Original Article

Impact of KIF6 Polymorphism rs20455 on Coronary Heart Disease Risk and Effectiveness of Statin Therapy in 100 Patients from Southern Iran

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Abstract

Background: The purpose of this study was to investigate the association between Trp719Arg (rs20455) and Coronary Heart Disease (CHD), and also Coronary Heart Disease reduction in individuals with this SNP during statin therapy in southern Iran. It has been shown that rs20455, which could affect the function of kinesin protein, is associated with Coronary Heart Disease and could be an effective factor for patients who take statin therapy.

Methods: Patients and control individuals were genotyped for rs20455 Single Nucleotide Polymorphism (SNP) using ARMS PCR (Amplification Refractory Mutation System Polymerase Chain Reaction) and RFLP (Restriction Fragment Length Polymorphism) analysis. The effect of kinesin family member 6 (*KIF6*) on statin therapy was also examined among patients who had a history of one or two heart attacks.

Results: It was found that rs20455 had a significant association with Coronary Heart Disease (Odds Ratio [OR] 3.17, 95% Confidence Interval [CI] 1.68 to 5.98). In addition, statin therapy was more effective in rs20455 carriers than non-carriers, with 80% of the carriers showed positive response to this treatment.

Conclusions: Trp 719Arg have an effect on development of Coronary Heart Disease but it is very useful for statin therapy. Overall, individuals with this Single Nucleotide Polymorphism can take statin therapy to prevent the catastrophic consequences of Coronary Heart Disease.

Keywords: Trp 719Arg, *KIF6,* rs20455, CHD, Statin Therapy

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Introduction

Today, heart diseases and their side effects are so integrated in modern life that they constitute a major health problem. Cardiovascular Disease (CVD) is a group of diseases that affect heart and blood vessels. Cardiovascular disease is divided into several branches including Coronary Heart Disease (CHD), cerebrovascular disease, peripheral arterial disease, rheumatic heart disease, congenital heart disease and deep vein thrombosis and pulmonary embolism.[1] The highest mortality rate due to CVD in the world belongs to the Middle East and parts of Eastern Europe (MEN).[2] According to statistics provided by the WHO Global Burden of Disease study, in 2002, 5% of cardiovascular disease deaths worldwide belong to low- and middle-income countries, and more than 35% of these mortalities are due to CVD.[3] In fact, specifically CVD-related mortality rate, in 2001, is equal to 671 thousand in MEN[4] with half of the mortality of the CVD and 16.9% of all mortalities worldwide is related to CHD.[3] According to the statistics provided by the WHO in 2008, 17.3 million people have died due to CVD[5,6] and 7.3 million die from CHD annually.[7]

In Iran, Coronary Heart Disease (CHD) is the main cause of disease-related death in both sexes. Based on the WHO factsheet published in 2002, the total of death in Iran was reported to be 385,000 in 2002. Among that, 268,000 deaths were related to CHD (Figure 1).

Coronary Heart Disease manifests as heart failure, angina, arrhythmia and Myocardial Infarction (MI)[8] which is permanent tissue damage caused by death of heart cells. Heart is a vital and indispensable organ in human life, pumping blood into all arteries to supply oxygen and nutrients to all cells. However, like any other organs in the body, it also needs oxygen and nutrients to survive and function, and this task falls on the coronary arteries. Blockage in these arteries with plaques (aggregations of fatty acids, calcium or connective tissue)diminishes the blood supply and oxygen to the heart and causes Myocardial Infarction or heart attack and in some cases, may lead to cardiac death.[9,10]

In addition to environmental factors, including high cholesterol, obesity, hypertension, smoking, metabolic syndrome, physical inactivity, age and diabetes mellitus which lead to Coronary Heart Disease,[9,11] genetic risk factors also contribute to this disorder which have been in the focus of various research institutes in the last few years and play a crucial role in the Coronary Heart Disease. The Cardiovascular Health Study (CHS),[12,13] the Women's Health Study (WHS),[14] the Cholesterol and Recurrent Events

