


# Association of 3 single nucleotide polymorphisms of the eighth chromosome with remodeling of the myocardium and carotid arteries in the Kazakh population

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## Abstract

Cardiovascular diseases are one of the key health issues in Kazakhstan. According to the WHO, the prevalence of arterial hypertension (AH) was 28% in males and 25% in females in 2015, which puts up vastly to premature mortality from non-communicable diseases.

The search for genetic features of target organ lesions processes in AH is relevant. The goal of this study was to search for the genetic markers of myocardial remodeling (MR) and carotid artery remodeling (CAR).

A total of 866 hypertensive individuals were recruited in Nur-Sultan, Kazakhstan. Their blood was genotyped for 9 single nucleotide polymorphisms (SNPs) of the eighth chromosome to find an association with remodeling. The analysis was carried out in the group pairs (control and CAR, control and MR, and control and CAR and MR). The genotype–phenotype association was assessed using 5 different inheritance models: dominant, codominant, recessive, overdominant, and log-additive.

Statistically significant results were found for 3 SNPs (rs2407103, rs11775334, rs2071518) which minor alleles enlarged risks of MR and CAR in AH in the studied population. Three polymorphisms have previously been associated with AH and some other traits like pulse pressure and blood glucose in other ethnic populations: rs2407103 – in Afro-American population, rs11775334 – in the European population, rs2071518 is well studied in various ethnic populations (European, South Asian, Afro-American, Hispanic, East Asian).

**Abbreviations:** AH = arterial hypertension, CAR = carotid artery remodeling, CCL2 = CC motif ligand 2, CHARGE = The Cohorts for Heart and Aging Research in Genomic Epidemiology, HWE = Hardy–Weinberg equilibrium, HyperGEN = African American population by the Hypertension Genetic Epidemiology Network, IL1B = Interleukin 1 Beta, KCNU1 = Potassium Calcium-Activated Channel Subfamily U Member 1, LVMI = left ventricular myocardial mass index, MAF = minor allele frequency, MMP2 = matrix metalloproteinases 2, MMP9 = matrix metalloproteinases 9, MR = myocardial remodeling, MR + CAR = a combination of carotid artery remodeling and myocardial remodeling, MsrA = methionine sulfoxide to methionine, NOV = overexpressed nephroblastoma protein, OR = odds ratio, SNP = single nucleotide polymorphism, TNF = tumor necrosis factor.

**Keywords:** carotid artery remodeling, carotid artery thickness, Kazakh, left ventricular hypertrophy, myocardial remodeling, single nucleotide polymorphisms

## 1. Introduction

Cardiovascular diseases in Kazakhstan are one of the main health problems. In the structure of the total morbidity registered in health care facilities, diseases of the circulatory system ranked

second in prevalence, immediately after diseases of the respiratory system.<sup>[1]</sup> According to the WHO, the prevalence of arterial hypertension (AH) was 28% in adult men and 25% in adult women in 2015, which contributes significantly to

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The datasets generated during and/or analyzed during the current study are not publicly available, but are available from the corresponding author on reasonable request.

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premature mortality from non-communicable diseases, which in Kazakhstan is one of the highest in the European region.<sup>[2]</sup>

AH, one of the main factors of cardiovascular risk leads to damage of target organs, which includes carotid artery remodeling (CAR) and myocardial remodeling (MR).<sup>[3]</sup>

Quite numerous studies have been carried out with the purpose to search for genetic markers of AH.<sup>[4,5]</sup> Some genetic features were found in regions located on the eighth chromosome. So in a study conducted on the Chinese population, Han Dongfeng Gu et al continued the study of 8p22, which was previously associated with AH and systolic blood pressure (SBP). The presence of a connection between the alpha1A adrenergic receptor gene, located on chromosome 8p21–p11.2, with AH was investigated. It turned out that 347Arg allele and 2547G alleles were more likely to have AH.<sup>[6]</sup> Another study conducted in the Chinese population found a link between the lipoprotein lipase gene and the presence of AH in a suburban Beijing population. This gene is located on the eighth chromosome too, and was previously detected in association with AH in the Taiwanese; the study was able to replicate the result obtained in Taiwanese population.<sup>[7]</sup>

