DISTRIBUTION OF rs4977574 AND rs10757274 POLYMORPHISMS OF CDKN2B-AS1 GENE, rs10911021 OF ZNF648 GENE, rs 266729 OF ADIPOQ GENE IN PATIENTS WITH CORONARY HEART DISEASE WHO UNDERWENT REVASCULARISATION WITH TYPE 2 DIABETES MELLITUS

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Abstract

Recent studies have addressed the question of at what stages of CHD development the polymorphisms of rs4977574 and rs10757274 of the CDKN2B-AS1 gene, rs10911021 of the ZNF648 gene, and rs 266729 of the ADIPOQ gene may be important. These studies were performed in populations with CHD (as opposed to comparing populations with and without CHD) who underwent coronary angiography at different time points to allow for follow-up. Individuals with 1 or 2 copies of the Chr9p21 risk allele had more severe CHD and progressed more rapidly compared with those with 0 copies. Moreover, Chr9p21 did not predispose to MI in patients with prior CHD or recurrent MI in patients with early MI. These studies confirmed earlier findings showing that Chr9p21 was not associated with incident MI, although it did predict the diagnosis of CHD. Taken together, these data provide the first evidence that Chr9p21 is important for the development of atherosclerosis leading to CHD, whereas it may not be important for the occurrence of MI. This concept is not inconsistent with the often reproducible association of Chr9p21 with MI, because recent associations have been obtained by comparing patients with MI with healthy controls, and MI almost always precedes CHD.

Keywords: rs4977574, rs10757274 CDKN2B-AS1, rs10911021 ZNF648 gene, rs 266729 ADIPOQ, type 2 diabetes mellitus, coronary heart disease

Cardiovascular diseases (CVDs) remain one of the main causes of morbidity, disability and mortality among the population worldwide, including in Uzbekistan. According to the World Health Organisation (WHO), 17.9 million people died from CVDs in 2016, with an increase of 9 million in 2019, accounting for 31% of all deaths worldwide [1]

The most common single nucleotide polymorphism (SNP) of this gene is rs266729(-11,377C>G), it is located in the proximal promoter region of the ADIPOQ gene. Literature data indicate that the ADIPOQ rs266729 polymorphism functionally regulates adiponectin promoter activity and adiponectin lev-

els.[2] Moreover, ADIPOQ rs266729 was found to correlate with circulating adiponectin levels in obesity. This ADIPOQ variant has been identified to be associated with high body mass index, insulin resistance and diabetic nephropathy. The impact of weight loss or dietary interventions on metabolic response given this SNP is scarce in the literature. For example, the Finnish Diabetes Prevention Study (Caucasian population) showed that a genetic variant of the ADIPOQ rs266729 gene contributes to changes in body weight and serum adiponectin concentration.[3]

The initial discovery of the 9p21 locus was made in cohorts of CHD and MI patients of predominantly European descent. This locus has now been replicated in many ethnic groups such as Koreans, Japanese, Chinese, Pakistanis and US Hispanics. [4] Although the association of 9p21 with CHD risk is consistent between these populations, the haplotype structure of this region and the association with CHD in black Africans differ. A recent meta-analysis of 35,872 cases and 95,837 controls showed that the effect of the Chr9p21 gene on the risk of IHD depends on age of onset: individuals aged 55 years and younger had an odds ratio (OR) of 1.35 (95% CI, 1.30-1.40) compared with those aged 56 to 75 years who had an OR of 1.21 (95% CI, 1.16-1.25), suggesting a stronger effect of Chr9p21 at an earlier age of IHD onset [5].

Recent work has also considered at what stages of CHD development Chr9p21 may be important. These studies were conducted in populations with CHD (as opposed to comparing populations with and without CHD) who underwent coronary angiography at different time points to allow for follow-up [6]. Individuals with 1 or 2 copies of the Chr9p21 risk allele had more severe CHD and progressed more rapidly compared with those with 0 copies. Moreover, Chr9p21 did not predispose to MI in patients with prior CHD or recurrent MI in patients with early MI. These studies confirmed earlier findings showing that Chr9p21 was not associated with incident MI, although it did predict the diagnosis of CHD. [7] Taken together, these data provide the first evidence that Chr9p21 is important for the development of atherosclerosis leading to CHD, whereas it may not be important for the occurrence of MI. [8] This concept is not inconsistent with the often reproducible association of Chr9p21 with MI, because recent associations have been obtained by comparing patients with MI with healthy controls, and MI almost always precedes CHD. It is important that these results are replicated in the future [9,10].

