Health Science Monitor
2024; 3(2): 84-89
Published online (http://hsm.umsu.ac.ir)

Original Article

Frequency of the R202Q mutation of *MEFV* gene in Iranian patients with premature coronary artery disease: a report from West Azerbaijan province of Iran

Morteza Bagheri [1,2,*], Kamal Khadem-Vatani [3], Isa Abdi Rad [2], MirHossein Seyed-Mohammadzad [3], Alireza Rostamzadeh [3], Behzad Rahimi [3], Negin Kavosi [1]

[1] *Student Research Committee, Urmia University of Medical Sciences, Urmia, Iran*

[2] *Cellular and Molecular Research Center, Cellular and Molecular Medicine Research Institute, Urmia University of Medical Sciences, Urmia, Iran*

[3] *Seyyed-al Shohada University Hospital, Urmia University of Medical Sciences, Urmia, Iran*

***Corresponding author:** Dr Morteza Bagheri, **Address**: Student Research Committee, Urmia University of Medical Sciences, Urmia, Iran, **Email**: mortezabagheri@umsu.ac.ir, **Tel**: +984431988000

Abstract

***Background & Aims*:** Premature coronary artery disease (CAD) is common in men and women under 45 and 55 years, respectively. It has been demonstrated that R202Q mutation of *MEFV* gene may increase the risk of cardiovascular disease in individuals with metabolic syndrome. The goal of the present investigation was to evaluate the frequency of *MEFV* gene mutation R202Q in exon 2 in Iranian patients with premature CAD (West Azerbaijan province of Iran).

***Materials & Methods*:** A total of 100 patients with premature CAD and 100 healthy individuals participated in this hospital-based study. Cases and controls were selected based on strict criteria, including a minimum of one documented angiography with at least 50% stenosis of the coronary artery. *Pvu*II based PCR-RFLP technique was used for the detection of R202Q mutation in the tested samples.

***Results*:** R202Q mutation was not found in any of the healthy controls; whereas 12 out of 100 patients with premature CAD were heterozygote for R202Q mutation (12%) (12% vs. 0%). Considering the heterozygosity of the R202Q mutation in the patients, the allele frequency was 0.06 (12 out of 200 chromosomes).

***Conclusion*:** Our results indicate that the R202Q mutation in the MEFV gene is frequent in patients with premature CAD. Further studies are necessary to analyze more details regarding variable expressivity or incomplete penetrance of R202Q mutation in the tested population.

***Keywords*:** Arg202Gln (605 G > A), MEFV Gene, Premature CAD, R202Q Mutation

Received 13 July 2023; accepted for publication 19 December 2023

Introduction

Premature coronary artery disease (CAD) is the most common heart disease, defined by an onset age of ≤ 45 years in males and ≤ 55 years in females; although cut-offs may vary between 45 and 65 years of age (1). Premature CAD occurs when the arteries that transport blood to the heart muscle are narrowed. These phenomena are under the control of plaque formation within the inner walls of the arteries during a process called "Atherosclerosis". The pathogenesis is the hardening and narrowing of the arteries, and consequently, hypoxemia of the myocardium that leads to chest pain, heart attack, stroke, and peripheral vascular disease (2).

Premature CAD has several genetic and environmental risk factors, which are different among individuals, families, and populations (3, 4). The role of *MEFV* gene mutations, tumor necrosis factor-alpha (TNFα) (3), TNF receptor type 1 and TNF receptor type 2 (5), IL-17 (10), IL-23, and TGF-β1 (6), as well as IL-18-137G/C SNP (rs187238) in patients with CAD have been studied (7).

Recent studies indicated that *MEFV* gene mutations are associated with CAD in patients over 50 years old (8). The most common *MEFV* gene mutations are M694V, M680I, M694I, and V726A in exon10 and E148Q and R202Q in exon 2 (9, 10). The E148Q and R202Q mutations were more frequent in patients with coronary heart disease than in healthy individuals (8). The R202Q (c.605G > A) mutation is a known disease-causing genetic factor in patients with Familial Mediterranean Fever (FMF) (11). It has been demonstrated that there is linkage disequilibrium between R202Q and M694V mutation (11). Basar et al. reported heterozygous and homozygous R202Q mutations in patients with premature CAD (8). Öztürk et al. revealed that Arg202Gln (605 G > A) (R202Q) alteration in the *MEFV* gene is a pathogenic variant (11).

It has been demonstrated that the R202Q mutation of the *MEFV* gene may increase the risk of metabolic syndrome (12). The association between metabolic syndrome and a high risk of cardiovascular disease in the general population is well defined (13).

The present study aimed to evaluate the frequency of 605 G > A (Arg202Gln) R202Q mutation of the *MEFV* gene in Iranian patients with premature CAD (in the west Azerbaijan province of Iran).