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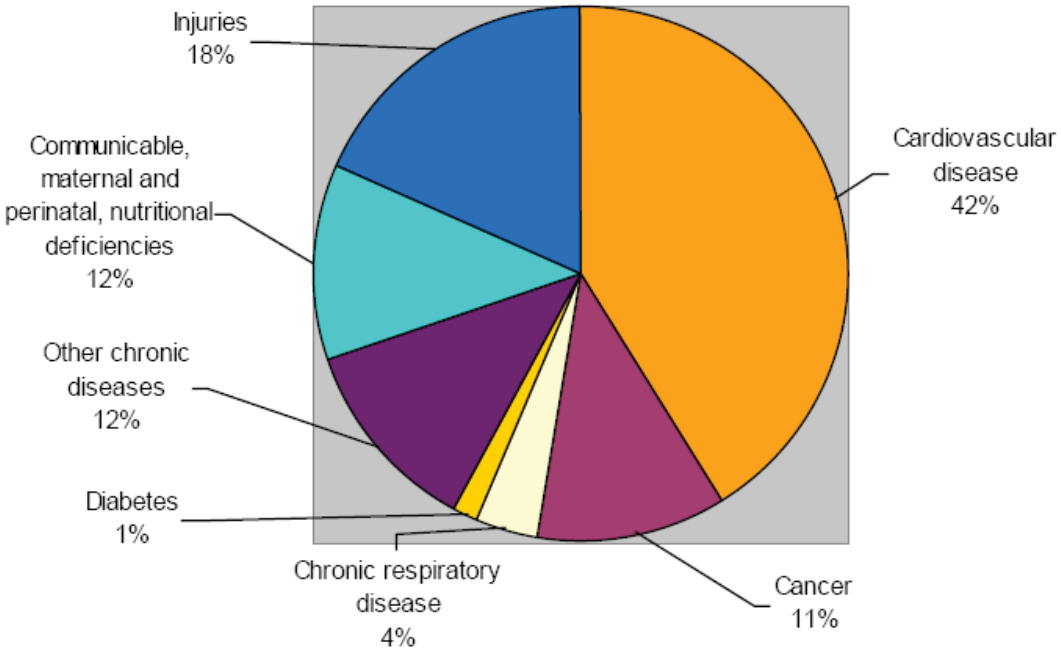

Figure 1. Deaths by cause, all ages, Islamic Republic of Iran, 2002. Published by WHO accessible on http://www.who.int/chp/chronic_disease_report/en/.

Table 1. PCR Primer and conditions used in PCR-RFLP.

	Restriction Site	Normal homozygote (Genotype: TT)	Carrier heterozygote (Genotype: TC)	Patient homozygote (Genotype: CC)
	HPYCH4 (Bst4c1)	252 bp	156+96+252 bp	156+96 bp
Primer pair	**F:** AAACTCCTTCTGGGGCCAACAG PCR product: 252 bp.		**R:** TCCTGCTGGATCATATGGCTTATC	

Table 2. Characteristics of Cases and Controls.

Characteristic	Controls (*n*=100)	Cases (*n*=100)	*P* Value [a]
Current Smoker, *n*	5	33	$P < 0.001$
History of diabetes, *n*	9	23	$P < 0.001$
History of kidney, *n*	10	17	$P = 0.147$
Family history of MI, *n*	31	37	$P =\cong 0.37$
Fisher's exact test for discrete variables (current smoker, history of diabetes, history of kidney)			

Table 3. Association between CHD and Trp719Arg.

Characteristic	Cases	Controls	OR**	95% CI	*P* Value
Arg/Arg + Arg/Trp	65	37	3.17	5.98–1.68	$P < 0.001$
Trp/Trp	35	63	ref		
Arg/Trp	48	27	3.20	5.99–1.71	$P < 0.001$
Arg/Arg	17	10	3.06	7.4–1.26	$P = 0.013$
** OR : odds ratio; CI : confidence interval; ref : reference group.					

Table 4. Relation of the rs20455, MI and Statin therapy.