The genetic markers of CAR and MR in AH have been less studied. MR due to AH is often characterized by left ventricular hypertrophy and an increase in myocardial mass. There are not plenty of publications on the connection of genetic markers and MR; however, one of the significant studies was a meta-analysis of genome-wide studies devoted to genetic variants associated with echocardiographic signs of heart function and structure. A total of 46,533 participants from 30 studies were taken into account in the meta-analysis, and genetic patterns of the remodeled myocardium were found, namely, several single nucleotide polymorphisms (SNPs) significantly associated with certain signs of RM.<sup>[8]</sup>

The study of the genetic patterns of CAR has also been studied insufficiently. The thickening of the intima-media complex of the carotid arteries is taken as the main sign of CAR. The association of the trait with genetic characteristics of 71,128 participants in 31 studies was the target of a meta-analysis conducted by Nora Franceschini et al. Consequently the promising results were obtained, according to which 9 new loci were found and 7 previously known were confirmed.<sup>[9]</sup>

From this perspective, the search for genetic patterns in the development of target organ lesions in AH is ongoing and relevant.

This study aimed to search for associations of SNPs associated with AH and/or its traits and MR and CAR.

## 2. Materials and methods

### 2.1. Study participants

This study was conducted following ethical standards and was approved by the Bioethics Committee of Karaganda State Medical University, permission note No. 305 dated May 19, 2017. All laboratory, physical, and instrumental methods were performed in accordance with the approved standard operating procedures of the Medical Centre Hospital of President's Affairs Administration of the Republic of Kazakhstan, Nur-Sultan, Kazakhstan. Participants in the study were surveyed; the questionnaires were approved for use after an ethical review. All study participants agreed to participate in the study voluntarily and signed the informed consent.

A total of 866 hypertensive patients were recruited. Of these, 56 had MR, 357 had CAR, 249 had both of the above remodeling (MR + CAR), and 204 had no remodeling. Patients were recruited

at the Medical Centre Hospital of President's Affairs Administration of the Republic of Kazakhstan.

AH was diagnosed when the mean systolic blood pressure was  $\geq 140$  mm Hg and/or mean diastolic blood pressure  $\geq 90$  mm Hg in daily monitoring of blood pressure or based on antihypertensive drugs receiving.

CAR was determined based on the results of color-coded duplex ultrasound performed on a Vivid E9 cardiovascular ultrasound scanner from GE Healthcare's (USA). The values of the thickness of the intima-media complex of the carotid arteries over 0.9 mm were taken as CAR.

MR was determined based on the results of echocardiography performed on a Vivid E9 cardiovascular ultrasound scanner from GE Healthcare's (USA). MR was established on the basis of left ventricular myocardial mass index (LVMI), calculated by the formula  $\text{left ventricular mass/body surface area}$ . The myocardium was considered remodeled when LVMI was greater than  $115 \text{ g/m}^2$  in males and more than  $95 \text{ g/m}^2$  in females, as well as in cases where the relative wall thickness (RWT) of the myocardium was  $\geq 0.43$ . RWT was calculated using the formula  $(\text{atrial septal thickness} + \text{LV posterior wall thickness})/\text{end-diastolic dimension}$ .<sup>[3]</sup>

### 2.2. DNA extraction and genotyping

**2.2.1. Isolation of DNA.** DNA extraction was performed automatically using the AutoMate Express Instrument. The kit for DNA extraction was iPrep Purelink gDna Blood Kit.

Firstly, the tubes were pre-numbered according to the DNA samples. Next, the Qubit working solution was prepared: the Qubit dsDNA BR Reagent was diluted in the Qubit dsDNA BR Buffer, 1:200 for 1 patient.

Then  $2 \mu\text{l}$  was removed from the buffer and reagent mix and  $2 \mu\text{l}$  of DNA was added. The concentration was measured on a Qubit 4 Fluorometer using the Qubit dsDNA BR Assay Kits.

**2.2.2. Genotyping.** The genotyping method is carried out using the OpenArray technology, which is a unique platform for reactions in nanoliter volumes. This technology uses special OpenArray slides. Each slide produces 3072 data points.