Objective: to investigate the distribution of gene polymorphisms rs4977574 and rs10757274 of CDKN2B-AS1 gene, rs10911021 of ZNF648 gene, rs 266729 of ADIPOQ gene in patients with CHD undergoing revascularisation with type 2 diabetes mellitus

In addition, there is an emerging need for a comprehensive study of gene polymorphisms in disease development for a more nuanced understanding of the pathogenesis of type 2 DM-associated CHD.52 patients suffering from CHD, angina II, III, who had undergone myocardial revascularisation in anamnesis were under observation. Depending on the presence of type 2 DM, the patients were divided into two groups. The first group consisted of 27 (50.9%) patients without type 2 DM. The second group consisted of 26 (49.1%) patients with type 2 DM. All patients were comparable in clinical, anthropometric, haemodynamic parameters. All patients had previously undergone myocardial revascularisation.

To detect polymorphisms rs4977574 and rs10757274 of CDKN2B-AS1 gene, rs10911021 of ZNF648 gene, rs 266729 of ADIPOQ gene, 53 blood samples of patients were obtained. DNA isolation from clinical samples was performed on spin-columns using the ArtBioTech reagent kit (Belarus). The concentration and purity of isolated DNA were measured using a BioSpec-nano spectrophotometer (Shimadzu Biotech, Japan). Determination of polymorphisms rs4977574 and rs10757274 of CDKN2B-AS1 gene, rs10911021 of ZNF648 gene, rs 266729 of ADIPOQ gene was performed by real-time PCR using QuantStudio 5 Applied Biosystems amplifier. The reaction was performed in a volume of 10μl using TaqMan® Genotyping Assays kit (Thermo Fisher Scientific, USA) according to the manufacturer's standard protocol. PCR amplification was performed on an Applied biosystems amplifier by Thermo Fisher scientific (USA).

Results and Discussion

In the distribution of genotypes of polymorphism rs4977574, rs10757271 of CDKN2B-AS1 gene, ST genotype 29 (55.8%) and 25 (48.1%) genotypes accounted for the largest number of patients (Tables 1, 2). When patients were divided into groups according to the presence of diabetes mellitus, patients with AA, AG, and GG genotypes accounted for approximately equal numbers in the group with type 2 DM, while in the group without type 2 DM, the predominant number was the AG genotype 17 (60.7%) and 19 (67.9%), respectively.

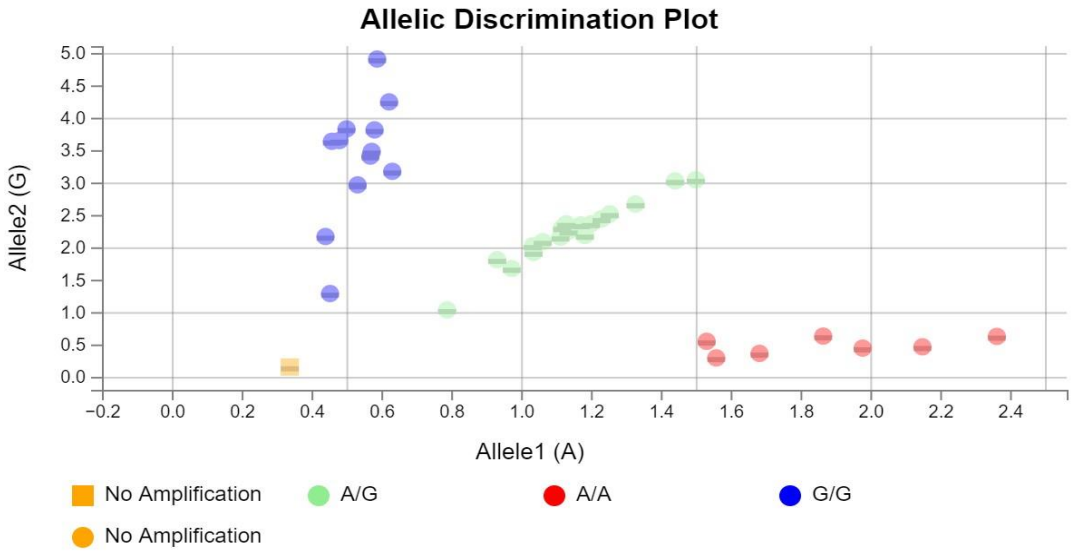

Figure 1.
Results of amplification of polymorphism rs4977574 of CDKN2B-AS1 gene. No amplification- negative control

