A substitution mutation in LRP8 gene is significantly associated with susceptibility to familial myocardial infarction

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Abstract

BACKGROUND: Myocardial infarction (MI) is a multifactorial disease caused by the suspension of blood circulation in a part of the myocardium. Understanding the genetic basis of MI can provide insight regarding the pathogenesis of the disease. The aim of this study was to investigate the association between pathogenic mutations and early-onset MI in five families with familial MI and without common MI risk factor.

METHODS: Patients with MI younger than 50 years with family history of MI and without common diagnostic criteria (obesity, diabetes, familial hypercholesterolemia, opium/alcohol use) were evaluated for pathogenic mutations by whole exome sequencing (WES) and mutation was confirmed by polymerase chain reaction (PCR)-Sanger sequencing.

RESULTS: The c.2855G > A missense mutation with homozygous autosomal recessive inheritance was identified in low-density lipoprotein receptor-related protein 8 (LRP8) gene in all patients of a family.

CONCLUSION: The c.2855G > A (R952Q) mutation in LRP8 gene in homozygous state could be considered as a possible etiology of early-onset familial MI.

Keywords: Myocardial Infarction; Low Density Lipoprotein Receptor-Related Protein 8; Whole Exome Sequencing

Date of submission: 20 May 2018, Date of acceptance: 30 Sep. 2019

Introduction

Myocardial infarction (MI) is one of the underlying causes of morbidity and mortality and it causes more than 30% of all deaths worldwide.1 According to the recent report of the heart and cardiovascular center of Iran Health Ministry, about 300 patients die each day due to cardiovascular disease (CVD).2 High incidence of CVD in Iranian population shows the importance of investigating the cause of MI in this population. MI is a complex multifactorial disease, which involves both environmental and genetic factors and their interactions.3,4 The coronary artery disease (CAD) risk factors include (but not limited to) diabetes, smoking, hypertension (HTN), hyperlipidemia, age, and gender.5 Lifestyle risk factors have an important role in the incidence of CAD and MI. However, the role of genetic factors cannot be ignored in etiology of the CAD and MI pathogenesis.6 The heritability of CAD and MI was estimated approximately 50 to 60 percent by the long-recognized familial clustering of CAD which suggests that genetics plays a critical role in the CAD and MI development.7 Genetic evaluation for finding pathogenic mutations in patients with early-onset CAD and MI can be useful.4 Early-onset MI in a first-degree relative which is younger than 55 years in men and younger than 65 years in women could

How to cite this article: Ghorbani MJ, Razmi N, Tabei SMB, Zibaenezhad MJ, Goodarzi HR. A substitution mutation in LRP8 gene is significantly associated with susceptibility to familial myocardial infarction. ARYA Atheroscler 2020; 16(6): 301-5.

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DOI: http://dx.doi.org/10.22122/arya.v16i6.1797
Published by Vesnu Publications
be defined as an independent risk factor for CAD. One-quarter of early-onset MIs are unrecognized MI (umi) and recognition is critical to minimize further cardiovascular complications. Whole exome sequencing (WES) is a valuable screening tool for clinical diagnosis of familial CAD, particularly for cases without common diagnostic criteria and sudden cardiac death. We aimed to identify the pathogenic mutations responsible for early-onset MI in five families with familial MI and without common MI risk factor.

