton P, Brilakis E, Kornman KS, Witztum J. Pro-inflammatory interleukin-1 genotypes potentiate the risk of coronary artery disease and cardiovascular events mediated by oxidized phospholipids and lipoprotein(a). J Am Coll Cardiol. 2014;63(7):1724–34.
14. Gibson CM, Cannon CP, Daley WL, Dodge JT Jr, Alexander B, Marble SJ, McCabe CH, Raymond L, Fortin T, Poole WK. TIMI frame count: a quantitative method of assessing coronary artery flow. Circulation. 1996;93(5):879–80.
15. Lozano-Calderón S, Anthony S, Ring D. The quality and strength of evidence for etiology: example of carpal tunnel syndrome. J Hand Surg. 2008;33(3):525–8.
16. Wiberg A, Ng M, Schmid AB, Smillie RW, Baskozos G, Holmes MV, Künnapa A, Mapi K, Bennett DL, Furniss D. A genome-wide association analysis identifies 16 novel susceptibility loci for carpal tunnel syndrome. Nat Commun. 2019;10(1):1–12.
17. The UCSC Genome Browser, http://genome.ucsc.edu/, accessed May 2019.
18. Werner RA, Andary M. Carpal tunnel syndrome: pathophysiology and clinical neurophysiology. Clin Neuropyschol. 2002;113(8):1373–81.
19. Camara-Lemarroy CR, Gonzalez-Moreno EL, Guzman-de la Garza FJ, Fernandez-Garza NE. Arachidonic acid derivatives and their role in...
