Genetic risk factors for restenosis after percutaneous coronary intervention in Kazakh population

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Abstract

Background: After coronary stenting, the risk of developing restenosis is from 20 to 35%. The aim of the present study is to investigate the association of genetic variation in candidate genes in patients diagnosed with restenosis in the Kazakh population.

Methods: Four hundred fifty-nine patients were recruited to the study; 91 patients were also diagnosed with diabetes and were excluded from the sampling. DNA was extracted with the salting-out method. The patients were genotyped for 53 single-nucleotide polymorphisms. Genotyping was performed on the QuantStudio 12K Flex (Life Technologies). Differences in distribution of BMI score among different genotype groups were compared by analysis of variance (ANOVA). Also, statistical analysis was performed using R and PLINK v.1.07. Haplotype frequencies and LD measures were estimated by using the software Haploview 4.2.

Results: A logistic regression analysis found a significant difference in restenosis rates for different genotypes. FGB (rs1800790) is significantly associated with restenosis after stenting (OR = 2.924, P = 2.3E−06, additive model) in the Kazakh population. CD14 (rs2569190) showed a significant association in the additive (OR = 0.08033, P = 2.11E−09) and dominant models (OR = 0.05359, P = 4.15E−11). NOS3 (rs1799983) was also highly associated with development of restenosis after stenting in additive (OR = 20.05, P = 2.74E−12) and recessive models (OR = 22.24, P = 6.811E−10).

Conclusions: Our results indicate that FGB (rs1800790), CD14 (rs2569190), and NOS3 (rs1799983) SNPs could be genetic markers for development of restenosis in Kazakh population. Adjustment for potential confounder factor BMI gave almost the same results.

Keywords: Coronary heart disease, Restenosis, SNP, Genotyping

Background

Coronary heart disease (CHD) is a disease characterized by reduced blood supply to the heart muscle. Narrowing of the coronary arterial lumen due to atherosclerosis is the primary cause in 97–98% of CHD cases. Coronary heart disease has the highest rate of death and serious complications among all forms of cardiovascular disease. An estimated 17.5 million people died from cardiovascular diseases in 2012, representing 31% of all global deaths. Of these deaths, an estimated 7.4 million were due to CHD and 6.7 million were due to stroke [1]. It should be noted that CHD usually affects the population aged between 35 and 65 years. In addition, CHD represents the most important cause of sudden cardiac deaths. Together with cerebrovascular diseases, CHD accounts for 64% of all cardiovascular deaths [2].

According to the Ministry of Healthcare and Social Development of the Republic of Kazakhstan prior to year 2012, CHD morbidity has been constantly increasing in 2009–2012 with exception of 2013 when a minor decrease in the morbidity was observed [3]. In 2013,
there were 59,799 new cases of CHD registered in Kazakhstan and the morbidity rate reached 500.6 cases per 100,000 population, compared to 445.6 cases in 2011 and 507.4 cases in 2012 [3].

Advances in medicine have led to the emergence of novel methods of CHD treatment, such as angioplasty or coronary stenting. The first use of coronary stenting in clinical practice was in 1986 [4]. Primary percutaneous coronary intervention (PCI) has become a well-established strategy for patients with coronary heart disease [5]. Nowadays, endovascular methods for the reestablishment of coronary blood flow preserve the lives and health of hundreds of thousands of people around the world. Nevertheless, there is a possibility that during the first 6 months to 1 year after successful coronary stenting, a symptomatic relapse of angina may occur due to the development of restenosis. Reoccurrence of stenosis is a major limitation to the effectiveness of the stenting, and even the use of drug-eluting stents does not solve the problem completely [6]. After coronary stenting, the restenosis rate is 20–30 %. The use of second generation drug-eluting stents has reduced this rate, but the development of restenosis after implantation remains a serious clinical problem [7].

