

Basic research

Genetic analysis of early onset familial coronary artery diseases

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Abstract

Introduction: Coronary artery diseases (CAD) are the most common causes of death. Myocardial infarction (MI) is a complex multifactorial and the most severe type of CAD. Early onset MI in a first-degree relative could be defined as an independent risk factor for CAD. This study was performed to investigate the genetic cause of early onset familial CAD.

Material and methods: In this study, the genetic cause of familial CAD was investigated in patients with a family history of CAD who underwent angiography before the age of 50 years. The patients did not have any diagnostic criteria for familial hypercholesterolemia, diabetes, or obesity, and also they were not opium or alcohol users. Whole exome sequencing in probands was performed and mutation was confirmed by PCR and Sanger sequencing.

Results: In our studied population, the c.501G>C (p.K167N) mutation in the *OLR1* gene was identified in a family. Mutation was confirmed by PCR and Sanger sequencing in the homozygous state (GG) in patients. Healthy individuals in this family were heterozygous (GC) and homozygous (CC).

Conclusions: This finding suggests that the *OLR1* gene could be a possible cause of early onset familial MI. Considering that parents of all affected individuals had a consanguineous marriage, it is important to perform carrier screening and genetic counseling in this family and their close relatives as a prevention strategy in populations at risk.

Key words: myocardial infarction, *OLR1* gene, whole exome sequencing.

Introduction

According to the Third Report by the World Health Organization (WHO), 12 million people die annually of coronary artery diseases (CAD) worldwide [1]. In developing countries, the number of adults who develop CAD at a young age is high [2]. In Iran, CAD are the most common causes of death [1]. Recently, the Director of the Heart and Cardiovascular Center of Iran's Health Ministry emphasized that about 300 people die in Iran due to cardiovascular disease every day [3]. These diseases lead to complications, significant disability, and reduced productivity of patients

[4]. The risk factors for CAD include age, gender, smoking, hypertension (HTN), hyperlipidemia, and diabetes, but are not limited to these factors [5]. Lifestyle factors are well-known risk factors for CAD but genetic factors also play an important role in the disease pathogenesis [6]. The American Heart Association reported in a recently update in 2017 that among adults ≥ 20 years of age, 12.2% reported having a parent or sibling with a heart attack or angina before the age of 50 years [7]. These statistics indicate the importance of investigating the genetic cause of early onset familial CAD. The long-recognized familial clustering of CAD suggests that genetics plays a central role in its development, with the heritability of CAD estimated at approximately 50% to 60% [8]. Genetic investigations on early onset CAD patients can be more fruitful [9]. Early onset CAD (< 55 in men and < 65 in women) in a first-degree relative could be defined as an independent risk factor for CAD [8]. Whole exome sequencing (WES) is an exceptionally valuable screening tool for its capability to establish the clinical diagnosis of inherited CAD, particularly for poorly defined cases of sudden cardiac death [10].

Due to the importance of early onset familial CAD, this study was performed to investigate the genetic cause of early onset CAD.

Material and methods

In this study, 40 patients with premature CAD and myocardial infarction (MI) attending the Angiography Center of Namazi Hospital, Shiraz University of Medical Sciences were evaluated and

referred for genetic counseling in Amin genetic counseling center, Marvdasht, Iran. The diagnosis of MI was referred to the Third Universal Definition of Myocardial Infarction [11] and patients were identified as having definite MI and premature CAD on the basis of coronary angiography. We selected the familial form of premature CAD and MI (at least 5 patients in a pedigree) and patients that did not have known risk factors for cardiovascular disease to increase the chances of finding effective genetic factors in the incidence of MI and premature CAD. Information about age, gender, family history of CAD (male and female first-degree relatives < 50 years old), dyslipidemia (high low-density lipoprotein (LDL) cholesterol based on ATP III or high-density lipoprotein (HDL) cholesterol < 40 mg/dl or triglycerides > 150 mg/dl) [12], diabetes mellitus (fasting blood glucose ≥ 126 mg/dl, 2 h postprandial glucose ≥ 200 mg/dl, or use of hypoglycemic agents or insulin), hypertension (positive past history of hypertension or use of antihypertensive drugs), smoking, and opium consumption were collected from all patient’s medical record and the coronary angiography report including the deceased patients. Patients and families who were diagnosed with dyslipidemia, diabetes mellitus, smoking or opium consumption were excluded from the study. After informed consent, 5–6 ml blood samples were taken from patients. Genomic DNA was extracted from peripheral blood samples using a High Pure PCR Template Preparation Kit (Roche Life Science, Germany). After qualitative and quantitative assessment using standard techniques, DNA was subjected to WES. We used the homozygosity strategy for consan-