Materials & Methods

The Research Ethics Committee of Urmia University of Medical Sciences has approved all stages of this study. This hospital-based study was designed and conducted at Seyedoshohada Hospital of Urmia University of Medical Sciences (Urmia, Iran) in 2015. The case (n = 100) and control (n = 100) groups were selected out of all the patients referred for coronary angiography at Seyedoshohada Teaching Hospital (Hospital of Cardiovascular Diseases of Urmia University) by a cardiologist, as described previously (4). Each patient was informed about the contents and aims of the study. The age of patients from 45 to 65 years was considered as the cutoff. Coronary angiography indications were performed based on clinical symptoms and non-invasive tests such as the exercise tolerance test, nuclear myocardial perfusion imaging, exercise stress echocardiography, and CT-angiography. Patients in the case group were chosen if they had at least 50% stenosis in one of their coronary arteries (14). The individuals selected for the control group had either a fully patent coronary lumen or only a luminal irregularity with less than a 50% diameter reduction. To prevent any research bias, patients who were referred in succession were selected and no intervention was performed. Exclusion criteria include patients with FMF, acute coronary syndrome, acute illness, or other inflammatory diseases (2, 3).

DNA isolation and PCR-RFLP:

For the extraction of genomic DNA, 3-4 ml of blood was obtained in EDTA tubes. The samples were conserved at -20°C. DNA was isolated via the "salting-out" method (15). The purity of DNA was confirmed by measuring absorbance at 260 nm and 280 nm in a Biophotometer (Eppendorf AG, Germany). The R202Q

mutation of the *MEFV* gene in exon 2 was determined through the RFLP-PCR method (16). A pair of primers, F: 5′- AGATGATTCCGCAGCGTCC- 3′ and R: 5′-GGGGTTCTGTTGCCGAGTCC-3′ were used for PCR with a thermal cycling program of 94°C for 3 minutes; 35 cycles: 94°C for 30 seconds, 58°C for 40 seconds, and 72°C for 50 seconds, and final a extension at 94°C for 3 minutes. Each PCR reaction was performed in a 25 µL final volume, containing 100 ng of DNA, 1x reaction buffer, 5 pmol of each primer, 200 µmol of each dNTPs, 0.3 units of Taq DNA polymerase, and 1.5 mmol $MgCl_2$. The presence of the R202Q mutation leads to the creation of a Thermo Scientific™ PvuII (#ER0631) cutting site, as well as the production of 196 bp and 304 bp fragments. However, in the absence of the R202Q mutation, PCR products with 500 bp were not digested by Thermo Scientific™ PvuII. The presence/absence of fragments after digestion was monitored by a UV transilluminator. PCR products were analyzed via electrophoresis on a 2.5% agarose gel stained with CinnaGen DNA safe Stain (CinnaGen Co., Tehran, Iran).

Statistical analysis:

The allelic and genotypic frequencies were calculated by direct counting, as well as the number of normal and mutated chromosomes. The average age of participants was reported based on mean (±) standard deviation (SD). All frequencies were reported as frequency or percentage. Excel 2016 was used for statistical analysis.

Results

In total, 100 patients with premature CAD (age: 46.11 ± 6.61) and 100 healthy controls (age: 61.36 ± 7.51) were tested in this study. The R202Q mutation was not found in healthy controls. However, 12 out of 100 patients with premature CAD were heterozygote for the R202Q mutation (12%) (12% vs. 0%). Considering the heterozygosity of the R202Q mutation in the patients, the allele frequency was 0.06 (12 out of 200 chromosomes). Figure 1 shows a gel electrophoresis image of R202Q mutation in eight samples.

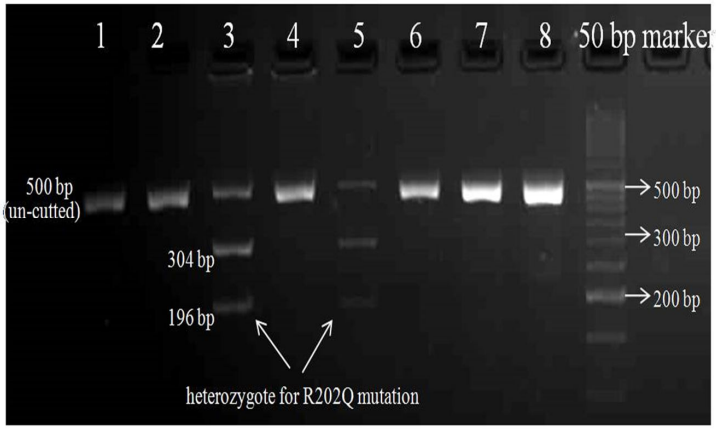

Fig. 1. Electrophoresis gel image regarding RFLP-PCR of R202Q mutation in eight samples.