For genotyping, the previously extracted DNA samples were combined with the reaction mixture in a 384 well sample plate. For 1 sample OpenArray Real-time master mix –  $3.0 \mu\text{l}$ ; DNA sample –  $2.0 \mu\text{l}$  (concentration  $50 \text{ ng}/\mu\text{l}$ ) is needed. The total volume of the reaction mixture per well is  $5 \mu\text{l}$ . Each sample is duplicated. The reaction mixture was mixed thoroughly in a plate with a shaker and centrifuge.

Next, the probes were designed using the QuantStudio OpenArray AccuFill Plate Configurator. Genotyping plates were supplied with dried assays in the indicated through holes. For the assay unique plate was used, there were 2 allele-specific probes, minor groove binder and 2 PCR primers, to ensure high reliability and accuracy of genotyping calls.

OpenArray technology employs nanoliter fluidics and can be customized with 3072 through holes in 6 different formats.

Then, a protocol for the applied samples with analysis information was created in the plate setup file. The protocol was loaded into QuantStudio 12K Flex software to create and run an experiment.

The prepared chips were loaded into the QuantStudio 12K Flex using replaceable blocks for genotyping. Next, the amplification reaction takes place using real-time PCR microfluidic technology.

Analysis of the data obtained as a result of the amplification reaction is performed using the online tools of the Thermo Fisher Cloud service. In accordance with the results of bioinformatic analysis, the studied genes were classified as homozygotes for the major allele, homozygotes for the minor allele, and heterozygotes.

### 2.3. SNP for analysis

In this study, 9 SNPs were genotyped. The list of SNPs, information on their location in chromosomes and genes is presented in Table 1.

These polymorphisms are located in the eighth chromosome. The presented polymorphisms are frequently linked to transcription variants of introns, or to sequences variants located between genes.

### 2.4. Statistical analysis

The analysis was carried out using the R statistics programs (compare Groups R packages <http://www.jstatsoft.org/>, Statistica 6.0 [Stat-Soft], and SPSS [IBM]).

Assessment of the association of the SNPs with phenotypes was carried out in pairs in the groups: control – MR, control – CAR, and control – MR + CAR, in accordance with the case–control design based on a generalized linear model. The genotype–phenotype association was assessed using 5 different inheritance models: dominant, codominant, recessive, overdominant, and log-additive inheritance models.

Based on the results of genotyping, for each SNP in each group, such indicators as the proportion of major and minor alleles, the minor allele frequency (MAF), relative values for genotypes, as well as the *P*-value when calculating the Hardy–Weinberg law (HWE – Hardy–Weinberg equilibrium). The data obtained is presented in Table 1.

## 3. Results

### 3.1. Study participants

The general characteristics of the studied groups are presented in Table 2. In total, data were collected from 866 participants, of which 439 (50.7%) were men. In the group with MR 56 people, which is 6.5% of the total number of participants, in the group with CAR 357 people (41.2%), in the group with a combination of 2 remodelings (MR + CAR) 249 people (28.8%), and in the control group 204 participants (23.5%). The median age values significantly differed and were 43 y.o. in the control group, 46 y.o. in the RM group, 52 y.o. in the CAR group and 55 y.o. in the RM + CAR group.

### 3.2. Hardy–Weinberg equilibrium and alleles of 9 SNPs

Nine SNPs of the eighth chromosome were genotyped, all in accordance with the HWE ( $P > .05$ ).

The minor allele was taken as a risk factor, the minor allele frequencies in the population of the studied SNPs are shown in Table 3. The odds ratios that revealed the SNPs associated with CAR were calculated (rs2407103, odds ratio [OR]=1.468 [1.053–2.064],  $P = .02370$ ) with a combination of CAR and MR (rs2407103, OR = 1.498 [1.05–2.15],  $P = .02550$ ), in addition, 1 SNP showed a *P*-value close to the significant (rs2071518, OR = 1.376 [0.964–1.972],  $P = .081$ ).