Table I. Sequence of forward and reverse PCR primers

Forward	Reverse
TCCTTGTCGCAAGACTGGAT	GGCATCAAAGGAGAACCGTC

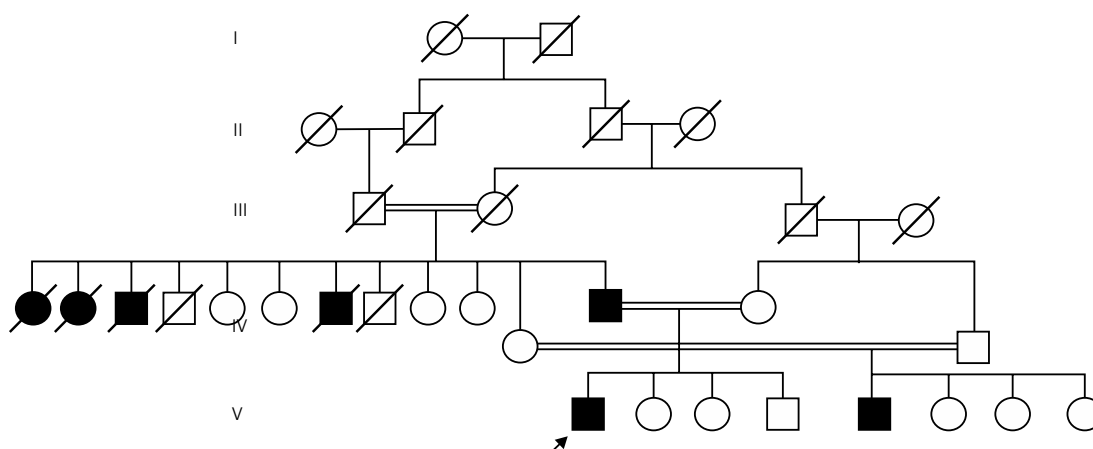


Figure 1. Pedigree of a family demonstrating autosomal recessive inheritance of early onset MI. Individuals with early onset MI are indicated by solid squares (males) or solid circles (females). Unaffected individuals are indicated by open symbols. Deceased individuals are indicated by a slash (/). The proband is indicated by an arrow

guineous pedigrees [13]. Considering that parents of all affected individuals had a consanguineous marriage, the pedigree suggested homozygous recessive mode of inheritance. We performed sequencing of a single affected person to identify a homozygous mutation in a gene located within a homozygous region. Paired-end 101 base pair sequencing was performed on Illumina's HiSeq2000 platform (Illumina, San Diego, CA, USA). Whole exome sequencing was performed in patients. In WES reports, 51180 variants were identified. Variants were first filtered based on quality criteria. Subsequently, variants outside the coding regions were filtered out, as well as synony-

mous coding variants. After analysis, we identified a suspected mutation. The c.501G>C (p.K167N) missense mutation was identified in the *OLR1* gene, in the homozygous state in a proband by WES, and PCR primers (Table I) were designed manually and using primer 3 and Primer-BLAST to amplify the mutations containing fragments. PCR amplification was carried out in a total volume of 25 μ l containing 30 ng of genomic DNA, 12.5 μ l PCR Master Mix (Promega, Madison, USA), 1 μ l of each primer and 5.5 μ l of DDW. The thermal profile included initial denaturation at 95°C for 5 min, followed by 34 cycles of denaturation at 95°C for 15 s, annealing and extension at 60°C for 60 s,