Characteristic	Carriers	Non-carriers	OR	95% CI	*P* Value
Cases with once MI, *n* (%)	52(%80)	14 (%40)	0.17	0.08 – 0.41	$P < 0.001$
Cases with twice MI, *n* (%)	13 (%20)	21 (%60)			

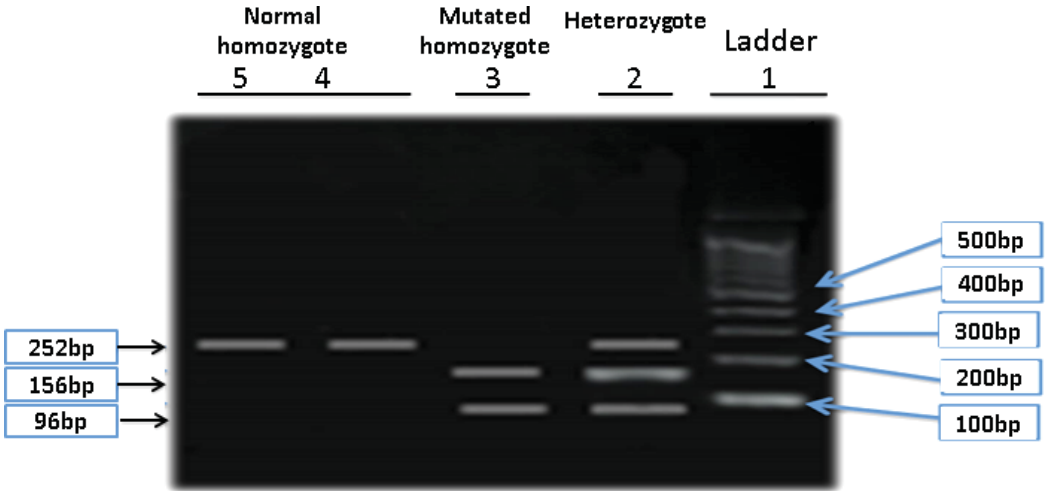

Figure 2. PCR-RFLP results. After Bst4CI restriction enzyme digestion, heterozygous carrier individuals showed 252bp, 156bp, and 96bp bands (line 2), individual with homozygote mutated allele 156bp and 96bp bands (line 3) and individuals with wild type allele following enzyme digestion showed a 252bp band (line 4, 5).

(CARE) study, and the West of Scotland Coronary Prevention Study (WOSCOPS)[15] include studies related to the association of single nucleotide polymorphisms (SNP) with Coronary Heart Disease.

One of the main genetic risk factors reported to be associate with increased risk of incident CHD is the single nucleotide polymorphism (SNP) in the kinesin family member 6 (*KIF6*) gene, Trp719Arg (rs20455).[16] Furthermore, some studies have reported that carrier individuals with *KIF6* Trp719Arg are at increased risk for Coronary Heart Disease and some researches have suggested that rs20455 may benefit from statin therapy.[17,18] *KIF6* (kinesin family member 6) gene which is located on 6p21.2 is related to the kinesin superfamily and expressed in vascular tissue, mainly in the coronary arteries. This gene consists of 22 exons and encodes a protein containing 814 amino acids with microtubule motor activity that transports cellular cargoes along microtubules.[19] These cargoes include membrane organelles, cellular vesicles, protein, and mRNAs.[20–24] Kinesin protein is made up of two conserved motor domains, a connecter neck region, non-conserved stem and also a tail domain. The rs20455 SNP which converts Trp codon 719 (TGG) to Arg codon (CGG) is located at position 39325078 in exon 20 which codes the tail region of the Kinesin. A substitution of thymine (T) to cytosine (C) in the nucleotide sequence at this position can affect the Kinesin ability to bind cargoes inside cells.[25] Different populations show a different distribution of allele frequency for Trp719Arg (rs20455) of *KIF6*. Until now, such studies have not been performed in Iran. The aim of this study was to investigate the association between Trp719Arg (rs20455) with Coronary Heart Disease and also Coronary Heart Disease reduction during statin therapy in southern Iran.

Materials and Methods

This research was an applied case-control study carried out in the geographic region of southern Iran. This work was approved by the Ethics Committee of Shiraz University of Medical Sciences. This project focuses on two statistical targets. We aimed to analyze the relationship of the rs20455 SNP in the *KIF6* gene and heart disease. Furthermore, we monitored the patients who underwent Statin therapy and analyzed the rate of second heart attack. We hypothesize that carriers of the rs20455 SNP respond more efficiently to the Statin therapy.

According to the information obtained from questionnaires, patients were placed into two groups: patients with a history of one myocardial infarction and those with two myocardial infarctions.