### 3.3. Association of the SNPs with MR and CAR

We continued the analysis by inheritance patterns (codominant, dominant, recessive, overdominant, and log-additive) with those SNPs that showed a significant or close to significant association with the remodeling. The results of this analysis are shown in Tables 4 and 5.

**Table 1**  
Basic information about the study SNPs.

RS	Location	Consequence	SYMBOL	Control (MAF)	CAR (MAF)	MR (MAF)	MR + CAR (MAF)	HWE
rs2407314	8:4220831-4220831	intron_variant	CSMD1	38%	36%	29.5%	34.7%	0.657
rs6601530	8:10813762-10813762	intron_variant	PINX1	37.7%	36.4%	41.1%	39.2%	0.881
rs11250135	8:11420637-11420637	downstream_gene_variant	FAM167A	28.4%	29.6%	25.9%	33.3%	0.607
rs7016717	8:77718131-77718131	intergenic_variant	–	2.2%	1.5%	5.4%	1.6%	1
rs2071518	8:119423572-119423572	3_prime_UTR_variant	CCN3	15.9%	19.2%	15.2%	20.7%	1
rs11781551	8:122395852-122395852	intergenic_variant	–	21.3%	21.8%	27.7%	21.7%	1
rs12541595	8:124845117-124845117	Upstream_gene_variant	LINC00964	32.8%	32.4%	28.6%	34.3%	0.874
rs11775334	8:10214110-9738012	intron_variant	MSRA	41.7%	49.3%	48.2%	44.6%	0.388
rs2407103	8:37094396-37094396	upstream_gene_variant	SMARCE1P4	15.4%	21.1%	20.5%	21.5%	0.179

CAR = carotid artery remodeling, HWE = Hardy–Weinberg equilibrium, MAF = minor allele frequency, MR = myocardial remodeling, MsrA = methionine sulfoxide to methionine, SNP = single nucleotide polymorphism.

**Table 2**  
General characteristics of the remodeling subjects and healthy controls.

	[ALL] N = 866	Control N = 204	CAR N = 357	MR N = 56	MR + CAR N = 249	<i>P</i> overall
Age	51.0 [43.0; 56.0]	43.0 [36.8; 49.2]	52.0 [45.0; 56.0]	46.0 [40.5; 55.2]	55.0 [49.0; 60.0]	<.001
Gender						.905
Female	427 (49.3%)	99 (48.5%)	181 (50.7%)	26 (46.4%)	121 (48.6%)	
Male	439 (50.7%)	105 (51.5%)	176 (49.3%)	30 (53.6%)	128 (51.4%)	

CAR = carotid artery remodeling, MR = myocardial remodeling, MR + CAR = a combination of carotid artery remodeling and myocardial remodeling.

**Table 3****Association between the SNPs and the remodelings.**

RS	Genotype	MR		CAR		MR + CAR	
		ORs (95%CI)	P	ORs (95%CI)	P	ORs (95%CI)	P
rs2407314	G/C	1.466 [0.915–2.385]	.12100	0.918 [0.708–1.191]	.54700	1.151 [0.868–1.525]	.34500
rs6601530	A/G	0.87 [0.557–1.368]	.59500	1.059 [0.816–1.372]	.70400	0.942 [0.713–1.244]	.71500
rs11250135	G/T	1.137 [0.694–1.899]	.68100	0.947 [0.716–1.249]	.74200	0.795 [0.591–1.066]	.13000
rs7016717	G/A	2.504 [0.717–8.081]	.14800	0.694 [0.259–1.912]	.56500	0.724 [0.241–2.136]	.67800
rs2071518	T/C	0.944 [0.495–1.724]	.96200	1.253 [0.897–1.762]	.19900	1.376 [0.964–1.972]	.08100
rs11781551	A/G	0.709 [0.431–1.185]	.19500	1.031 [0.76–1.405]	.89600	0.979 [0.702–1.362]	.95900
rs12541595	G/C	0.818 [0.499–1.319]	.45700	0.978 [0.749–1.28]	.91800	1.069 [0.803–1.425]	.68700
rs11775334	A/G	0.768 [0.494–1.195]	.25800	0.735 [0.57–0.946]	.01620	0.888 [0.676–1.167]	.41600
rs2407103	A/G	1.414 [0.792–2.464]	.25400	1.468 [1.053–2.064]	.02370	1.498 [1.05–2.15]	.02550

CAR=carotid artery remodeling, MR=myocardial remodeling, MR+CAR=a combination of carotid artery remodeling and myocardial remodeling, SNP=single nucleotide polymorphism.