**Materials and Methods**

Forty patients with early-onset MI and premature CAD were evaluated in the Angiography Center of Namazi Hospital, Shiraz University of Medical Sciences, Shiraz, Iran, and five families with at least five definite diagnosis of patients with MI were selected and referred to the third universal definition of MI. Patients with definite MI and premature CAD on the basis of coronary angiography were referred for genetic counseling in Amin Genetic Counseling Center, Marvdasht, Iran. Both environmental and hereditary factors can cause CAD and MI. Therefore, we selected the familial form of CAD and MI disease with at least five patients in a pedigree to increase the chances of identifying genetic factors, and excluded patients and families with environmental risk factors of CVD (such as diabetes, familial hypercholesterolemia, and opium consumption) from the study. In this study, five families with these characteristics were investigated. Information about family history of CAD and MI, diabetes (2 hours postprandial glucose ≥ 200 mg/dl, fasting blood glucose ≥ 126 mg/dl, or use of insulin or hypoglycemic agents), dyslipidemia [triglycerides (TGs) > 150 mg/dl, high-density lipoprotein cholesterol (HDL-C) < 40 mg/dl, or high low-density lipoprotein cholesterol (LDL-C) based on Adult Treatment Panel III (ATP III)], HTN (use of antihypertensive drugs or positive past history of HTN), smoking or opium consumption, age, and gender were collected from probands and their family members. Genomic deoxyribonucleic acid (DNA) was extracted from peripheral blood of patients and their family members using a High Pure PCR Template Preparation Kit (“Roche Life Science”, Germany) (this research was approved by the local institutional review board). All DNA samples’ concentration was 30 µg and A260/280 ratio was ~ 1.8. After quantitative and qualitative assessment using standard techniques, DNA was subjected to WES. WES was carried out for five probands. Paired-end sequencing with 101-base reads was performed on Illumina’s HiSeq2000 platform (Illumina, San Diego, CA, USA). In a proband, WES result showed 1048 variants. After data and variants analysis, a missense mutation (c.2855G > A) was identified in exon 19 of lipoprotein receptor-related protein 8 (LRP8) gene in homozygous state. For suspected mutation (LRP8 gene c.2855G > A variant), polymerase chain reaction (PCR) primers (Table 1) were designed manually and using Primer-BLAST and Primer3 to amplify the mutation containing fragment. PCR amplification was carried out in a total volume of 25 µl containing 12.5 µl PCR Master Mix (Promega, Madison, USA), 30 ng of genomic DNA, 0.5 µM of each primer, and 5.5 µl double-distilled water (DDW). Sanger sequencing was performed in proband and other patients in pedigree.

**Results**

In this study, we collected information about probands and their family members and excluded families with cardiovascular risk factors from the study as explained above. WES was carried out for five probands. The c.2855G > A (p.R952Q) (rs5174) missense mutation was identified in exon 19 of LRP8 gene, chr1:2,315,167 in homozygous state in proband of a family by WES (Table 2). Pedigree analysis of the family was consistent with autosomal recessive inheritance of CAD (Figure 1). Proband (V.1) was a 39-year-old man with early-onset MI at the age of 35. Family history showed that his father (IV.8), grandfathers (III.2, III.9), uncle and aunt (IV.9, IV.11), and his male cousin (V.6) were diagnosed with early-onset MI and premature CAD. Although next generation sequencing (NGS) is a high throughput sequencing method, it has not been approved as a clinical diagnostic test; therefore, we designed PCR primers and amplified the fragment containing the c.2855G > A mutation point. Sanger sequencing was done which confirmed the sequencing data.

**Table 1. Sequence of forward and reverse polymerase chain reaction (PCR) primers**

| Gene     | Primer                        | Primer length (bp) | Tm  | GC% |
|----------|-------------------------------|--------------------|-----|-----|
| LRP8     | Forward: TTTGCCAAGCTAACCCACTG | 21                 | 59  | 47  |
|          | Reverse: CCTCATGGTAGTGAAACC | 20                 | 59  | 55  |

LRP8: Low-density lipoprotein receptor-related protein 8
Table 2. The characteristics of c.2855G > A (p.R952Q) mutation in low-density lipoprotein receptor-related protein 8 (LRP8) gene

| Gene and transcript | Variant | Location | Zygosity | Inheritance | Associated disease | OMIM | CADD score | Polyphen |
|---------------------|---------|----------|----------|-------------|-------------------|------|------------|----------|
| LRP8 NM_004631      | c.2855G>A p.R952Q | 1p32     | HOM      | AR          | Type 1 MI         | 602600 | 34         | Probably damaging |

OMIM: Online Mendelian Inheritance in Man; CADD: Combined annotation dependent depletion; LRP8: Low-density lipoprotein receptor-related protein 8; NM: NCBI reference sequence (locus); HOM: Homozygous; AR: Autosomal recessive; MI: Myocardial infarction

Figure 1. Pedigree of a family demonstrating autosomal recessive inheritance of early-onset myocardial infarction (MI); individuals with early-onset MI are indicated by solid squares (men) or solid circles (women). Unaffected individuals are indicated by open symbols. Deceased individuals are indicated by a slash (/). The proband is indicated by an arrow. Genetic status: M/M indicates the presence of mutation (homozygous); M/- indicates heterozygous status, and --/-- indicates the absence of the mutation.

Mutation was confirmed in homozygous state in proband by PCR-Sanger sequencing and segregation analysis revealed mutation in heterozygous and homozygous states in pedigree (Figure 2). There are no data about first and second generations.

Proband’s youngest brother had the c.2855G > A mutation in LRP8 gene in homozygous state without CAD that may be due to his younger age.