Wells 1, 2, 4, 6-8: negative for the tested mutation (500 bp uncut fragments); Wells 3 and 5: positive for R202Q mutation (heterozygote regarding 500,304, 196 bp fragments).

Discussion

It has been demonstrated that premature CAD has several conventional and modifiable risk factors among

people. The study of conventional risk factors indicated that age, sex, and family history play an important role in CAD predisposition. Older men have an increased risk of CAD. In the case of young individuals, multiple risk factors have been found for CAD.

The R202Q mutation of the MEFV gene has been associated with an increased risk of cardiovascular disease in individuals with metabolic syndrome (12, 13). In this study, the presence of the R202Q mutation in the MEFV gene was analyzed in patients with premature CAD and healthy individuals as controls.

Approximately 90–97% of young cases with CAD have one or more risk factors of atherosclerosis (17). Atherosclerosis is the most important factor responsible for CAD. In this respect, the results of several studies indicated that inflammatory genes and related polymorphisms have a key role in the pathogenesis of atherogenesis (17). Atherosclerotic lesions are focal thickenings of the intima. Myocardial infarction happens while the atheromatous progression impedes blood flow within the coronary artery. Angiographic tests have recognized that plaque activation and endothelial dysfunction more than stenosis lead to myocardial ischemia and infarction (18).

It has been demonstrated that the presence of *MEFV* gene mutations increases the risk of inflammatory diseases and makes individuals susceptible to developing the disease (19). Up to now, more than 314 gene mutations or polymorphisms have been revealed in the *MEFV* gene (19). The *MEFV* gene has 10 exons and encodes a protein, entitled pyrin or marenostrin, with 781 amino acids. *MEFV* gene mutations lead to malfunctioning pyrin protein. Mutated pyrin cannot suppress inflammatory processes that are normally inhibited by functional pyrin. The frequent mutations of the *MEFV* gene are M680I, M694I, M694V, K695R, V726A, A744S, and R761H in exon 10, and E148Q and R202Q in exon 2.

In this study, we studied the R202Q mutation, which indicated that this mutation was frequent among tested patients. Numerous known risk factors appear in concert with premature CAD.

There is a relationship between smoking and cardiovascular disease (20). Smoking cigarettes raises the risk of atherosclerotic disorders by at least 50% and doubles the rates of coronary heart disease (20).

Our study had some limitations. The overall male-to-female ratio was not equal in this study. However, large studies with more details, such as gene-gene interaction and environmental risk factors, are required to analyze the relationship between the R202Q mutation and the pathogenesis of inflammatory-related disorders. Our findings implied that the R202Q mutation was more frequent among premature CAD patients, which may be considered as a predictive factor in the development of this disorder.

Conclusion

Our results indicate that the R202Q mutation in the MEFV gene is frequent in patients with premature CAD. Further studies are necessary to analyze more details regarding the variable expressivity or incomplete penetrance of the R202Q mutation in the tested population.

Acknowledgments

All individuals who participated in this project are thanked and appreciated.

Conflict of interest

There is no conflict of interest.

Ethical statement

Research Ethics Committee of Urmia University of Medical Sciences has approved all stages of this study (IR.UMSU.REC.1394.138).

Funding/support

The authors would like to thank the Student Research Committee, Urmia University of Medical Sciences, Urmia, Iran, for supporting this project (Grant No: 1748).

Data availability

The raw data supporting the conclusions of this article are available from the authors upon reasonable request.