In Table 4, it is shown the results of the analysis in the control group and the group of participants with CAR. In the codominant model, the genotype A/A and in the dominant model the genotype A/G, A/A, were significantly associated with an increase of the chances of CAR (rs11775334, OR=1.94 [1.15–3.27],  $P=.03755$  and OR=1.53 [1.05–2.24],  $P=.02936$ , respectively). This association persisted after adjustments for gender and age. In the log-additive model, the minor allele was also associated with an increase in the chances of CAR (OR=1.39 [1.08–1.8],  $P=.01042$ ), after adjustments for sex and age, too.

The GG genotype in the codominant and recessive inheritance model was associated with a significant increase in the chances of CAR + MR (rs2407103, OR=5.45 [1.24–23.95],  $P=.01503$  and OR=5.05 [1.15–22.08],  $P=.00902$ , respectively). In the log-additive model, the minor allele was also associated with an

increase in the chances of CAR (OR=1.48 [1.07–2.05]), the significance remained after adjustments for sex and age.

In Table 5, showing the results of the analysis in the control group and in the group of participants with a combination of CAR and MR, the G/G genotype in the codominant and recessive inheritance models was associated with greater chances of CAR and MR (rs2407103, OR=5.14 [1.12–23.59],  $P=.02423$  and OR=4.67 [1.02–21.3],  $P=.020985$ , respectively). The A/G–G/G genotype in the dominant model and the minor allele in the log-additive inheritance models were also associated with high chances of CAR and MR (rs2407103, OR=1.47 [0.99–2.18],  $P=.05348$  and OR=1.53 [1.07–2.18],  $P=.017$ , respectively), gender and age had little effect.

In the dominant model of inheritance, the C/T–T/T genotype was associated with greater chances of CAR and MR, as in the