Restenosis can occur for many different reasons. The pathophysiological mechanisms of restenosis have not yet been fully explained, but it is believed that those mechanisms include inflammation, proliferation, and matrix remodeling. Over the years, many predictive clinical, biological, genetic, epigenetic, lesion-related, and procedural risk factors for restenosis have been identified. Those factors are useful in the risk stratification of patients and also contribute to our understanding of this condition [8]. In this sense, the search for new predictor factors in the development of restenosis is topical.

Currently, the genetic factors of restenosis have been studied mostly in European populations. The ethnic variability of genetic markers is well known, as shown in the results of the GENetic DEterminants of Restenosis (GENDER) study discussed in the article by Verschuren et al. The GENDER databank contains the genotypic data of 2,571,586 single-nucleotide polymorphism (SNPs) from 295 cases with restenosis and 571 matched controls. The set that included all 36 reported genes in the literature was indeed significantly associated with restenosis in the GENDER study \( (P = 0.024) \). Subsequent analyses of the individual genes demonstrated that this association was determined by 6 of the 36 genes [9]. As a result of the GENDER study, the selected SNPs have been associated with the risk of developing restenosis in European populations.

Based on literature review, candidate genes for restenosis were not studied or validated in Kazakh population. That is why the purpose of the current study is to look for associations of genetic variation in candidate genes in patients diagnosed with restenosis after percutaneous coronary intervention in the Kazakh population.

**Methods**

**Study population**

There were initially 459 patients with diagnosed CHD recruited to the study. Of these patients, 91 were also diagnosed with diabetes and were excluded from the sampling, since several studies have shown that diabetes is an independent risk factor for restenosis and may introduce bias to the interpretation of results [10]. Anthropometrical and biochemical characteristics were gathered for the population sample comprising 368 patients (299 males and 69 females). There were 99 case subjects, those who manifested in-stent restenosis within 6 months after stenting and 269 control subjects and those who did not developed restenosis after stenting. The study protocol was approved by the Ethics Committee of the National Center for Biotechnology. All subjects were ethnic Kazakhs.

**Genotyping**

Whole blood samples of 368 patients (9 ml) were collected into tubes containing 50 mmol/l ethylenediamine-tetraacetic acid (disodium salt). DNA was extracted with the salting-out method. [11]. Genotyping of the extended panel of polymorphisms of candidate genes was performed on the QuantStudio 12K Flex (Life Technologies). The total reaction volume was 5 μl, containing 2.5 μl of 2× OpenArray Real-time master mix, and 2.5 μl of DNA concentration of 50 ng/μl. Temperature conditions were 10 min at 93 °C, cycling for 45 s at 93 °C, 13 s at 94 °C, and 2.14 min at 53.5 °C for 50 cycles, followed by incubation at 25 °C for 2 min. Data analysis was performed using the software package TaqMan Genotyper Software v.1.3.

Table 1 shows a panel of 53 SNPs used in the current study. All SNPs were selected based on the results of the GENDER study.

**Statistical analysis**

A pairwise correlation matrix was made to check for multicollinearity between measured variables. A stepwise regression was performed to evaluate significance of potential confounders. A logistic regression analysis with adjustment for potential confounder was used to test for differences between statuses of restenosis according to genotyping. Associations between each SNP and development of the restenosis were tested according to three genetic models: additive (cumulative effect of each additional variant allele), dominant (homozygous wild-type vs. variant allele-carrying genotype) and recessive (wild-type allele carrying genotype vs. homozygous variant genotype). Every SNP that reached statistically significant level of \( P < 0.0001 \) were reported. Significant SNPs were further annotated using RegulomeDB that harbors information for known
and predicted regulatory elements [12]. Annotation of functional variation in personal genomes using RegulomeDB.

Additionally, differences in distribution of BMI score among different genotype groups were compared by one-way analysis of variance (one-way ANOVA). For ANOVA test a default threshold of 0.05 was used to report significance. Statistical analysis was performed using R and PLINK [13, 14].

LD statistical analysis was performed using Haploview 4.2. For block generations, Hardy-Weinberg P value cutoff 0.001 was used [15]. We ignored SNPs that minor allele frequencies (MAF) of less than 0.001. For block generations, the confidence intervals default algorithm was used.