References

1. Khoja A, Andraweera PH, Lassi ZS, Zheng M, Pathirana MM, Ali A, et al. Risk factors for premature coronary artery disease (PCAD) in adults: a systematic review protocol. F1000 Research 2021;10:1228. https://doi.org/10.12688/f1000research.74926.1
2. Rasmi Y, Bagheri M, Faramarz-Gaznagh S, Nemati M, Khadem-Ansari MH, Saboory E, et al. Transcriptional activity of tumor necrosis factor-alpha gene in peripheral blood mononuclear cells in patients with coronary slow flow. ARYA Atherosclerosis 2017; 13(4):196-201.
3. Hassan-Nejhad M, Bagheri M, Khadem-Vatani K, Seyed Mohammad Zad MH, Abdi Rad I, Rahimi B, et al. Tumor Necrosis Factor-alpha Gene Expression in PBMCs of Iranian Azeri Turkish Patients with Premature Coronary Artery Disease (Age.50 Years). Maedica (Bucharest) 2018; 13(1):12-16. https://doi.org/10.26574/maedica.2018.13.1.12
4. Bagheri M, Khadem-Vatani K, Mohammad Zad MHS, Abdi Rad I, Rahimi B, Rostamzadeh A, et al. Analysis of the mutations in exon 10 of MEFV gene in patients with premature coronary heart disease in west Azerbaijan province of Iran. Journal of Cardiovascular and Thoracic Research 2018; 10(1):20-23 https://doi.org/10.15171/jcvtr.2018.03
5. Bagheri M, Khadem-Vatani K, Seyed-Mohammad-Zad MH, Rad IA, Rostamzadeh A, Rahimi B, et al. TNF receptor type 1 and TNF receptor type 2 mRNA expression was not associated with coronary artery disease in a group of Iranian Turks. Bratislava Medical Journal 2019; 120(2):144-147. https://doi.org/10.4149/BLL_2019_023
6. Khojasteh-Fard M, Abolhalaj M, Amiri P, Zaki M, Taheri Z, Qorbani M, et al. IL-23 gene expression in PBMCs of patients with coronary artery disease. Disease Markers 2012; 33(6): 289-93. https://doi.org/10.1155/2012/629814
7. Hoseini F, Mahmazi S, Mahmoodi K, Jafari GA, Soltanpour MS. Evaluation of the Role of -137G/C Single Nucleotide Polymorphism (rs187238) and Gene Expression Levels of the IL-18 in Patients with Coronary Artery Disease. Oman Medical Journal 2018; 33(2): 118-125. https://doi.org/10.5001/omj.2018.23
8. Basar N, Kisacik B, Ercan S, Pehlivan Y, Yilmaz S, Simsek I,et al. Familial Mediterranean fever gene mutations as a risk factor for early coronary artery disease. International Journal of Rheumatic Diseases 2017; 20(12): 2113-2117. https://doi.org/10.1111/1756-185X.12356
9. Bagheri M, Rad IA. Analysis of the Most Common Three MEFV Mutations in 630 Patients with Familial Mediterranean Fever in Iranian Azeri Turkish Population. Maedica (Bucharest) 2017; 12(3):169-173.
10. Nikibakhsh AA, Houshmand M, Bagheri M, Zadeh HM, Rad IA. MEFV gene mutations (M694V, V726A, M680I, andA744S) in Iranian children with Henoch-Schönlein purpura. Pneumologia 2012; 61(2):84-7.
11. Öztürk A, Özçakar B, Ekim M, Akar N. Is MEFVGene Arg202Gln (605 G> A) A Disease-Causing Mutation? Turkish Journal of Medical Sciences 2008; 38(3):205-8.
12. Balkarli A, Akyol M, Tepeli E, Elmas L, Cobankara V. MEFV gene variation R202Q is associated with metabolic syndrome. European Review for Medical and Pharmacological Sciences 2016; 20(15):3255-61.
13. Ju SY, Lee JY, Kim DH. Association of metabolic syndrome and its components with all-cause and cardiovascular mortality in the elderly: A meta-analysis of prospective cohort studies. Medicine (Baltimore) 2017; 96(45):e8491. https://doi.org/10.1097/MD.0000000000008491
14. Jiangping S, Zhe Z, Wei W, Yunhu S, Jie H, Hongyue W, et al. Assessment of coronary artery stenosis by coronary angiography: a head-to-head comparison with pathological coronary artery anatomy. Circulation: Cardiovascular Interventions 2013; 6(3): 262-8. https://doi.org/10.1161/CIRCINTERVENTIONS.112.000205

15. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Research 1988; 16:1215 https://doi.org/10.1093/nar/16.3.1215
16. Farivar Sh, Hasani M, Shiari R. R202Q Mutation of Mediterranean Fever Gene in Iranian patients with Systemic-onset Juvenile Idiopathic Arthritis. Research in Molecular Medicine 2014; 2 (4): 30-32. https://doi.org/10.18869/acadpub.rmm.2.4.30
17. Chouhan L, Hajar HA, Pomposiello JC. Comparison of thrombolytic therapy for acute myocardial infarction in patients aged < 35 and > 55 years. American Journal of Cardiology 1993; 71(2):157-9. https://doi.org/10.1016/0002-9149(93)90731-Q
18. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. New England Journal of Medicine 2005; 352(16):1685-95. https://doi.org/10.1056/NEJMra043430
19. Mejtoute T, Sayel H, El-Akhal J, Moufid FZ, Bouguenouch L, El Bouchikhi I, et al. The detection of a novel insertion mutation in exon 2 of the MEFV gene associated with familial mediterranean fever in a moroccan family. Human Genome Variation 2017; 4(1):1-4. https://doi.org/10.1038/hgv.2017.23
20. Doll R, Hill AB. Lung cancer and other causes of death in relation to smoking. British Medical Journal 1956; 2(5001):1071. https://doi.org/10.1136/bmj.2.5001.1071