In this study, all samples (case and control) were collected at Fars Heart Central Hospital. All participants were Caucasian and residents of Fars Province. The criteria for including patients in the sample group were; 1) Age above 18 years old, 2) Persian ethnicity and Caucasian race, 3) Patients in the sample group should have at least one history of Myocardial Infarction, 4) In patients with two incidences of Myocardial Infarction, the interval of two Myocardial Infarctions should be at least 3 months, 5) Patients with two incidences of Myocardial Infarction should have received regular doses (20 mg/day) of atorvastatin between the two Myocardial Infarctions. Patients without any of these criteria were excluded from the sample group. For the control group, individuals should have had no previous histories of Myocardial Infarction and fulfill the criteria 1 and 2.

For this purpose, 5 mL of peripheral whole blood was collected in the presence of EDTA (Ethylene Di-amine Tetra Acetic Acid) (2 mg/mL) from 100 patients and 100 controls.

All patients and controls underwent genetic counseling and genetic testing which was performed after obtaining informed consent.

Genomic DNA was extracted using the cinnapure DNA extraction kit according to manufacturer's instructions and stored at -20°C until use.

Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) was used to genotype the patients and normal controls. Table 1 shows PCR primers and the condition used in this study. The PCR for the DNA fragment was carried out by ABI thermocycler in a total volume of 50 µL containing 3 µL genomic DNA (150 ng), 1 µL of each primer (20 pmol/µL), 0.5 µL dNTPs (10 mM), 1.5 µL $MgCl_2$ (50 mM), 5 µL PCR buffer 0.5 µL of Taq polymerase (5 U/µL, Cinnagen), and 37.5 µL H_2O. The PCR amplification was 95°C for 5 min, followed by 35 cycles of 94°C for 30 s, 62°C for 30 s, 72°C for 30 s, and further

final extension step at 72°C for 7 min. PCR amplified a fragment of 252 bp containing HPYCH4 restriction site and then the PCR products were purified by ethanol precipitation and digested using HPYCH4 (Bst4c1, Vivantis Cat.no RE1204) according to the conditions recommended by the manufacturer (Vivantis). The enzyme digestion reaction was performed in a total volume of 15 µL containing 11.5 µL of the purified PCR product, 2 µL diluted enzyme (Vivantis, Malaysia), and 1.5 µL buffer V (Vivantis) and incubated at 65°C for 1 hr. The reaction was then visualized on 2% agarose gel containing ethidium bromide. The possible genotype expected from RFLP PCR is shown in Table 1. All experiments were carried out at Medical Genetics Department, Shiraz University of Medical sciences and the data obtained from questionnaires and genetic testing were analyzed by SPSS statistical software version 19.0.

Statistical analysis

For sample collection, sex and age were matched by group matching method between the sample and control groups. For matching, sex variant was analyzed by Chi-square and age variant was analyzed by *t*-test. According to the data obtained, the average age was 57.8 ± 13.6 years in the control group and 59.7 ± 11.3 years in the case group. *t*-test showed that there is no significant difference between the two groups ($P = 0.284$). Moreover, there were 66 (66%) men in the control group and 62 (62%) men in the case group which, according to Fisher test's results, no significant difference were observed ($P = 0.65$).

The variables of smoking, family history of MI and also variables influenced by other diseases such as diabetes and kidney problems were analyzed between the case and control groups by Chi-square. Logistic regression models were used to assess the association between genotype and risk of MI and also the effect of statin therapy on patients. *P* values (*P*) less than 0.05 were considered to be statistically significant. All data are presented as mean ± SD or number (percentages).

Results

All information related to cases and controls from southern Iran used in the current study are shown in Table 2. According to the data obtained, the average age is 57.8 ± 13.6 years in the control group and 59.7 ± 11.3 years in the case group and the results of *t*-test showed that there is no significant difference between the two groups ($P = 0.284$). Also, for sex variable, there are 66 (66%) men in the control group and 62 (62%) men in the case group which, according to Fisher test's results, there is no significant difference between the two groups ($P = 0.65$). Thus, these results give us confidence that matching was done properly.

Several risk factors for Coronary Heart Disease such as smoking, history of diabetes, history of kidney and family history of Myocardial Infarction (MI) were higher in cases in comparison with controls; in fact, smoking and history of diabetes variables have significant differences between the case and control groups (Table 2).