**Table 4****Relationship between the SNPs and CAR under multiple models of inheritance.**

RS	Model inheritance	Control group	CAR group	OR 95%CI	P	OR 95%CI adj. by age	P value adj. by age	OR 95%CI adj. by gender	P value adj. by gender	OR 95%CI adj. by age and gender	P value adj. by age and gender
rs11775334	Codominant										
	G/G	66 (32.4%)	85 (23.8%)	1	.03755	1	.07474	1	.03368	1	.06156
	A/G	106 (52%)	192 (53.8%)	1.41 [0.94–2.1]		1.38 [0.89–2.12]		1.44 [0.96–2.15]		1.41 [0.91–2.18]	
	A/A	32 (15.7%)	80 (22.4%)	1.94 [1.15–3.27]		1.89 [1.08–3.31]		1.96 [1.16–3.3]		1.93 [1.1–3.39]	
	Dominant										
	G/G	66 (32.4%)	85 (23.8%)	1	.02936	1	.05585	1	.02345	1	.04337
	A/G–A/A	138 (67.6%)	272 (76.2%)	1.53 [1.05–2.24]		1.5 [0.99–2.26]		1.56 [1.06–2.28]		1.53 [1.01–2.32]	
	Recessive										
	G/G–A/G	172 (84.3%)	277 (77.6%)	1	.05201	1	.07894	1	.05635	1	.0755
	A/A	32 (15.7%)	80 (22.4%)	1.55 [0.99–2.44]		1.53 [0.94–2.49]		1.54 [0.98–2.43]		1.54 [0.95–2.51]	
	Overdominant										
	G/G–A/A	98 (48%)	165 (46.2%)	1	.67768	1	.74641	1	.60177	1	.68618
rs2407103	A/G	106 (52%)	192 (53.8%)	1.08 [0.76–1.52]		1.06 [0.73–1.54]		1.1 [0.78–1.55]		1.08 [0.74–1.57]	
	Log-additive										
	0,1,2	204 (36.4%)	357 (63.6%)	1.39 [1.08–1.8]	.01042	1.38 [1.04–1.81]	.02275	1.4 [1.08–1.82]	.00933	1.39 [1.06–1.84]	.01827
	Codominant										
	A/A	143 (70.1%)	223 (62.5%)	1	.01503	1	.01859	1	.01554	1	.01651
	A/G	59 (28.9%)	117 (32.8%)	1.27 [0.87–1.85]		1.29 [0.86–1.95]		1.29 [0.88–1.88]		1.31 [0.87–1.98]	
	G/G	2 (1%)	17 (4.8%)	5.45 [1.24–23.95]		6.01 [1.25–28.85]		5.37 [1.22–23.6]		6.09 [1.27–29.2]	
	Dominant										
	A/A	143 (70.1%)	223 (62.5%)	1	.06627	1	.07206	1	.06038	1	.06261
	A/G–G/G	61 (29.9%)	134 (37.5%)	1.41 [0.97–2.04]		1.44 [0.97–2.14]		1.42 [0.98–2.06]		1.46 [0.98–2.18]	
	Recessive										
	A/A–A/G	202 (99%)	340 (95.2%)	1	.00902	1	.01132	1	.0101	1	.01076
rs2407103	G/G	2 (1%)	17 (4.8%)	5.05 [1.15–22.08]		5.53 [1.16–26.42]		4.95 [1.13–21.69]		5.59 [1.17–26.64]	
	Overdominant										
	A/A–G/G	145 (71.1%)	240 (67.2%)	1	.34267	1	.33737	1	.31492	1	.31097
	A/G	59 (28.9%)	117 (32.8%)	1.2 [0.82–1.74]		1.22 [0.81–1.83]		1.21 [0.83–1.77]		1.23 [0.82–1.85]	
	Log-additive										
	0,1,2	204 (36.4%)	357 (63.6%)	1.48 [1.07–2.05]	.01678	1.52 [1.06–2.16]	.01975	1.49 [1.07–2.07]	.01566	1.54 [1.07–2.2]	.01668

CAR=carotid artery remodeling, OR=odds ratio, SNP=single nucleotide polymorphism.



