Acta Scientific NUTRITIONAL HEALTH (ISSN:2582-1423)

Volume 4 Issue 8 August 2020

Research Article

ACTA2 Mutations and Risk of Premature Myocardial Infarction

Fatemeh Hajibabaie[1,2], Mohammad Hossein Modarressi[3], Masoumeh Sadeghi[4]* and Laleh Shariati[5,6]*

[1]*Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran*

[2]*Applied Physiology Research Center, Cardiovascular Research Institute, Isfahan University of Medical Sciences, Isfahan, Iran*

[3]*Department of Medical Genetics, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran*

[4]*Cardiac Rehabilitation Research Center, Isfahan Cardiovascular Research Institute, Isfahan University of Medical Sciences, Isfahan, Iran*

[5]*Isfahan Cardiovascular Research Center, Cardiovascular Research Institute, Isfahan University of Medical Sciences, Isfahan, Iran*

[6]*Department of Biomaterials, Nanotechnology and Tissue Engineering, School of Advanced Technologies in Medicine, Isfahan University of Medical Sciences, Isfahan, Iran*

***Corresponding Author:** Masoumeh Sadeghi, Cardiac Rehabilitation Research Center, Isfahan Cardiovascular Research Institute, Isfahan University of Medical Sciences, Isfahan, Iran and Laleh Shariati, Isfahan Cardiovascular Research Center, Cardiovascular Research Institute, Isfahan University of Medical Sciences and Cardiac Rehabilitation Research Center, Isfahan Cardiovascular Research Institute, Isfahan University of Medical Sciences, Isfahan, Iran.

Received: July 07, 2020

Published: July 30, 2020

Abstract

Introduction: Premature myocardial infarction (MI) is characterized by high re-infarction rates, the occurrence of severe heart failure and therefore significant cardiovascular long-term mortality at a young age. Previous studies demonstrated that one of the most important risk factors in younger patients is family history and genetic factors. This research was launched to assess the association of *ACTA2* genetic variations with premature MI.

Materials and Methods: Out of eighty unrelated patients with premature MI referred to health center, patients with autosomal dominant premature MI were included in the study. Exclusion criteria included hypercholesterolemia, hyperlipidemia, diabetes and smoking. Genomic DNA was extracted from the whole peripheral blood. Eight exons and intron/exon boundaries of the *ACTA2* gene were amplified, and all the amplicons were subject to Sanger sequencing.

Results: According to the criteria, 16 patients were included in our research. No mutations were found in *ACTA2* gene in our probands.

Keyword: Premature Myocardial Infarction; *ACTA2*; Pathogenesis

Abbreviations

MI: Myocardial Infarction; CVD: Cardiovascular Disease; NCDs: Non-communicable Diseases; GWAS; Genome-Wide Association Studies; SMC: Smooth Muscle Cell; TAAD: Thoracic Aortic Aneurysm and Dissection; CGH: Comparative Genomic Hybridization; MI: Myocardial Infarction

Introduction

Cardiovascular disease (CVD) is the leading cause of mortality worldwide [1-4], accounted for an estimated 44% of all global deaths due to non-communicable diseases (NCDs) in 2018. In Iran, CVDs are the most common causes of death [5]. Myocardial infarction (MI), as a heart attack happens when blood flow stops or decreases in one part of the heart, causing injury to the muscle of the heart [6,7]. Premature MI is characterized by high rates of re-infarction, severe heart failure incidence, and significant CV mortality in < 50 years old. The prevalence of AMI in Iran is high and has increased in recent years [5]. Premature MI prevalence was high in men, and smoking was the most common risk factor among young people [5]. The latest studies revealed that genetics and family history are major risk factors at a young age. Previous studies showed several genes are involved in the premature MI. Twenty-seven genetic variants have been found using Genome-wide association studies (GWAS) that are related to increased risk of MI. There are some genes associating with MI including *PCSK9, SORT1, MIA3, WDR12, MRAS, PHACTR1, LPA, TCF21, MTHFDSL, ZC3HC1, CDKN2A, 2B, ABO, PDGF0, APOA5, MNF1ASM283, COL4A1, HHIPC1, SMAD3, ADAMTS7, RAS1, SMG6, SNF8, LDLR, SLC5A3, MRPS6, and KCNE2.*

The vascular smooth muscle cell (SMC)-specific isoform of a-actin (*ACTA2*) is a main element of the contractile apparatus in SMCs, placed in the artery system [8-10]. The contraction, in response to the tension induced by pulsatile blood flow, is the main function of SMC [8].