PCR-RFLP was carried out for 100 cases with Coronary Heart Disease and 100 controls. Following Bst4CI restriction enzyme digestion, 252bp, 156bp, and 96bp bands were obtained from heterozygous carrier individuals and also 156bp and 96bp bands from individual with homozygous mutated allele (Arg/Arg,CGG/CGG). A 252bp band was observed in individuals with wild type allele (Trp/Trp, TGG/TGG) following enzyme digestion (Figure 2).

According to our data, the *KIF6* 719 Arg polymorphism in one or both alleles of the chromosome were seen more in patients comparison with non-controls. The odds of CHD in carriers (Arg/Arg + Arg/Trp) was 3.17 times higher compared to non-carriers (Trp/Trp). Moreover, the Trp/Arg heterozygotes had 3.2 times more likelihood to have CHD than Trp/Trp homozygotes and also the odds of CHD in Arg/Arg homozygotes was 3.06 times higher than non-carriers (Trp/Trp) (Table 3).

Among the carriers patients, the odds of second heart attack was 83% lower than non-carrier patients (OR = 0.17, 95% CI: 0.08 – 0.41). On the other hand, 80% of patients with rs20455 polymorphism (carrier patients) had only one time MI and also 60% of non-carriers patients had two times MI during statin therapy (atorvastatin, 20 mg/day) (Table 4).

Discussion

Cardiovascular diseases (CVD) have become a major health concern worldwide, especially in developing countries. The incidence of the disease is growing and several studies have focused on the genetic characteristics that are related to heart disease. Various research have been done on genes such as *ITGA2*, *THBS4*, *SERPINE1*, *LTA*, and *F2* which are related to MI, and *APOE* related to CHD and MI.[26-36] SNPs in some genes are shown to have an impact on the progression of the disease and response to treatment. SNPs have been investigated in these genes and shown to be related to the heart disease. For example, in a research done by Santoso, *et al.*[26] and Antoniades, *et al.*[27] the impact of re1126643 was investigated in the *ITGA2* gene and shown to be related to nonfatal MI in younger population. Topol, *et al.*[28] and Wessel, *et al.*[29] showed that rs1866389 SNP in the *THBS4* gene causes premature MI and a research by Eriksson, *et al.*[30] and Margaglione, *et al.*[31] investigated the rs1799768 in the *SERPINE1* gene and showed that this SNP is related to MI and high plasminogen Activator Inhibit-1 activity. Rs1799963 SNP in *F2* gene is related to MI in young women.[32,33] SNPs in *APOE*, for example rs429358 and rs405509, have also been shown to have an impact on the CHD [34,35] and MI.[36]

In the recent years, *KIF6* gene's SNPs are shown to have a great impact on CHD.[37] SNPs in *KIF6* gene have been investigated in CARE clinical trial and WOSCOPS clinical trial and were proven to affect the course of treatment of MI and CHD. It has been suggested that SNPs in *KIF6* gene greatly affect the course of MI treatment.[15] Thus, in this research we have aimed to investigate the effect of SNPs in *KIF6* gene.

The main finding of this case-control study was that the *KIF6* 719Arg polymorphism was seen more in patients suffering from CHD when compared to the control group, which can suggest that this polymorphism could have an effect on development of CHD.

A thymine (T) to cytosine (C) transition at position 39325078 of *KIF6* gene results in an altered amino acid at position 719 in the Kinesin protein. In fact, the substitution of a non-polar amino acid (tryptophan) with basic amino acid (arginine) in the tail region affects the protein mobility and activity.[25] Several SNPs were useful in the treatment strategies, for instance, some studies suggested that *KIF6* 719 Arg carriers respond better to the Statin treatment than non-carriers by 83%. This was in alliance with results obtained by other researchers[37] who found that pravastatin therapy (40 mg/day) reduces the risk of cardiovascular disease by 37%,

50% and 34% in carriers of the rs20455 SNP in the *KIF6* gene.

Our findings from the *KIF6* test can be useful for recognizing individuals at a higher risk of CHD and also individuals who can benefit from statins therapies.

Consequently, in our subjects collected from southern Iran, it was found that smokers and diabetic individuals were at a higher risk of heart attack. Also, we found that *KIF6* 719Arg polymorphism is seen more in patients with CHD in comparison with non-carriers and CHD reduction in individuals with this SNP during statin therapy in the studied population of southern Iran.