Results

A total of 368 patients participated in the study. Body mass index (BMI), the blood levels of cholesterol, low-density lipoproteins (LDL), and high-density lipoproteins (HDL) were recorded for every patient at the time of participation. Table 2 shows summary statistics of the measured variables.

Anthropometrical and measured biochemical characteristics for potential confounders were evaluated before performing a test for association between genotype distribution and restenosis status. The distributions of the measured traits fitted the normality assumption and were included in the analysis as is without any transformation. Construction of the correlation matrix revealed that in our dataset, cholesterol and LDL are

### Table 1 Description of SNPs included in the study (Continued)

| Gene | Polymorphism | Locus |
|------|--------------|-------|
| Tumor protein p53 (TP53) | rs1042522 | 17p13.1 |
| Transferrin (TF) | rs1799899 | 3p |
| Uncoupling protein 3 (UCP3) | rs1800849 | 11q13.4 |
| Vitamin D receptor (VDR) | rs11568820 | 12q13.11 |
| Vitamin D receptor (VDR) | rs11574027 | 12q13.11 |
| Vitamin D receptor (VDR) | rs11574077 | 12q13.11 |
| Tumor necrosis factor α (TNFa) | rs1800629 | 6p21.3 |
| Tumor necrosis factor α (TNFa) | rs361525 | 6p21.3 |
| Interleukin 1 receptor antagonist (IL1RN) | rs419598 | 2q14.2 |
| Interleukin 1a (IL1A) | rs1800587 | 2q12–q21 |
| Interleukin 1B (IL1B) | rs1143627 | 2q13–q21 |
| Interleukin 1 (IL4) | rs2243250 | 5q23–31 |
| Interleukin 6 (IL6) | rs1800796 | 7p21 |
| Interleukin 8 (IL8) | rs 4073 | 4q12–q13 |
| Interleukin 10 (IL10) | rs1800871 | 1q31–q32 |
| Interleukin 10 (IL10) | rs1800872 | 1q31–q32 |
| Interleukin 10 (IL10) | rs1800896 | 1q31–q32 |
| Interleukin 10 (IL10) | rs30/4498 | 1q31–q32 |
for the results of logistic regression analysis is shown in Table 4.

Association of genotype of the candidate genes 53 SNP with the restenosis was examined with one-way ANOVA by comparing the mean scores of BMI to the genotype. According to ANOVA, BMI distribution had significant differences with genotypes of rs6025 SNP in coagulation factor V also known as F5 (P = 0.00643) in control group. rs419598 SNP mapped to IL1RN was positively associated with BMI in the control group (P = 0.0299). Finally, rs4795895 SNPs mapped to CCL11 gene showed significant differences with BMI in group of patients (P = 0.012). Genotypes of other SNPs were not significantly different.

Using Haploview 4.2 software, LD statistics results for the Kazakh population were obtained (Fig. 1) (HW P cut-off, 0.001; MAF, 0.001). As a result, two haplotype blocks were defined: one block consisting of two SNPs, i.e., rs5182 and rs5186 (block 1, chromosome 3); one block consisting of two SNPs, i.e., rs1800871 and rs1800896 (block 2, chromosome 1). The haplotype frequencies in the studied population are presented in Table 5. Haplotype CC (rs5182-rs5186, block 1) was associated with the risk of developing restenosis (OR 2.17; 95 % CI; 1.33–3.53, P = 0.002) and the haplotype GC (rs1800871-rs1800896, block 2) was associated with restenosis (OR 1.51; CI, 1.01–2.26, P = 0.04).

Discussion

The present study included Kazaks that are Turkic people of the northern parts of Central Asia (largely Kazakhstan). From the historic point of view and because of scarce genetic data, it was concluded that Kazakh population was formed as a result of admixture of the European and Asian populations [16]. Case and controls were diagnosed with CHD. BMI was increased in both groups. Our results showed that BMI is a single confounding factor that may affect the association between genotype and restenosis status. In turn, genotype polymorphisms of F5, IL1RN, CCL11 genes significantly influence on increased body mass index.