Recent studies showed that there is a mutation in the *ACTA2* and β-myosin heavy chain (*MYH11*) genes in families with thoracic aortic aneurysm and dissection (TAAD). These genes encode the SMC contractile protein that acts through ATP-fueled cyclic interaction of the myosin motor with actin filaments [11-13].

Early-onset coronary artery disease (CAD), TAA, stroke, aortic dissections, and moyamoya disease, as diverse and diffuse vascular diseases, are due to heterozygous missense mutations in the *ACTA2* [8,12], but fifty percent of the mutation carriers have an aortic disease, and the rate of penetrance is lower than that detected in genes, causing familial TAAD [8].

The *ACTA2* mutations cause 14% of non-syndromic TAAD [12] and reports show that those variants are responsible for premature CAD [12,14]. Significant genetic heterogeneity in CVD and their interactions with environmental and other factors, contribute to the disease pathogenesis, including diabetes mellitus, smoker, obesity, and hypertensive [15], leading to slow progress in the identification of vascular disease genes [16].

Aim of the Study

The aim of this study was to determine the *ACTA2* mutations in patients with early MI, referred to as Isfahan Health Centers. Determination of the role of *ACTA2* gene mutations in the development of premature myocardial infarction. Help diagnose, determine the status of the carrier, the carrier of genetic counseling and prevention in future generations.

Materials and Methods

Family characterization and sample collection

This investigation was approved by the Ethics Committee of the National Ethics Committee for Biomedical Research, Islamic Azad University, Science and Research Branch (Approval ID: IR.IAU.SRB.REC.1397.106).

Eighty unrelated families with premature MI were recruited and characterized referred to Chamran hospital, Isfahan. Premature MI is when MI occurs in men at age 55 or earlier and in women at age 65 or earlier. Demographic data and medical records related to vascular diseases and risk factors were obtained from all family members. MI was validated based on documentation of a myocardial infarct or 75% narrowing of one or more main coronary arteries distinguished during cardiac catheterization. The patients who had a positive family history, including personal history, clinical examination, and family history for premature MI with autosomal dominant inheritance, were recruited. Pedigree data was drawing by Cyrillic.210 software (Cherwell. CO, Scientific publishing, USA) (Figure 1). Serum total cholesterol (TC) and high-density lipoprotein cholesterol (HDLC), as well as triglycerides (TG), were measured enzymatically. Diabetes mellitus, a chronic illness characterized by hyperglycemia, is typically diagnosed when fasting plasma glucose levels exceed 125 mg/dL. The patients with hypercholesterolemia, hyperlipidemia, diabetes, and smoking habit were excluded from the study. After signing an informed consent form, about 5 mL of peripheral blood was collected in tubes containing EDTA (0.5M) from all patients.

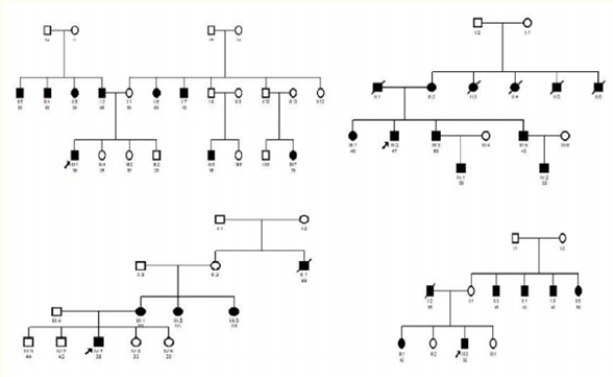

Figure 1: The autosomal dominant pedigree pattern in probands.

