



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

Mohammad Javad Ghorbani<sup>(1)</sup> , Nematollah Razmi<sup>(2)</sup>, Seyed Mohammad Bagher Tabei<sup>(3)</sup> ,  
Mohammad Javad Zibaenezhad<sup>(4)</sup>, Hamid Reza Goodarzi<sup>(5)</sup>

### Short Communication

#### 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

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#### Introduction

Myocardial infarction (MI) is one of the underlying causes of morbidity and mortality and it causes more than 30% of all deaths worldwide.<sup>1</sup> 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).<sup>2</sup> 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.<sup>3,4</sup> The coronary artery disease (CAD) risk factors include (but not limited to) diabetes, smoking, hypertension (HTN), hyperlipidemia, age, and gender.<sup>5</sup> 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.<sup>6</sup> 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.<sup>7</sup> Genetic evaluation for finding pathogenic mutations in patients with early-onset CAD and MI can be useful.<sup>4</sup> Early-onset MI in a first-degree relative which is younger than 55 years in men and younger than 65 years in women could

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1- PhD Candidate, Department of Genetics, Fars Science and Research Branch AND Islamic Azad University, Marvdasht Branch, Marvdasht, Iran

2- Associate Professor, Department of Biochemistry, Islamic Azad University, Shiraz Branch, Shiraz, Iran

3- Associate Professor, Maternal-Fetal Medicine Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

4- Professor, Cardiovascular Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

5- Assistant Professor, Department of Genetics, Islamic Azad University, Marvdasht Branch, Marvdasht, Iran

Address for correspondence: Seyed Mohammad Bagher Tabei; Associate Professor, Maternal-Fetal Medicine Research Center, Shiraz University of Medical Sciences, Shiraz, Iran; Email: [tabeismb@sums.ac.ir](mailto:tabeismb@sums.ac.ir)

be defined as an independent risk factor for CAD.<sup>7</sup> One-quarter of early-onset MIs are unrecognized MI (UMI) and recognition is critical to minimize further cardiovascular complications.<sup>8</sup> 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.<sup>9</sup> 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.<sup>10</sup> 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  $\geq$  200 mg/dl, fasting blood glucose  $\geq$  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)],<sup>11</sup> 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  $\mu$ g and A260/280 ratio was  $\sim$ 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 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  $\mu$ l containing 12.5  $\mu$ l PCR Master Mix (Promega, Madison, USA), 30 ng of genomic DNA, 0.5  $\mu$ M of each primer, and 5.5  $\mu$ l 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

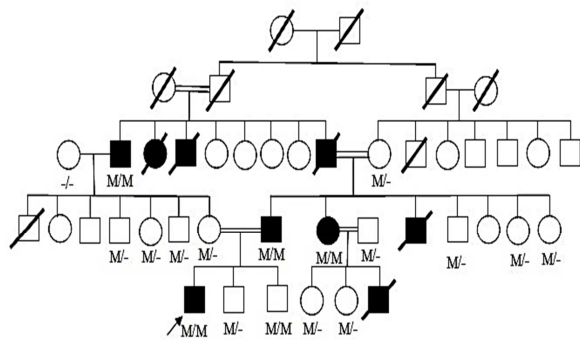
Gene	Primer	Primer length (bp)	Tm	GC%
LRP8	Forward: TTTGCCAAAGCTAACCCACTG	21	59	47
	Reverse: CCTCATGGGTAGTGCAACCA	20	59	55

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	Zygoty	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



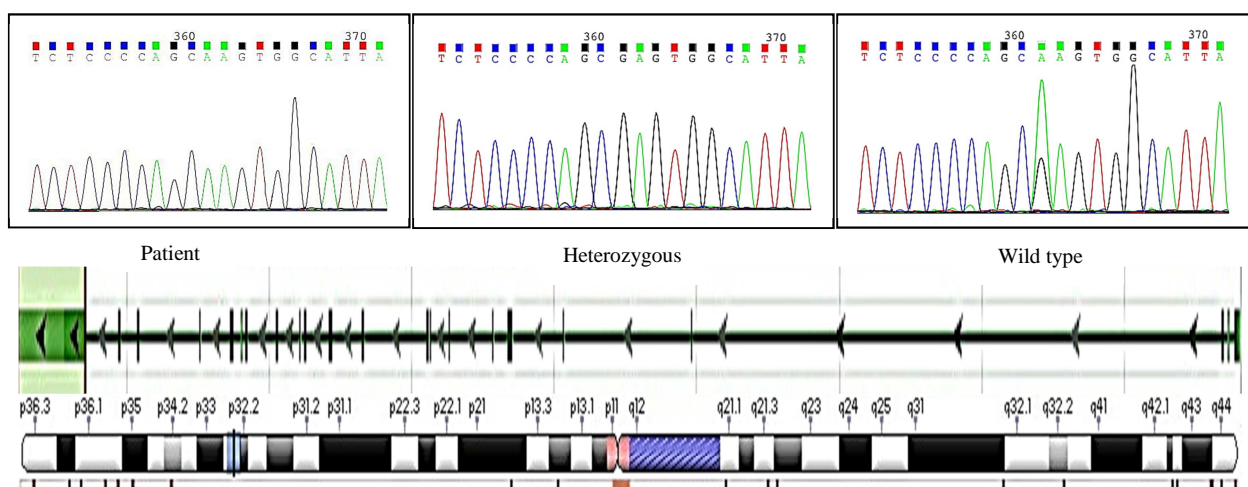
**Figure 1.** Pedigree of a family demonstrating autosomal recessive inheritance of early-onset myocardial infarction (MI); individuals with early-onset MI are indicated by solid squares (men) or solid circles (women). Unaffected individuals are indicated by open symbols. Deceased individuals are indicated 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.<sup>12</sup> In Iranian population, CAD is the most common cause of death,<sup>12</sup> and MI is the most severe type of CAD, which is ranked as the leading cause of death worldwide.<sup>13</sup> Recent update of American Heart Association in 2017 showed that 12.2% of patients aged  $\geq 20$  years had a parent or sibling with angina or heart attack before age of 50 years.<sup>14</sup> As mentioned above, early-onset MI in a first-degree relative could be considered as an independent risk factor for CAD,<sup>7</sup> and WES is a valuable screening tool to evaluate patients with suspected inherited CAD.<sup>9</sup> 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).<sup>15</sup>



**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.<sup>9</sup> 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,<sup>3</sup> and is reported to be associated with early-onset and familial MI.<sup>16,17</sup> 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.<sup>18</sup> 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,<sup>19</sup> and may be a marker for complex psychiatric disorders.<sup>20</sup> 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.<sup>21</sup> 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.<sup>18</sup> 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.<sup>17</sup> 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.<sup>3</sup> 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.

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### Conflict of Interests

Authors have no conflict of interests.

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