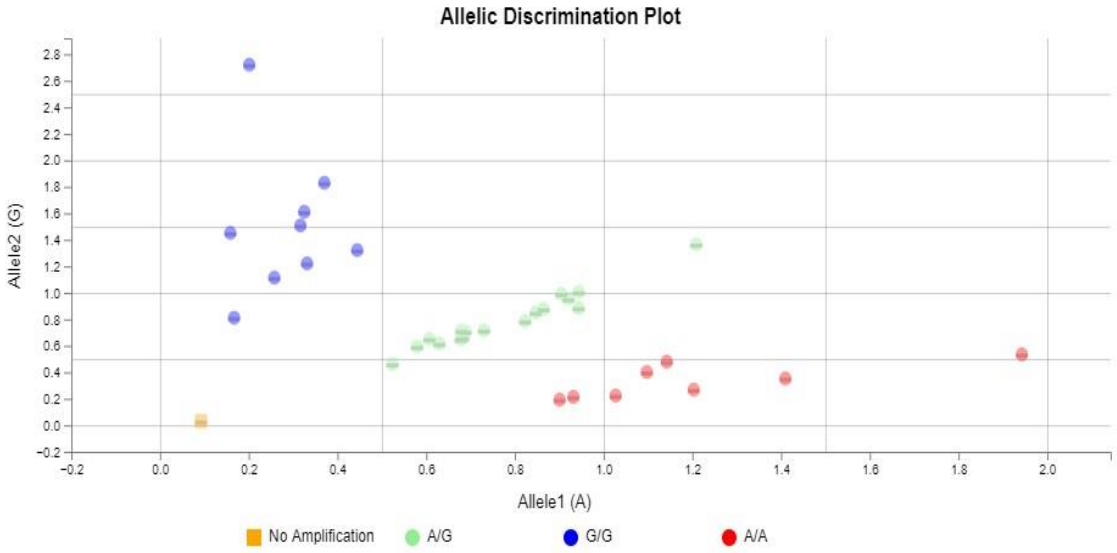

Figure 2.
Results of amplification of polymorphism rs10757274 of CDKN2B-AS1 gene. No amplification- negative control.

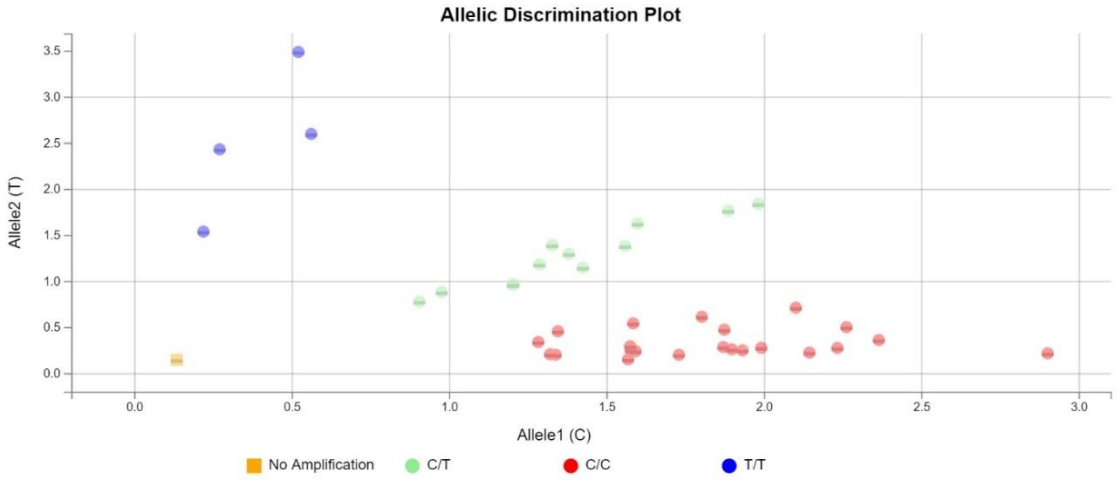

Figure 3.
Amplification results of the rs10911021 polymorphism of the ZNF648 gene. No amplification- negative control.