The present study focuses on 48 SNPs in 36 candidate genes that were previously reported as genetic risk factors for restenosis. Association of hemostatic gene polymorphisms with restenosis after coronary stent placement was the first genetic risk to be described [17, 18]. The association of selected SNPs in inflammation-related genes with restenosis is also well documented [19–21]. In addition, a number of candidate genes in the renin-angiotensin hormone system and the endothelial nitric oxide synthase (eNOS, Glu298Aps and −786T>C) are also involved in this process [22–24]. New molecular markers of restenosis are constantly emerging. For example, in a recent study, SNPs in VDR (vitamin D-dependent receptor) gene were highly correlated with each other (Table 3). In order to avoid multicollinearity, cholesterol was excluded from further analysis.

The remaining traits were used to build a statistical model that predicts the development of restenosis as a main outcome. Age, LDL, and HDL contribute insignificantly to restenosis variability in our dataset based on the results from stepwise regression analysis. The final statistical model included BMI as a single confounding factor that may affect the association between genotype and restenosis status.

There were 368 patients available to collect whole blood for further genetic analysis. After applying quality control filters, the final dataset contained 268 patients with genotype information for 48 SNPs. Four SNPs were excluded based on the Hardy Weinberg equilibrium test (P ≤ 0.001). One SNP was excluded because of its low genotyping rate.

SNPs that reached a significant level P < 0.0001 were reported. The results from logistic regression revealed that FGB (rs1800790) SNP was associated with restenosis after stenting. Notably that homozygous A allele carriers of the rs1800790 SNP are 24 times less likely to develop restenosis after stenting compared to other allele carriers. CD14 (rs2569190) SNP showed highly significant correlation development of restenosis. Based on results, carrying homozygous G allele is a risk factor for development of restenosis. Finally, a missense mutation rs1799983 SNP was associated with restenosis after stenting compared to other allele carries. Both additive and recessive models showed similar results, suggesting that carrying additional G allele for rs1799983 SNP is a protective factor against developing restenosis. Summary

Table 2 Anthropometrical and biochemical characteristics of the population sample (n = 368)

| Restenosis (case/control) | Gender (male/female) | Age (years) | BMI (kg/m²) | Cholesterol (mmol/l) | LDL (mmol/l) | HDL (mmol/l) |
|--------------------------|----------------------|-------------|-------------|---------------------|-------------|-------------|
| 99/269                   | 299/69               | 58.61 ± 11.67 | 28.35 ± 4.53 | 4.93 ± 1.24         | 3.05 ± 1.18 | 1.24 ± 0.63 |

BMI body mass index, LDL low-density lipoproteins, HDL high-density lipoproteins

Table 3 Pairwise correlation matrix of measured variables

|         | BMI    | HDL    | LDL    | Cho    | Age    |
|---------|--------|--------|--------|--------|--------|
| Age     | −0.072 | −0.067 | −0.061 | −0.081 | 1      |
| Cho     | 0.402  | 0.364  | 0.739  | 1      | 1      |
| LDL     | 0.322  | 0.492  | 1      |        |        |
| HDL     | 0.163  | 1      |        |        |        |
| BMI     | 1      |        |        |        |        |
considered as risk markers of restenosis [25]. Fragoso et al.
reported that transforming growth factor-β (rs1800469) was associated with the risk of developing restenosis after coronary stenting in Mexican patients [26]. This SNP was not investigated in our study.

Our study has revealed that blood coagulation fibrinogen factor I (FGB), monocyte differentiation antigen CD14 (CD14), and nitric oxide synthase 3 (NOS3) genes are among the factors associated with the risk of restenosis in the studied population.

Monocytes play a central role in restenosis after balloon angioplasty and stent implantation. Monocytes migrate into the damaged area either as a direct response or through the release of platelet-derived factors. Activated monocytes release large amounts of proinflammatory cytokines, which cause vasoconstriction and non-specific recruitment, proliferation, and activation of other cells including vascular smooth muscle cells in the vascular wall. The activation of monocytes/macrophages, endothelial cells, and smooth muscle cells mediated by CD14 and/or CD14 may play an important role in the restenosis processes [27].