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References

1. WHO. Available form: URL: http://www.who.int/mediacentre/factsheets/fs317/en/.
2. Motlagh B, O'Donnell M, Yusuf S. Prevalence of cardiovascular risk factors in the Middle East: a systematic review. *Eur J Cardiovasc Prev Rehabil.* 2009; 16(3): 268-80.
3. Lopez AD. Global burden of disease and risk factors [electronic resource]: Oxford University Press, World Bank; 2006.
4. Murray CJ, Lopez AD. Mortality by cause for eight regions of the world: Global Burden of Disease Study. *Lancet.* 1997; 349(9061): 1269-76.
5. Alwan A. Global status report on noncommunicable diseases 2010: World Health Organization; 2011.
6. Organization WH. Global tuberculosis control: WHO report 2010: World Health Organization; 2010.
7. Mendis S, Puska P, Norrving B. Global atlas on cardiovascular disease prevention and control: World Health Organization; 2011.
8. Allingham-Hawkins D, Lea A, Levine S. *KIF6* p.Trp719Arg Testing to Assess Risk of Coronary Artery Disease and/or Statin Response. *PLoS Curr.* 2010; 2: RRN1191.
9. Kannel WB, Dawber TR, Kagan A, Revotskie N, Stokes J, 3rd. Factors of risk in the development of coronary heart disease--six year follow-up experience. The Framingham Study. *Ann Intern Med.* 1961; 55: 33-50.
10. NCBI. Available form: URL: http://www.nhlbi.nih.gov/health/health-topics/topics/atherosclerosis.
11. (DHHS). USDoHHS. National Heart, Lung and Blood Institute. September 18, 2010.
12. Tell GS, Fried LP, Hermanson B, Manolio TA, Newman AB, Borhani NO. Recruitment of adults 65 years and older as participants in the Cardiovascular Health Study. *Ann Epidemiol.* 1993; 3(4): 358-66.
13. Fried LP, Borhani NO, Enright P, Furberg CD, Gardin JM, Kronmal RA, et al. The Cardiovascular Health Study: design and rationale. *Ann Epidemiol.* 1991; 1(3): 263-76.
14. Shiffman D, Chasman DI, Zee RY, Iakoubova OA, Louie JZ, Devlin JJ, et al. A kinesin family member 6 variant is associated with coronary heart disease in the Women's Health Study. *J Am Coll Cardiol.* 2008; 51(4): 444-8.
15. Iakoubova OA, Tong CH, Rowland CM, Kirchgessner TG, Young BA, Arellano AR, et al. Association of the Trp719Arg polymorphism in kinesin-like protein 6 with myocardial infarction and coronary heart disease in 2 prospective trials: the CARE and WOSCOPS trials. *J Am Coll Cardiol.* 2008; 51(4): 435-43.
16. Bare LA, Morrison AC, Rowland CM, Shiffman D, Luke MM, Iakoubova OA, et al. Five common gene variants identify elevated genetic risk for coronary heart disease. *Genet Med.* 2007; 9(10): 682-9.
17. Iakoubova OA, Robertson M, Tong CH, Rowland CM, Catanese JJ, Blauw GJ, et al. *KIF6* Trp719Arg polymorphism and the effect of statin therapy in elderly patients: results from the PROSPER study. *Eur J Cardiovasc Prev Rehabil.* 2010; 17(4): 455-61.
18. Shiffman D, Sabatine MS, Louie JZ, Kirchgessner TG, Iakoubova OA, Campos H, et al. Effect of pravastatin therapy on coronary events in carriers of the *KIF6* 719Arg allele from the cholesterol and recurrent events trial. *Am J Cardiol.* 2010; 105(9): 1300-5.
19. Rosenfeld SS, Fordyce PM, Jefferson GM, King PH, Block SM. Stepping and stretching. How kinesin uses internal strain to walk processively. *J Biol Chem.* 2003; 278(20): 18550-6.
20. Brendza RP, Serbus LR, Duffy JB, Saxton WM. A function for kinesin I in the posterior transport of oskar mRNA and Staufen protein. *Science.* 2000; 289(5487): 2120-2.
21. Hollenbeck PJ, Swanson JA. Radial extension of macrophage tubular lysosomes supported by kinesin. *Nature.* 1990; 346(6287): 864-6.