DNA extraction

DNA extraction was performed using a DNA extraction kit (DENAGIR, Tehran, Iran). The concentration and quality of DNA were checked with the Nano-drop spectrophotometer (Thermo Scientific) and agarose gel electrophoresis (Figure 2).

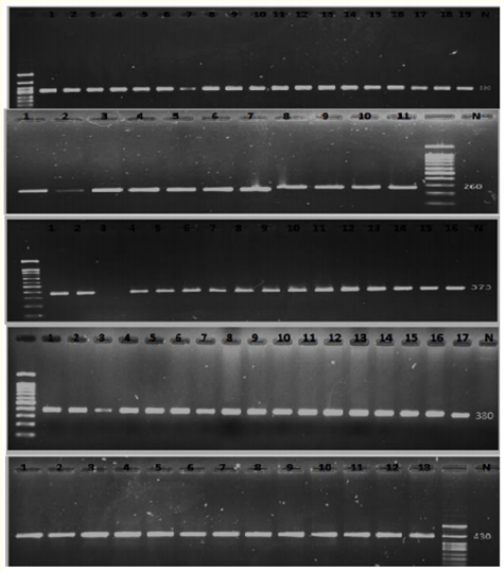

Figure 2: Agarose gel electrophoresis of exons *ACTA2* gene.

Mutation screening of *ACTA2*

The primers were designed using Oligo (version 6.7.1.0 National Biosciences Inc.) for all exons of *ACTA2* gene to include exon-intron boundaries (Table 1). The exons were amplified using PCR Master Mix (Biofact, Daejeon, Korea) by standard PCR programs. PCR products were investigated using agarose gel electrophoresis and sequenced bi-directionally using ABI 3130XL automated sequencer (Applied Biosystems, Foster City, California, USA). After that, sequences were matched with the reference genomic sequence NG_011541.1 for variant detection using the SnapGene software (version® 3.2.1 GSL Biotech).

Exon Number	Primer		TM (°C)	Template Size (bp)
Exon2	Forward	GGTGGGAGTTGTCAGGTAAG	60	390
	Reverse	GGTTACATAACTTCTGGGCAG	59.5	
Exon3	Forward	TGTGGCTTGGCTGTAATTG	58	395
	Reverse	AGTTGAGCAATGTGAGCCAG	59	
Exon4	Forward	AGCTTCTGGTCCCTTTTTG	55.2	265
	Reverse	GTGCTGCATAGCCTCCTTC	59.5	
Exon5	Forward	TCAACCAGGTGTGCTCTCC	59.5	260
	Reverse	CCACGTGTTAACGACCATTC	58.4	
Exon6	Forward	AGGCTTCCCTCTACTTGTCC	60.5	320
	Reverse	TCCTTGGATAGTGAGGATGG	58.4	
Exon7	Forward	TCTTGAGGGAGAGACTGCAG	60.5	373
	Reverse	AACCGTCACTTGTCTCCATG	58.4	
Exon8	Forward	TCCACTGCAAGAATCATCCAG	58.4	380
	Reverse	CCCACAATTGCATGTCACC	57.3	
Exon9	Forward	ATGGTAGAACATCCAGGCTC	58.4	430
	Reverse	AATTGCCATGTGCTCAGC	53.9	

Table 1: Primer sequences for PCR.

Results

Smooth muscle cells (SMCs) contract to perform many physiological functions, including regulation of blood flow and pressure in arteries, contraction of the pupils, peristalsis of the gut, and voiding of the bladder [10]. Previous studies reported mutations in *ACTA2* gene causes a syndrome characterized by dysfunction of SMCs throughout the body, leading to Aortic and Cerebrovascular disease, Fixed dilated pupils, Hypotonic Bladder, Malrotation, and hypoperistalsis of the gut and pulmonary hypertension. According to the criteria, 16 pro-bands with premature MI were selected, and DNA was extracted. After amplification, all of 16 samples showed satisfactory results based on the results of Nano-drop spectrophotometer and agarose gel electrophoresis (Figure 2).

The pathological and demographic features of the samples are shown (Table 2). All of the patients had a positive family history.