Functional C(-260) → T polymorphism in the promoter of the CD14 gene has been reported to be associated with CHD but data have yielded conflicting results. In the meta-analysis of Zhang et al., TT genotype is associated with ischemic heart disease in the East Asian population but not in the European or Indian populations [28]. Two previous studies have investigated the role of CD14 in the development of restenosis, one being a prospective study by Zee et al. [29] in 779 patients and the other being a prospective study by Shimada et al. [27] in 129 Japanese patients. They found the C→T genotype to be a risk factor for restenosis. But the GENDER study found that T allele was not associated with restenosis in the European population [9]. Also, it was showed that the CD14+CD16+CX3CR1+monocytes might have a role in-stent restenosis following coronary implantation of bare-metal stents in patients with acute myocardial infarction [30].

The results of our study have revealed that CD14 promoter polymorphism remained statistically significant in the additive (OR = 0.08033, P = 2.11E−09) and dominant models (OR = 0.05359, P = 4.15E−11). Therefore, the T allele at position −260 of CD14 gene is a risk allele for restenosis in Kazakh population. Population stratification based on ethnicities may lead to inconsistency, especially when both allele frequencies and incidence rates of the diseases vary across ethnic groups.

Fibrinogen (factor I) is a glycoprotein synthesized by the liver. It consists of three polypeptides Aα, Bβ, and γ coded by the alpha (FGA), beta (FGB), and gamma (FGG) genes, respectively. Fibrinogen is an important component of the coagulation cascade and a major determinant of

| CHR | SNP      | BP   | Type        | Genes | Genotype | Mean | N | Additive | Dominante | Recessive |
|-----|----------|------|-------------|-------|----------|------|---|---------|-----------|-----------|
| 4   | rs1800790| 155483708 | Intron | FGB    | GG     | 0.18 | 201 | 2.967 | 1.62E-06 | 24.95 | 7.74E-07 |
|     |          |       |           |       | GA      | 0.17 | 42  | 2.924 | 2.31E-06 | 2.725 | 0.001843 |
|     |          |       |           |       | AA      | 0.86 | 21  | 2.725 | 2.31E-06 | 2.725 | 0.001843 |
| 5   | rs2569190| 140012916 | Intron | CD14   | GG     | 0.47 | 115 | 0.08694 | 2.20E-09 | 0.05848 | 5.31E-11 |
|     |          |       |           |       | GA      | 0.08 | 88  | 0.08033 | 2.11E-09 | 0.05359 | 4.15E-11 |
|     |          |       |           |       | AA      | 0.07 | 57  | 2.11E-09 | 0.05359 | 4.15E-11 |
| 7   | rs1799983 | 150696111 | Missense mutation | NOS3 | TT     | 0.81 | 31  | 20.56  | 1.85E-12 | 23.6   | 2.87E-10 |
|     |          |       |           |       | GT      | 0.35 | 93  | 20.05  | 2.74E-12 | 22.24  | 6.81E-10 |
|     |          |       |           |       | GG      | 0    | 137 | 20.05  | 2.74E-12 | 22.24  | 6.81E-10 |