**Table 5****Relationship between the SNP and MR + CAR under multiple models of inheritance.**

RS	Model inheritance	Control group	CAR + MR group	OR 95%CI	P	OR 95%CI adj. by age	P value adj by age	OR 95%CI adj. by gender	P value adj by gender	OR 95%CI adj. by age and gender	P value adj by age and gender
rs2407103	Codominant										
	A/A	143 (70.1%)	153 (61.4%)	1	.02423	1	.01526	1	.0244	1	.01121
	A/G	59 (28.9%)	85 (34.1%)	1.35 [0.9–2.01]		1.54 [0.95–2.48]		1.33 [0.88–1.99]		1.59 [0.98–2.58]	
	G/G	2 (1%)	11 (4.4%)	5.14 [1.12–23.59]		7.66 [1.19–49.48]		5.26 [1.15–24.14]		8.28 [1.25–54.7]	
	Dominant										
	A/A	143 (70.1%)	153 (61.4%)	1	.05348	1	.02668	1	.06181	1	.0192
	A/G–G/G	61 (29.9%)	96 (38.6%)	1.47 [0.99–2.18]		1.69 [1.06–2.71]		1.46 [0.98–2.16]		1.75 [1.09–2.81]	
	Recessive										
	A/A–A/G	202 (99%)	238 (95.6%)	1	.02095	1	.02192	1	.01847	1	.02034
	G/G	2 (1%)	11 (4.4%)	4.67 [1.02–21.3]		6.62 [1.04–42.12]		4.81 [1.05–21.94]		7.02 [1.08–45.49]	
	Overdominant										
	A/A–G/G	145 (71.1%)	164 (65.9%)	1	.23477	1	.13182	1	.27018	1	.10135
rs2071518	A/G	59 (28.9%)	85 (34.1%)	1.27 [0.85–1.9]		1.44 [0.89–2.32]		1.25 [0.84–1.87]		1.49 [0.92–2.41]	
	Log-additive										
	0,1,2	204 (45%)	249 (55%)	1.53 [1.07–2.18]	.017	1.75 [1.14–2.69]	.00859	1.52 [1.07–2.16]	.01905	1.81 [1.17–2.79]	.006
	Codominant										
	C/C	144 (70.6%)	156 (62.7%)	1	.18065	1	.10378	1	.16924	1	.07754
	C/T	55 (27%)	83 (33.3%)	1.39 [0.93–2.1]		1.57 [0.97–2.56]		1.4 [0.93–2.11]		1.66 [1.02–2.72]	
	T/T	5 (2.5%)	10 (4%)	1.85 [0.62–5.53]		2.28 [0.63–8.17]		1.9 [0.63–5.7]		2.17 [0.61–7.78]	
	Dominant										
	C/C	144 (70.6%)	156 (62.7%)	1	.07471	1	.03994	1	.07094	1	.02604
	C/T–T/T	60 (29.4%)	93 (37.3%)	1.43 [0.96–2.13]		1.63 [1.02–2.61]		1.44 [0.97–2.15]		1.71 [1.06–2.75]	
	Recessive										
	C/C–C/T	199 (97.5%)	239 (96%)	1	.34835	1	.28602	1	.32028	1	.32937
	T/T	5 (2.5%)	10 (4%)	1.67 [0.56–4.95]		1.97 [0.56–6.96]		1.72 [0.58–5.11]		1.86 [0.52–6.57]	
	Overdominant										
	C/C–T/T	149 (73%)	166 (66.7%)	1	.14154	1	.09025	1	.14095	1	.05635
	C/T	55 (27%)	83 (33.3%)	1.35 [0.9–2.03]		1.51 [0.93–2.45]		1.36 [0.9–2.05]		1.6 [0.98–2.61]	
	Log-additive										
	0,1,2	204 (45%)	249 (55%)	1.38 [0.98–1.95]	.06454	1.55 [1.03–2.34]	.03352	1.39 [0.98–1.97]	.05951	1.59 [1.05–2.41]	.0251

CAR = carotid artery remodeling, MR = myocardial remodeling, OR = odds ratio, SNP = single nucleotide polymorphism.

log-additive model (rs2071518, OR = 1.43 [0.96–2.13],  $P = .07471$  and OR = 1.38 [0.98–1.95],  $P = .06454$ , respectively). When using corrections for gender and age, the  $P$  value became less than .05, namely .02604 and .0251.

#### 4. Discussion

Nine SNPs located on the eighth chromosome were genotyped, 3 of them were significantly associated with CAR and/or a combination of CAR and MR.

It was found that SNP rs2407103 showed a significant association with a higher chance of CAR and a combination of 2 remodelings, which makes the association significant in 2 pairs. The polymorphism was previously mentioned in a genome-wide study performed on an African American population by the Hypertension Genetic Epidemiology Network (HyperGEN) study. HyperGEN was conducted to establish the presence of genome-wide associations with blood glucose and insulin resistance. rs2407103 showed an association with both studied indicators with 15% effect, but the significance did not reach the accepted threshold and was  $P = 2 \times 10^{-6}$ .<sup>[10]</sup> The SNP is located in the region encoding Potassium Calcium-Activated Channel Subfamily U Member 1 (KCNU1). A testis-specific potassium channel, activated by both intracellular pH and membrane tension, which mediates potassium export. The protein is critical for fertility.<sup>[11]</sup> It has been linked to type 2 diabetes in other studies, namely, in a large meta-analysis of individuals from Southeast Asia, protein gene markers were found to be significantly associated with type 2 diabetes. In another study of new analytical approaches conducted on data from the European population, an association of KCNU1 with blood lipid parameters (low-density lipoprotein cholesterol, high-density

lipoprotein cholesterol, triglycerides, and total cholesterol) was indicated.<sup>[12]</sup> Thus, we can assume that rs2407103 has the effect on CAR through influencing blood glucose and lipids levels. Likewise, the association of rs2407103 with type 2 diabetes, which has been proven to independently increase cardiovascular risk,<sup>[3]</sup> supports the hypothesis of the effect SNP through glucose level. Moreover, in persons with diabetes mellitus, such target organ damage as left ventricular hypertrophy, as well as signs of atherosclerosis, is more often observed. This is probably an important part of explaining the reason for the association of polymorphisms associated with diabetes and the remodeling of target organs in AH.<sup>[13]</sup>