Demographic			
Age	Male	(<45 years old)	
	Female	(<50years old)	
Sex	Male	(n = 60)	Total = 80
	Female	(n = 20)	
Patient history			
Family history of CAD		91.25%	
History of hypertention		6.25%	
Diabet mellitus		32.5%	
Atherosclerosis		43.75%	
Prior cardiac surgery (any)		93.75%	
Hypercholesterolemia / hyperlipidemia		22.5%	
Smoking		75%	
Obesity		3%	

Table 2: History and Demographics of Patients With initial and recurrent myocardial infarction.

Sequencing results for the *ACTA2* gene

Sequencing analysis of 8 exons and splice junctions of the *ACTA2* gene did not reveal any pathogenic variants (Figure 3).

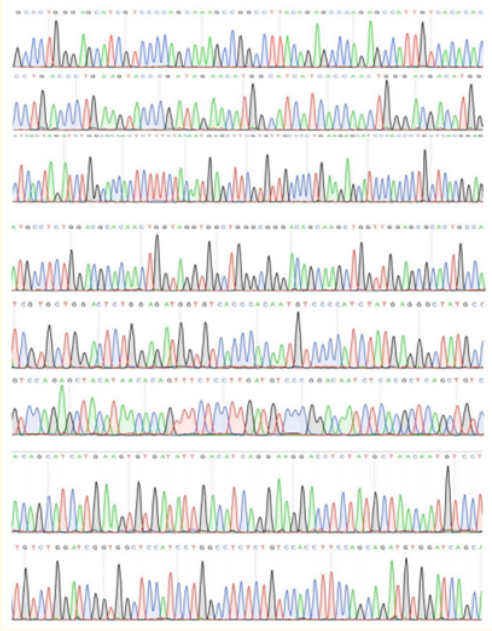

Figure 3: Sequencing analysis of 8 exons and splice junctions of the *ACTA2* gene.

Discussion

Non-communicable diseases kill 41 million people each year, equivalent to 71% of all deaths globally. CVDs as the heart and blood vessel disorders include coronary heart, rheumatic heart and cerebrovascular disease, accounted for an estimated 44% of all global deaths due to non-communicable diseases (NCDs) in 2018 [6]. The first study at the University of Texas Health Science Center in 2007 Families with multiple members with TAAD who did not have a known genetic syndrome was recruited carried out a mutational analysis of *ACTA2* gene by bidirectional direct sequencing of amplified genomic DNA fragments with intron-based, exon specific primers. N117T and R118Q in exon 4, R149C and Y135H in exon 5, V154A in exon 6, R258H and R258C in exon 7, R292G in exon 8 and T353N in exon 9 identified in *ACTA2* gene as pathogenic mutation [12].

Guo., *et al*. also in 2009, identified *ACTA2* gene mutations including P72Q in exon 3, G160D in exon 6, R39H in exon 2, R118Q in

exon 4, R185Q in exon 6, R149C in exon 5, R258C and R258H in exon 7, in related with Coronary Artery Disease, Stroke, and Moyamoya Disease, Along with Thoracic Aortic Disease [8]. However, they suggested Heterozygous missense mutations in *ACTA2* cause diffuse and diverse vascular diseases, including thoracic aortic aneurysms, aortic dissections, early-onset coronary artery disease, stroke, and Moyamoya disease, but only half of the mutation carriers have Aortic disease, a penetrance lower than that typically observed for genes leading to familial TAAD [8,12].