Discussion

According to the latest statistics of World Health Organization (WHO), twelve million people die each year due to CAD worldwide. In Iranian population, CAD is the most common cause of death, and MI is the most severe type of CAD, which is ranked as the leading cause of death worldwide. Recent update of American Heart Association in 2017 showed that 12.2% of patients aged ≥ 20 years had a parent or sibling with angina or heart attack before age of 50 years. As mentioned above, early-onset MI in a first-degree relative could be considered as an independent risk factor for CAD, and WES is a valuable screening tool to evaluate patients with suspected inherited CAD. According to the NGS-based study on early-onset CAD that was carried out on approximately 5000 cases with early-onset CAD in order to find genes of significant associations with CAD in 2015, 2% of studied patients with early-onset CAD harbored at least a rare variant on LDL receptor (LDLR).

Figure 2. Truncated sequencing chromatogram of low-density lipoprotein receptor-related protein 8 (LRP8) gene of patients; the mutation point is indicated by an arrow.
In this study, WES helped us identify the cause of early-onset MI. The utility of exome sequencing as a fast and cost-effective technique in diagnosis of hereditary CAD, where the clinical diagnosis is uncertain, has been discussed. The proband and other patients in our studied pedigree showed the missense mutation p.R952Q in LRP8 gene. LRP8 gene encodes LDLR which plays a critical role in lipoprotein metabolism and facilitates the clearance of LDL and very-low-density lipoprotein (VLDL) from plasma, and is reported to be associated with early-onset and familial MI. The LRP8 gene is highly expressed in the testes and brain; however, it is also expressed in the vascular smooth muscle cells, platelets, endothelial cells, and heart. LRP8 gene encodes a member of the LDLRs family which play role as cell surface proteins. Signal transduction and receptor-mediated endocytosis of specific ligands for lysosomal degradation are the main role of LDLRs. Also, LDLRs play a critical role in the migration of neurons during development by mediating Reelin signaling, and may be a marker for complex psychiatric disorders. In work-up of patients in the present study, we found that patients had stressful lifestyle. However, we could not find history of psychiatric disorders in patients. Findings of this report show that the c.2855G > A (R952Q) mutation in LRP8 gene in homozygous state could be considered as a possible cause of early-onset familial MI. In this study, we found nine patients with early-onset CAD and MI that five patients died before the age of 50 years old. Parents in this family had consanguineous marriage. Shen et al. genotyped and analyzed a single-nucleotide polymorphism (SNP) (rs5174) of LRP8 in 381 patients with familial early-onset CAD, 183 patients with MI, and 560 controls. Results of their study showed that the c.2855G > A (R952Q) mutation in LRP8 gene conferred a significant risk of familial early-onset CAD/MI. Also, Shen et al. studied multiple independent populations in 2007, which showed that genetic variants in LRP8 might contribute to the development of premature CAD and MI in familial form of the disease. A case-control study by Martinelli et al. in the Italian cohort suggested that the c.2855G > A (R952Q) variant might have an additive effect to apolipoprotein E (APOE) genotype in determining APOE concentrations and risk of premature CAD and MI. However, Asif et al. sequenced regions of a SNP (rs5174) of LRP8 in 100 patients with MI and 100 age-matched controls. Results of their study showed that the c.2855G > A (R952Q) mutation in LRP8 gene was not significantly associated with MI. To better understand the association between c.2855G > A mutation in LRP8 gene and familial MI, we need large population studies on familial MI.

**Conclusion**

There was a significant association between c.2855G > A (p.R952Q) mutation and premature CAD and familial MI. However, further research is required to identify other unknown genes that cause premature CAD and familial MI in patients without common premature CAD and MI risk factor.

**Acknowledgments**

Special sincere thanks to all the patients and their families who participated in this research. This article is based on the PhD thesis with project number: 2542881 in Marvdasht Branch, Islamic Azad University, Marvdasht.

**Conflict of Interests**

Authors have no conflict of interests.