**Table 4** Results from logistic regression analysis

**bold** – snp that also had functional effect

**red** – p values unadjusted

**green** – p values adjusted for BMI

**Bold snp that also had functional effect, red P values unadjusted, green P values adjusted for BMI**

Fig. 1 LD SNP plot. The LD is displayed according to standard color schemes, with bright red for very strong LD (LOD > 2, D’ = 1), light red (LOD > 2, D’ < 1) and blue (LOD < 2, D’ = 1) for intermediate LD, and white (LOD < 2, D’ < 1) for no LD.
blood viscosity and platelet aggregation [31]. Polymorphisms of FGB was shown to be associated with coronary heart disease [32, 33]. In a study by Völzke in 2004, there was no association between the β-fibrinogen −455G/A and the risk of restenosis after PTCA or recurrent restenosis after re-PTCA [34]. In the GENDER study, a multicenter prospective study, the association of gene polymorphism FGB −455G>A (rs1800790) with the risk of restenosis was not found in the European population [9]. In the study by Oguri in 2007, the association of FGB −455G>A (rs1800790) with restenosis was shown in the Japanese population [35]. Our results suggest that genotype FGB −455G>A (rs1800790) is significantly associated with restenosis after stenting (OR = 2.924, P = 2.3E−06, additive model) in the Kazakh population. Interestingly that the same SNP in dominant model gave close P value, but much higher OR = 24.36, suggesting that allele A is a recessive protective factor against development of restenosis. Thus, it can be concluded that the relationship between genetic polymorphisms of FGB −455G>A (rs1800790) and the development of restenosis is not universal between different ethnic groups. It is also interesting to observe that Japanese and Kazakhs are alike in turns of effect from polymorphisms of FGB −455G>A (rs1800790).

Nitric oxide synthase 3 (NOS3) locates at chromosome 7q36, and it encodes endothelial nitric oxide synthase (eNOS), which can generate nitric oxide (NO) in endothelial cells. Endothelial NO is a key determinant of vascular homeostasis, and it can also participate in vascular repair. Dysfunction of any of these processes may result in atherosclerotic and thrombotic diseases [36–38]. The association between several polymorphisms of the NOS3 gene and CAD and restenosis risks has been previously studied [39–41]. In the meta-analysis by Zhang et al., 18 case-control studies with 2994 cases and 3130 controls, including 13 studies of East-Asia descendents, 5 studies of non-East-Asian descendents indicated that eNOS 894G/T polymorphism may play an important role in CHD development among Asian population [42]. In 2010, Li et al. performed a meta-analysis involving 20 studies relating non-Asian population and 3 studies relating Asian population and found significant association of T allele in eNOS 894G/T with CHD in non-Asian population [43]. Other studies showed that homozygosity of Glu298Asp and 786T/C polymorphisms of the NOS3 gene represented an independent risk factor for in-stent restenosis, and the 894G/T polymorphism of NOS3 gene was associated with an increased risk of death and/or myocardial infarction within 1 year after stent placement [24, 44, 45]. Shuvalova et al. showed that minor allele of polymorphism 298G/T of the eNOS gene (rs1799983) is associated with an increased risk of in-stent restenosis [46].

A number of polymorphisms have been identified in the NOS3 gene among which two polymorphisms in the promoter region (−786C/T), and one in the exon (894G/T or Glu298Asp) was studied. Only one of these polymorphisms NOS3 (rs1799983) has been found to be significantly associated with restenosis in our study OR = 20.05, P = 2.74E−12 in additive and OR = 22.24, P = 6.81E−10 in recessive models. Thus, the present results demonstrated that there might be a significant association between the NOS3 polymorphism (rs1799983) and restenosis after PCI in the Kazakh population.

In the linkage disequilibrium analysis, AGTR1 risk (CC) haplotype and IL10 (GC) for developing restenosis were detected in our study. Su et al. studied 16 AGTR1 polymorphisms. Based on the linkage disequilibrium pattern among these SNPs, six polymorphisms were selected as haplotype tagging SNPs and further were genotyped. SNP analyses indicated that GTC haplotype (rs275650, rs2276736, rs5182) associated with the risk of developing myocardial infarction [47]. Koch et al. investigated the possibility that single-nucleotide polymorphisms of the genes encoding TNF (_863C/A, _308G/A), LT−a (252G/A), and IL10 (_1082G/A, _819C/T, and _592C/A) are associated with the incidence of restenosis, death, or myocardial infarction (MI) after coronary stenting [48]. With regard to the IL10 polymorphisms, they observed three different haplotypes, _1082G_/ _819C/_592C (GCC), ACC, and ATA, with relative frequencies of 0.45, 0.29, and 0.26, respectively. Koch et al. have not detected a significant correlation between restenosis and the frequency of the