Also in another study, Morisaki., *et al*. identified three mutations in *ACTA2* gene, which two novel p.G152_T205del and p.R212Q, and a novel mutation Y145C of *ACTA2* as sporadic mutation, each of which is considered to be causative for TAAD [15]. Three novel mutations in the *ACTA2* gene identified in German patients with thoracic aortic aneurysms and dissections in 2011. Two mutations affect residues within (M49V) or adjacent to (R39C), the DNase-I-binding loop within subdomain 2 of alpha-actin. They were observed in families with recurrent aortic aneurysm (R39C) or aortic dissection (M49V). The third mutation causes an exchange in the vicinity of the ATP-binding site (G304R) in a patient thought to have isolated TAAD [17]. Munot., *et al*. showed a novel distinctive cerebrovascular phenotype is associated with heterozygous Arg179 *ACTA2* mutations [18]. Five mutations in the familial TAAD group in other study were identified that were absent in controls. The known p.Arg149Cys and the novel p.Asp82Glu, p.Glu243Lys, and p.Val45Leu mutations affected evolutionarily conserved residues. The IVS4+1G>A mutation was novel [19]. Tortoraa., *et al*. recruited 20 patients who underwent surgery for BAV and TAA; Clinical genetic evaluation and *ACTA2* mutation analysis were performed on each patient, along with next-generation sequencing analysis of BAV-related genes [27]. No mutations were found in *ACTA2* or in BAV-related genes in pro-bands nor any common clinical signs possibly related to their heart disease. Results of previous studies *ACTA2* mutations were found in 14-21% of familial cases, but only 2.5 - 3.8% of sporadic TAAD [15]. None of the mutations identified is fully penetrant for any of these vascular diseases [10].

In none of the previous studies, did not say anything about the relation between *ACTA2* mutations and premature mi, directly.

Risk factors of cardiovascular diseases included hypercholesterolemia, hyperlipidemia, diabetes, and smoking. The generation of higher cholesterol blood levels is proposed as a possible link between chronic periodontal inflammation and Atherosclerosis [20]. Smoking enhances the risk of Atherosclerosis, like Ischemic stroke and Myocardial infarction [21]. Type 2 diabetes exacerbates mechanisms underlying Atherosclerosis and heart failure [22]. Previous contrary studies, in our study, patients with hypercholesterolemia, hyperlipidemia, diabetes, and smoking habit were excluded from genetic examination. Epigenetic mechanisms, including CpG sites methylation, histone modification, and miRNA-mediated gene regulation, take apart in controlling gene expression of common diseases such as Atherosclerosis [23]. MicroRNAs are a vital player in a different range of biological processes; thus, their expression level is regulated in pathophysiological conditions [23]. Accumulating evidence has revealed microRNAs as fine-tuners of gene expression, regulation of signaling, and lipid homeostasis pathways which could affect atherosclerotic plaque destiny [24]. Overexpression of microRNA-21 (miR-21) decreases myocardial infarction area [25], and miR-1 and mir-206 are up-regulated in myocardial infarction [26], with miR-1 also being a potential biomarker for acute myocardial infarction [27]. miR-29 is a repressor of myocardial fibrosis after infarction, as well as miR-24, which modulates myocardial fibroblast functions after infarction via the furin-TGF-β pathway [28]. MiR-208b was down-regulated in hearts of model rats ($P < 0.01$). Overexpressing miR-208b improved myocardial functions, such as reducing the infarction area ($P < 0.05$) and promoting LVEF and LVFS ($P < 0.01$), and inhibited COL1 and ACTA2 ($P < 0.01$). Luciferase reporter assay proved *Gata4* to be the direct target of miR-208b, with the target sequence in the 3′UTR. Inhibiting *GATA4* resulted in the down-regulation of *COL1* and *ACTA2*, suggesting that the role of miR-208b was achieved via regulating *GATA4* [27]. In a recent study, the comparison of DNA methylation profile of atherosclerotic and healthy individuals revealed different miRNAs expression patterns while at the same time, the similarity of miRNA cluster expression recognized among the same tissues of different subjects [28]. A substantial portion of heritable atherosclerosis risk has not been identified by established approaches that, in general, leave a troublesome gap between genetics and clinical phenotype. The abnormal gene expression pattern may comprise associated with a heritable risk of cardiovascular disease such as premature MI.

Nevertheless, there is no biomarker to predict the type of complications that CV patients may develop, such as familial cases of MI. Detection of gene mutations and other biomarkers [2], in families with CAD leads to earlier diagnosis of premature MI in high-risk members of the family. Deep insight into the molecular mech-

anisms of disease can help in personalizing and optimizing the follow-up for individual patients [30], besides, reduces the health-related costs [31]. The most mutated gene in the familial TAAD is the *ACTA2* that may be related to premature MI [32]; therefore, this investigation was designed to determine if the *ACTA2* variants do contribute significantly to the common phenotype of premature MI in a cohort of 16 consecutive premature MI patients our data did not any change in pathogenic variant in the *ACTA2* in 16 premature MI prob-ands.