22. Nakata T, Hirokawa N. Point mutation of adenosine triphosphate-binding motif generated rigor kinesin that selectively blocks anterograde lysosome membrane transport. *J Cell Biol.* 1995; 131(4): 1039-53.
23. Tanaka Y, Kanai Y, Okada Y, Nonaka S, Takeda S, Harada A, et al. Targeted disruption of mouse conventional kinesin heavy chain, kif5B, results in abnormal perinuclear clustering of mitochondria. *Cell.* 1998; 93(7): 1147-58.
24. Terada S, Kinjo M, Hirokawa N. Oligomeric tubulin in large transporting complex is transported via kinesin in squid giant axons. *Cell.* 2000; 103(1): 141-55.
25. Marked C. *KIF6* Genotyping Assay. Available from: URL: www.abbottmolecular.com/products/genetics/realtime-pcr/*kif6*-genotyping-assay.html.
26. Santoso S, Kunicki T, Kroll H, Haberbosch W, Gardemann A. Association of the platelet glycoprotein Ia C807T gene polymorphism with nonfatal myocardial infarction in younger patients. *Blood.* 1999; 93(8): 2449-53.
27. Antoniades C, Tousoulis D, Vasiliadou C, Stefanadi E, Marinou K, Stefanadis C. Genetic polymorphisms of platelet glycoprotein Ia and the risk for premature myocardial infarction: effects on the release of sCD40L during the acute phase of premature myocardial infarction. *J Am Coll Cardiol.* 2006; 47(10): 1959-66.
28. Topol EJ, McCarthy J, Gabriel S, Moliterno DJ, Rogers WJ, Newby LK, et al. Single nucleotide polymorphisms in multiple novel thrombospondin genes may be associated with familial premature myocardial infarction. *Circulation.* 2001; 104(22): 2641-4.
29. Wessel J, Topol EJ, Ji M, Meyer J, McCarthy JJ. Replication of the association between the thrombospondin-4 A387P polymorphism and myocardial infarction. *Am Heart J.* 2004; 147(5): 905-9.
30. Eriksson P, Kallin B, Van't Hooft FM, Båvenholm P, Hamsten A. Allele-specific increase in basal transcription of the plasminogen-activator inhibitor 1 gene is associated with myocardial infarction. *Proc Natl Acad Sci U S A.* 1995; 92(6): 1851-5.
31. Margaglione M, Cappucci G, Colaizzo D, Giuliani N, Vecchione G, Grandone E, et al. The PAI-1 gene locus 4G/5G polymorphism is associated with a family history of coronary artery disease. *Arterioscler Thromb Vasc Biol.* 1998; 18(2): 152-6.
32. Rosendaal F, Siscovick D, Schwartz S, Psaty B, Raghunathan T, Vos H. A common prothrombin variant (20210 G to A) increases the risk of myocardial infarction in young women. *Blood.* 1997; 90(5): 1747-50.
33. Doggen CJ, Cats VM, Bertina RM, Rosendaal FR. Interaction of coagulation defects and cardiovascular risk factors increased risk of myocardial infarction associated with factor V Leiden or prothrombin 20210A. *Circulation.* 1998; 97(11): 1037-41.
34. Ye S, Dunleavey L, Bannister W, Day LB, Tapper W, Collins AR, et al. Independent effects of the− 219 G> T and ε 2/ε 3/ε 4 polymorphisms in the apolipoprotein E gene on coronary artery disease: The Southampton Atherosclerosis Study. *Eur J Hum Genet.* 2003; 11(6): 437-43.
35. Humphries SE, Talmud PJ, Hawe E, Bolla M, Day IN, Miller GJ. Apolipoprotein E4 and coronary heart disease in middle-aged men who smoke: a prospective study. *The Lancet.* 2001; 358(9276): 115-9.
36. Lambert J-C, Brousseau T, Defosse V, Evans A, Arveiler D, Ruidavets J-B, et al. Independent association of an APOE gene promoter polymorphism with increased risk of myocardial infarction and decreased APOE plasma concentrations—the ECTIM study. *Hum Mol Genet.* 2000; 9(1): 57-61.
37. Pera Ł, Białek S, Sygitowicz G, Marszałek A, Sitkiewicz D. *KIF6* rs20455 polymorphism as a genetic risk factor of coronary heart disease: a systematic review. *Diagnostyka.* 2012; 48(3): 271-7.