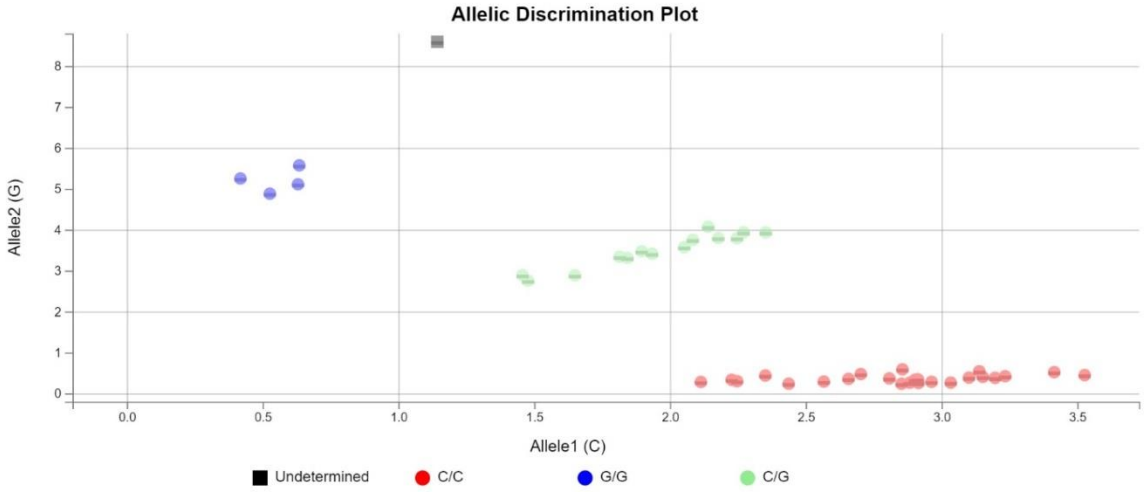

Figure 4.
Results of amplification of polymorphism rs266729 of ADIPOQ gene. No amplification- negative control.

Table 1

Distribution of genotypes and alleles of polymorphism rs10757274 of CDKN2B-AS1 gene

Genotypes	Number	Frequency, %
AA	10	19,2
AG	29	55,8
GG	13	25
Alleles	Number	Frequency, %
A	49	47,1
G	55	52,9

Table 2

Distribution of genotypes and alleles of polymorphism rs4977574 of CDKN2B-AS1 gene.

Genotypes	Number	**Частота, %**
AA	11	21,15
AG	25	48,1
GG	16	30,8
Alleles	Количество	Частота, %
A	47	45,2
G	57	54,8

Table 3

Distribution of genotypes and alleles of polymorphism rs10911021 of ZNF648 gene.

Genotypes	Number	Frequency, %
CC	28	53,8
CT	20	38,5
TT	4	7,7
Alleles	Number	Frequency, %
C	76	73,1
T	28	26,9

Table 4

Distribution of genotypes and alleles of polymorphism rs266729 of ADIPOQ gene.

Genotypes	Number	Frequency, %
CC	28	53,8
CG	20	38,5
GG	4	7,7
Alleles	Number	Frequency, %
C	76	73,1
G	28	26,9

Table 5

Genotype distribution of polymorphisms rs10757274 of CDKN2B-AS1 gene, rs4977574 of CDKN2B-AS1 gene, rs10911021 of ZNF648 gene, rs266729 of ADIPOQ gene according to the presence of type 2 diabetes mellitus.

Genotypes of gene polymorphisms	**with DM**, n=26		**without DM**, n=27	
	n	%	n	%
rs4977574 CDKN2B-AS1				
AA	7	29,2	4	14,3
AG	8	33,3	17	60,7
GG	9	37,5	7	25,0
rs10757271 CDKN2B-AS1				
AA	7	29,2	3	10,7
AG	8	33,3	19	67,9
GG	9	37,5	6	21,4
rs 266729 ADIPOQ				
CC	16	66,7	12	42,9
CG	6	25	14	50,0
GG	2	8,3	2	7,1
rs10911021 ZNF648				
CC	15	62,5	13	46,4
CT	6	25,0	14	50,0
TT	3	12,5	1	3,6

At distribution of genotypes of ADIPOQ gene polymorphism rs266729 genotype CC genotype was the largest in quantity, but at distribution of IBS patients who underwent revascularisation by presence of type 2 diabetes mellitus and its absence the distribution of patients was 16 (66,7%) and 12 (42,9%), respectively, by CC genotype. In the group without type 2 DM, the CG genotype accounted for 14 (50%) of the total number of patients without type 2 DM for this gene polymorphism. A similar pattern was observed in the genotype distribution of the rs10911021 ZNF648 polymorphism. In the initial determination of genotypes in the total group, the CC genotype dominated 28, 20, 4 for the CC, CT, TT genotypes, respectively. When patients were divided into groups according to the presence of type 2 DM, the CT genotype also accounted for 14 (50%) of the total number of patients without type 2 DM for this gene polymorphism.

Conclusions. Thus, the results of our research show that further studies are needed to investigate genetic aspects of pathogenesis of IBS associated with type 2 DM. It is expedient to investigate the prevalence and associations of polymorphisms of the above genes in different ethnic groups taking into account the ethnic features of the population under study, the interaction of mutation with phenotype for the subsequent formation of causal relationships in the development of the disease.

In addition, there is an emerging need for a comprehensive study of gene polymorphisms in disease development for a more nuanced understanding of the pathogenesis of type 2 DM-associated CHD.

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