Leisure development made in recognizing vascular disease genes is felt to be due to significant genetic heterogeneity in these diseases, along with the complex interactions between genes and environmental factors that contribute to the disease pathogenesis, including smoker, diabetes mellitus, obesity, and hypertensive [16].

Perhaps, the presence of pathogenic variations in the promoter regions of the *ACTA2* has been associated with early MI in patients residing in Isfahan, while other areas have not been involved in this study.

The different effects of the environment on the genes in different ethnic groups, which are indicative of epigenetic issues, and other genes involved in the pathogenesis of premature MI also influence the pathogenesis of this disease.

Conclusion

Based on the high prevalence of premature MI in our population, and reports on *ACTA2*-related familial TAAD, and only very rare mutation-positive individuals presented with premature MI, the results of this pilot study seems to justify the conclusion that the *ACTA2* does not play a significant role in the pathogenesis of premature MI. The genetic mechanism involved in the pathogenesis of CAD remains to be explored. The promising tools to identify the novel causative genes are array comparative genomic hybridization (CGH) and exome sequencing or whole-genome sequencing.

Conflict of Interest

This investigation was approved by the Ethics Committee of the National Ethics Committee for Biomedical Research, Islamic Azad University, Science and Research Branch (Approval ID: IR.IAU.SRB.REC.1397.106). This article does not contain any studies with human participants or animals performed by any of the authors.

Funding

There was no funding to support this study.

Acknowledgements

We thank our colleagues for their association and helpful discussions in this study.

Bibliography

1. Benjamin EJ., *et al*. "Heart Disease and Stroke Statistics-2017 Update: A Report From the American Heart Association". *Circulation* 135.10 (2017): e146-e603.
2. Hajibabaie F., *et al*. "MicroRNAs as the actors in the atherosclerosis scenario". *Journal of Physiology and Biochemistry* (2019).
3. Dai X., *et al*. "Genetics of coronary artery disease and myocardial infarction". *World Journal of Cardiology* 8.1 (2016): 1-23.
4. Zipes D., *et al*. "Braunwald Eugene Braunwald's Heart Disease: A Text book of Cardiovascular Medicine". Philadelphia (2005).
5. Mohseni J., *et al*. "A Systematic Review on the Prevalence of Acute Myocardial Infarction in Iran". *Heart Views* 18.4 (2017): 125-32.
6. Organization WH. World health statistics 2018: monitoring health for the SDGs, sustainable development goals (2018).
7. Ayani S., *et al*. "Survey and classification of functional characteristics in neural network technique for the diagnosis of ischemic heart disease: A systematic review". *Journal of Isfahan Medical School* 35.460 (2018): 1830-1839.
8. Guo DC., *et al*. "Mutations in smooth muscle alpha-actin (ACTA2) cause coronary artery disease, stroke, and Moyamoya disease, along with thoracic aortic disease". *American Journal of Human Genetics* 84.5 (2009): 617-627.
9. Gabbiani G., *et al*. "Vascular smooth muscle cells differ from other smooth muscle cells: predominance of vimentin filaments and a specific alpha-type actin". *Proceedings of the National Academy of Sciences of the United States of America* 78.1 (1981): 298-302.
10. Milewicz DM., *et al*. "De novo ACTA2 mutation causes a novel syndrome of multisystemic smooth muscle dysfunction". *American Journal of Medical Genetics* 152A.10 (2010): 2437-2443.
11. Zhu L., *et al*. "Mutations in myosin heavy chain 11 cause a syndrome associating thoracic aortic aneurysm/aortic dissection and patent ductus arteriosus". *Nature Genetics* 38.3 (2006): 343-349.

12. Guo DC., *et al*. "Mutations in smooth muscle alpha-actin (ACTA2) lead to thoracic aortic aneurysms and dissections". *Nature Genetics* 39.12 (2007): 1488-1493.