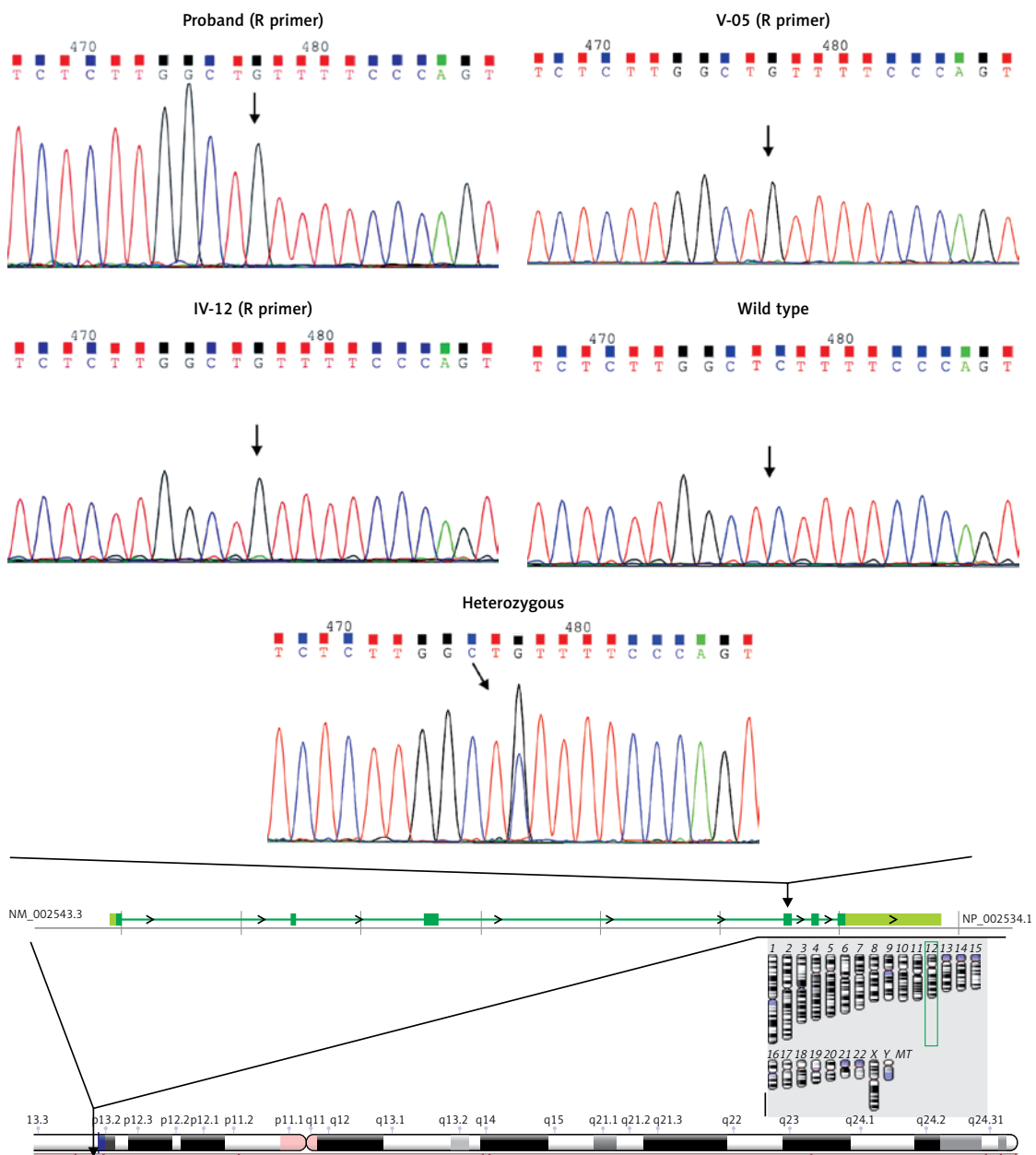


Figure 2. Truncated sequencing chromatogram of *OLR1* gene of patients. The mutation point is indicated by an arrow

Table II. Clinical characteristics of proband and patients in his family

Patients	Age of onset [years]	Gender	Smoking	Opium consumption	Alcohol	Hypertension	Diabetes mellitus		Dyslipidemia			
							FBS [mg/dl]	Use of hypoglycemic agents or insulin	LDL [mg/dl]	HDL [mg/dl]	Triglyceride [mg/dl]	Cholesterol total [mg/dl]
Proband	38	Male	No	No	No	Negative	102	No	68	42	103	138
V-05	45	Male	No	No	No	Negative	107	No	63	40	128	129
IV-12	48	Male	No	No	No	Negative	85	No	87	30	124	146

and a final extension at 72°C for 10 min. Sanger sequencing was performed to confirm mutations in probands and their family members.