| Locus | Haplotype | Block 1 | Block 2 | rs1800871|rs1800896 | 819 C>T|1082 A>G | 573C>T|1166A>C |
|-------|-----------|---------|---------|----------|-----------|---------|---------|---------|---------|
|       |           | genotype| genotype|          |           |         |         |         |         |
| FGB   | TA        | 0.634   | 0.455   | 0.340    | 0.198     | 0.205   | 0.050   | 0.205   | 0.110   |
|       | CA        | 0.162   |         |          |           |         |         |         |         |
|       | CC        | 0.205   |         |          |           |         |         |         |         |
|       | AT        | 0.455   |         |          |           |         |         |         |         |
|       | GC        | 0.340   |         |          |           |         |         |         |         |
|       | GT        | 0.198   |         |          |           |         |         |         |         |

**Table 5** Frequencies (%) of AGTR haplotypes [rs5182(573C>T), rs5186 (1166A>C)] and IL10 haplotypes [rs1800871(−819 C>T), and rs1800896 (−1082 A>G)] in patient with and without restenosis

| Locus | Haplotype | With restenosis (n = 99) | Without restenosis (n = 269) | OR | 95 % CI | P value |
|-------|-----------|-------------------------|-------------------------------|----|---------|---------|
|        |           |                         |                               |    |         |         |
| Block 1| TA        | 0.669                   | 0.669                         | -  | -       | NS      |
|        | CA        | 0.221                   | 0.221                         | -  | -       | NS      |
|        | CC        | 0.110                   | 0.110                         | 2.17| 1.33−3.53| 0.002   |
|        | AT        | 0.493                   | 0.493                         | -  | -       | NS      |
|        | GC        | 0.238                   | 0.238                         | 1.51| 1.01−2.26| 0.04    |
|        | GT        | 0.266                   | 0.266                         | -  | -       | NS      |
haplotypes [48]. But in our study, it was identified that GC haplotype of \textit{IL10} (819 C>T, −1082 A>G) was associated with restenosis. Thus, individuals with the risk haplotype have compromised function of inflammatory reactions.

**Conclusion**

In conclusion, the present study examined the association between 48 SNPs and restenosis in the Kazakh population. Our results indicate that BMI is a single confounding factor and \textit{FGB} (rs1800790), \textit{CD14} (rs2569190), and \textit{NOS3} (rs1799983). SNPs could be genetic markers for the development of restenosis in the Kazakh population. Genotyping of these polymorphisms may be used in predicting the risk of restenosis in the Kazakh population.

**Abbreviations**

ANOVA, analysis of variance; BMI, body mass index; CHD, coronary heart disease; GENDER, GENetic Determinants of Restenosis; HDL, high-density lipoproteins; LDL, low-density lipoproteins; PCI, percutaneous coronary intervention; SNP, single-nucleotide polymorphism

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**Availability of data and materials**

The dataset supporting the conclusions of this article is available in the Zenodo repository DOI 10.5281/zenodo.51651 and hyperlink to dataset(s) in https://zenodo.org/record/51651.

**Authors’ contributions**

EVZh and YMR conceived and designed the study, analyzed the data, interpreted the results, and wrote the paper. AMA and PVT carried out experimental work. AUD, OAV, and DZhT were involved in the clinical data collection and sampling. YAT, GNK, and ANI performed the statistical analysis. All authors have read and approved the final manuscript.

**Competing interests**

All authors declare no support from any organization or the submitted work, no financial relationships with any organizations that might have an interest in the submitted work in the previous 3 years, and no other relationships or activities that could appear to have influenced the submitted work.

**Consent for publication**

Not applicable.

**Ethics approval and consent to participate**

The study was approved by the local Ethics Committee of the National Center for Biotechnology, Republic of Kazakhstan (No. 2, 12.03.2012). The investigation was conducted in accordance with the humane and ethical research principles of the Declaration of Helsinki. All participants completed a questionnaire and informed consent that has been approved by the ethics committee.

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