13. Dillon PF., *et al*. "Myosin phosphorylation and the cross-bridge cycle in arterial smooth muscle". *Science* 211.4481 (1981): 495-497.

14. Morisaki H., *et al*. "Mutation of ACTA2 gene as an important cause of familial and nonfamilial nonsyndromatic thoracic aortic aneurysm and/or dissection (TAAD)". *Human Mutation* 30.10 (2009): 1406-1411.

15. Morisaki H., *et al*. "Mutation of ACTA2 gene as an important cause of familial and nonfamilial nonsyndromatic thoracic aortic aneurysm and/or dissection (TAAD)". *Human Mutation* 30.10 (2009): 1406-1411.

16. Wang Q. "Molecular genetics of coronary artery disease". *Current Opinion on Cardiology* 20.3 (2005): 182-188.

17. Hoffjan S., *et al*. "Three novel mutations in the ACTA2 gene in German patients with thoracic aortic aneurysms and dissections". *European Journal of Human Genetics* 19.5 (2011): 520-524.

18. Munot P., *et al*. "A novel distinctive cerebrovascular phenotype is associated with heterozygous Arg179 ACTA2 mutations". *Brain* 2012 135 (2012): 2506-2514.

19. Disabella E., *et al*. "Risk of dissection in thoracic aneurysms associated with mutations of smooth muscle alpha-actin 2 (ACTA2)". *Heart* 97.4 (2011): 321-326.

20. Katz J., *et al*. "On the association between hypercholesterolemia, cardiovascular disease and severe periodontal disease". *Journal of Clinical Periodontology* 28.9 (2001): 865-868.

21. Yokoyama Y., *et al*. "MicroRNAs as Potential Mediators for Cigarette Smoking Induced Atherosclerosis". *International Journal of Molecular Sciences* 19.4 (2018).

22. Low Wang CC., *et al*. "Clinical Update: Cardiovascular Disease in Diabetes Mellitus: Atherosclerotic Cardiovascular Disease and Heart Failure in Type 2 Diabetes Mellitus - Mechanisms, Management, and Clinical Considerations". *Circulation* 133.24 (2016): 2459-2502.

23. Hajibabaie F., *et al*. "MicroRNAs as the actors in the atherosclerosis scenario". *Journal of Physiology and Biochemistry* 76.1 (2020): 1-12.

24. Huang Y., *et al*. "Biological functions of microRNAs: a review". *Journal of Physiology and Biochemistry* 67.1 (2011): 129-139.

25. Dong S., *et al*. "MicroRNA expression signature and the role of microRNA-21 in the early phase of acute myocardial infarction". *Journal of Biological Chemistry* 284.43 (2009): 29514-29525.

26. Shan ZX., *et al*. "Upregulated expression of miR-1/miR-206 in a rat model of myocardial infarction". *Biochemical and Biophysical Research Communications* 381.4 (2009): 597-601.

27. Zhou C., *et al*. "MicroRNA-208b Alleviates Post-Infarction Myocardial Fibrosis in a Rat Model by Inhibiting GATA4". *Medical Science Monitor* 22 (2016): 1808-1816.

28. Dosenko VE., *et al*. "Mature and immature microRNA ratios in cultured rat cardiomyocytes during anoxia-reoxygenation". *Experimental and Clinical Cardiology* 17.2 (2012): 84-87.

29. Aavik E., *et al*. "Global DNA methylation analysis of human atherosclerotic plaques reveals extensive genomic hypomethylation and reactivation at imprinted locus 14q32 involving induction of a miRNA cluster". *European Heart Journal* 36.16 (2015): 993-1000.

30. Sadatian A. "Major protests and treatment of heart disease". Tehran: samat (2001).

31. Yusuf S., *et al*. "Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study". *Lancet* 364.9438 (2004): 937-952.

32. Tortora G., *et al*. "Search for genetic factors in bicuspid aortic valve disease: ACTA2 mutations do not play a major role". *Interactive CardioVascular and Thoracic Surgery* 25.5 (2017): 813-817.