Results

The present study identified an Iranian family containing 32 members (16 members deceased) with a history of early onset MI and premature CAD (Figure 1). The family consisted of 18 females and 14 males distributed in five generations; seven members were diagnosed with premature CAD and MI (four of whom were deceased). The proband (V.1) with two-vessel disease of the left anterior descending (LAD) and right coronary artery (RCA) was identified at the Department of Cardiology, Namazi Hospital, Shiraz University of Medical Sciences at 38 years of age. Subsequently, the patient’s father, paternal uncles and aunts, and his male cousin developed symptoms of MI. As shown in Table II, a few members of the family (V.4 and IV.11) had hypertension, but all had normal serum levels of low-density and high-density lipoproteins and serum levels of total cholesterol and triglycerides, and none of the family members were cigarette smokers or had hypertension, diabetes or obesity. These clinical manifestations strongly suggested heritable CAD in this family. Pedigree analysis of the family suggested autosomal recessive inheritance of CAD (Figure 1 A). The c.501G>C (p.K167N) missense mutation was identified in the *OLR1* gene in the homozygous state in patients by WES. Although WES is a method of high throughput sequencing, it has not been approved for clinical and diagnostic use; therefore mutation was confirmed by PCR and Sanger sequencing (Figure 2). There are no data about the first, second and third generation. Segregation analysis revealed that V.5’s parents and IV.13 were heterozygous. Also, all the proband’s siblings and one of V.5’s sisters were heterozygous.

Discussion

The *OLR1* gene encodes a cell-surface endocytosis receptor for oxidized low-density lipoprotein (OxLDL) which is termed LOX1. LOX1 is expressed on vascular smooth muscle cells and the plasma membrane of differentiated macrophages [14]. Hypoxia increases lipid uptake into macrophages and differentially regulates the expression of oxLDL receptors. Lox-1 plays a major role in hypoxia-induced foam cell formation [15]. We found a family in our studied population with missense mutation c.501G>C (p.K167N) in the *OLR1* gene in the homozygous state. The K167N polymorphism causes an amino acid substitution at codon 167 (Lys to Asn) which is located within the C-terminal domain of the protein. Replacement of this Lys residue causes reduced binding and internal-

ization of oxLDL [16]. The association reported in the present study confirms the previously reported association of the OLR1 gene product in the pathogenesis of MI. Tatsuguchi *et al.* reported a positive association of the K167N polymorphism with the risk of myocardial infarction in a sample of Japanese patients [17]. Wang *et al.* reported that the p.K167N missense mutation in the *OLR1* gene causes plaque formation and Predazzi *et al.* studied a population that included 2141 patients and concluded that the p.K167N missense mutation in the *OLR1* gene causes plaque formation in the carotid arteries [18, 19]. However, Sakowicz *et al.* and Trabetti *et al.* cannot find any relation between G501C genotype and acute myocardial infarction [14, 20]. This discrepancy might be due to differences in the genetic background between populations.

In conclusion, in the work-up of patients, we found that patients have hypertension before the age of onset and control it after MI by use of antihypertensive drugs. LOX1 plays diverse roles in cardiovascular diseases, such as atherosclerosis and hypertension. The basal expression of LOX-1 is low, but can be enhanced by hypertension [21]. Hou *et al.* showed that polymorphisms at G501C in the LOX-1 gene is associated with susceptibility to hypertension [22]. They studied 280 patients with hypertension and 284 control subjects in China. They reported that the CC genotype of OLR-1 G501C polymorphism is associated with susceptibility to hypertension in the Chinese population. To the best of our knowledge, this is the first report to identify an *OLR1* mutation in a family with premature CAD. In this study, we found seven patients with MI among whom four patients died before the age of 50 years. Considering that parents of all affected individuals had consanguineous marriages, it is important to perform carrier screening and genetic counseling in this family and their close relatives as a prevention strategy in populations at risk. The findings in this report show that mutations in the *OLR1* gene could possibly be a common cause of early onset familial CAD and MI in patients.

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Conflict of interest

The authors declare no conflict of interest.

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