Another SNP that has been associated with CAR is rs11775334 encoding the protein methionine sulfoxide reductase A. Methionine sulfoxide reductases are thioredoxin-associated enzymes involved in the enzymatic conversion of methionine sulfoxide to methionine (MsrA). MsrA protects cells from oxidative stress. Overexpression of MsrA increases resistance to cell death, while silence or suppression of MsrA decreases cell survival.<sup>[14]</sup> The antioxidant activity of the protein seems to underlie its functioning.<sup>[15]</sup> The essential role of oxidative stress in the development of atherosclerosis is beyond doubt<sup>[16,17]</sup> and probably sheds light on the reasons for the association of rs11775334 with CAR.

Regarding the SNP, it has been associated with AH in a genome-wide study by The Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium which included 5 prospective cohort studies from the US and Europe (the Age, Gene/Environment Susceptibility – Reykjavik Study, the Atherosclerosis Risk in Communities Study, the Cardiovascular Health Study, the Framingham Heart Study, and the Rotterdam Study). Most of the participants were from a

European population.<sup>[18]</sup> However, in a later study that shown work on the model predicting mean arterial pressure, it was found that the effect of SNPs included in the model did not increase its predictive power, among these polymorphisms were rs11775334.<sup>[19]</sup> However, in our study, the polymorphism turned out to be associated with CAR.

rs2071518 was found in association with cardiovascular disease or its risk factors/symptoms in a number of studies. The meta-analysis looking for genetic markers of pulse pressure and mean arterial pressure resulted in a significant association of pulse pressure with rs2071518.<sup>[20]</sup>

The SNP is located in the region that codes overexpressed nephroblastoma protein (NOV). NOV showed the highest expression levels of the genes evaluated for expression in human aortic samples at new pulse pressure loci. It is a multifunctional matrix cell regulator protein that is associated with processes of angiogenesis, proliferation, and inhibition of vascular smooth muscle cell growth and migration,<sup>[21]</sup> as well as reduced neointimal thickening in mice with NOV injury.<sup>[22]</sup> Truncated NOV mice show abnormal cardiac development, which manifests itself as signs of myocardial remodeling.<sup>[23]</sup> In addition, the gene plays the role of a negative regulator of pro-inflammatory activation of the endothelium, reducing the adhesion of monocytes, its anti-inflammatory effects occur secondary to inhibition of the NF-kappaB signaling pathway.<sup>[24]</sup> It contributes to the control and coordination of inflammatory processes in atherosclerosis (according to similarity). Also, it attenuates inflammatory pain by regulating Interleukin 1 Beta (IL1B) and tumor necrosis factor (TNF)-induced matrix metalloproteinases 9 (MMP9), matrix metalloproteinases 2 (MMP2), and CC motif ligand 2 (CCL2) expression.<sup>[25]</sup>

Thus, SNP seems to affect the processes of remodeling, as well as the processes of inflammation, which have a direct effect on the progression of CAR<sup>[26]</sup> and MR.<sup>[27]</sup>

There are several limitations in our study. Firstly, patients were recruited from 1 health facility, and study participants may not be representative of the general population. Secondly, the SNPs that were used in the study are few in number, and there was no analysis based on the haplotype since the selected polymorphisms are located at a significant distance from each other. The advantages of the study were that it was conducted on representatives of the Kazakh ethnic group, which has been studied scarcely; the sample was also large enough to divide the main group into 3 subgroups; in addition, the topic of genetic markers of MR and CAR was covered rather poorly.

Promising results were found for 3 polymorphisms (rs2407103, rs11775334, rs2071518) which minor alleles significantly increased the chances of remodeling target organs in AH in the studied population. Further search for genetic characteristics of AH progression with a larger sample and an expanded panel of genetic markers seems relevant.

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