**References**

1. Shanmugam K, Ravindran S, Kurian GA, Rajesh M, Fisetin confers cardioprotection against myocardial ischemia reperfusion injury by suppressing mitochondrial oxidative stress and mitochondrial dysfunction and inhibiting glycogen synthase kinase 3beta Activity. Oxid Med Cell Longev 2018; 2018: 9173436.
2. Biglu MH, Ghavami M, Biglu S. Cardiovascular diseases in the mirror of science. J Cardiovasc Thorac Res 2016; 8(4): 158-63.
3. Asif M, Bhat S, Nizamuddin S, Mustak MS. TG haplotype in the LRP8 is associated with myocardial infarction in south Indian population. Gene 2018; 642: 225-9.
4. InanlooRahatloo K, Parsa AF, Huse K, Rasooli P, Davaran S, Platzter M, et al. Mutation in ST6GALNAC5 identified in family with coronary artery disease. Sci Rep 2014; 4: 3595.
5. Chen QF, Wang W, Huang Z, Huang DL, Li T, Wang F, et al. Correlation of rs1122608 SNP with acute myocardial infarction susceptibility and clinical characteristics in a Chinese Han population: A case-control study. Anatol J Cardiol 2018; 19(4): 249-58.
6. Kelloniemi A, Szabo Z, Serpi R, Napankangas J, Ohukainen P, Tenhunen O, et al. The Early-Onset Myocardial Infarction Associated PHACTR1 Gene Regulates Skeletal and Cardiac Alpha-Actin Gene Expression. PLoS One 2015; 10(6): e0130502.
7. Dai X, Wiernek S, Evans JP, Runge MS. Genetics of coronary artery disease and myocardial
infarction. World J Cardiol 2016; 8(1): 1-23.
8. Stacey RB, Leaverton PE, Schokken DD, Perejofy JA, Bertoni AG. Prediabetes and the association with unrecognized myocardial infarction in the multi-ethnic study of atherosclerosis. Am Heart J 2015; 170(5): 923-8.
9. Seidelmann SB, Smith E, Subrahmanyam L, Dykas D, Abou Ziki MD, Azari B, et al. Application of Whole Exome Sequencing in the Clinical Diagnosis and Management of Inherited Cardiovascular Diseases in Adults. Circ Cardiovasc Genet 2017; 10(1).
10. Thygesen K, Alpert JS, Jaffe AS, Simoons ML, Chaitman BR, White HD, et al. Third universal definition of myocardial infarction. J Am Coll Cardiol 2012; 60(16): 1581-98.
11. Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. Circulation 2002; 106(25): 3143-421.
12. Mohseni J, Kazemi T, Maleki MH, Beydokhti H. A systematic review on the prevalence of acute myocardial infarction in Iran. Heart Views 2017; 18(4): 125-32.
13. Gao Y, Lee C, Song J, Li S, Cui Y, Liu Y, et al. Digenic mutations on SCAP and AGXT2 predispose to premature myocardial infarction. Oncotarget 2017; 8(49): 100141-9.
14. Benjamin EJ, Blaha MJ, Chiue SE, Cushman M, Das SR, Deo R, et al. Heart disease and stroke statistics-2017 update: a report from the American Heart Association. Circulation 2017; 135(10): e146-e603.
15. Do R, Stiitziel NO, Won HH, Jorgensen AB, Duga S, Angelica Merlino P, et al. Exome sequencing identifies rare LDLR and APOA5 alleles conferring risk for myocardial infarction. Nature 2015; 518(7537): 102-6.
16. Shen GQ, Li L, Wang QK. Genetic variant R952Q in LRP8 is associated with increased plasma triglyceride levels in patients with early-onset CAD and MI. Ann Hum Genet 2012; 76(3): 193-9.
17. Martinelli N, Olivieri O, Shen GQ, Trabetti E, Pizzolo F, Busti F, et al. Additive effect of LRP8/APOER2 R952Q variant to APOE epsilon2/epsilon3/epsilon4 genotype in modulating apolipoprotein E concentration and the risk of myocardial infarction: a case-control study. BMC Med Genet 2009; 10: 41.
18. Shen GQ, Li L, Girelli D, Seidelmann SB, Rao S, Fan C, et al. An LRP8 variant is associated with familial and premature coronary artery disease and myocardial infarction. Am J Hum Genet 2007; 81(4): 780-91.
19. Hirai H, Yasui N, Yamashita K, Tabata S, Yamamoto M, Takagi J, et al. Structural basis for ligand capture and release by the endocytic receptor ApoER2. EMBO Rep 2017; 18(6): 982-99.
20. Li M, Huang L, Grigoroiu-Serbanescu M, Bergen SE, Landen M, Hultman CM, et al. Convergent Lines of Evidence Support LRP8 as a Susceptibility Gene for Psychosis. Mol Neurobiol 2016; 53(10): 6608-19.
21. Shen GQ, Girelli D, Li L, Rao S, Archacki S, Olivieri O, et al. A novel molecular diagnostic marker for familial and early-onset coronary artery disease and myocardial infarction in the LRP8 gene. Circ Cardiovasc Genet 2014; 7(4): 514-20.