E101K and M123V alpha-cardiac actin gene mutations are not associated with cardiomyopathy in Iranian population

Asiyeh Jebelli(1), Eshrat Beyranvand(1), Hakimeh Sadeghian(2), Mohammad Ali Boroumand(2), Mehrdad Behmanesh(1)

Abstract

BACKGROUND: Cardiomyopathies are myocardial disorders in which the heart muscle is structurally and functionally abnormal. Several mutations in sarcomere protein coding genes are responsible for different types of cardiomyopathies. ACTC1 is one of the main sarcomere components in heart muscle. Two mutations of E101K and M123V in this gene are shown to be associated with cardiomyopathies.

METHODS: In this case and control study, a sample of contains 30 hypertrophic cardiomyopathy and 100 dilated cardiomyopathy patients, as well as 130 healthy individuals were screened for two mutations of E101K and M123V. The genotypes of samples were determined in whole blood genomic DNA by restriction fragment length polymorphism polymerase chain reaction (RFLP-PCR) and mismatched-PCR-RLFP techniques.

RESULTS: All patients and healthy peoples had wild type genotype for both locations and even no heterozygous was detected.

CONCLUSION: Despite previous reports, no association was observed between both mutations with cardiomyopathy. Our results indicated that two mutations of E101K and M123V of ACTC1 gene may are not associated with cardiomyopathy in Iranian population.

Keywords: ACTC1, Cardiomyopathy, Mutation, E101K, M123V

Introduction

Cardiovascular diseases (CVDs) are the main causes of death worldwide, accounting for over 16 million deaths each year. CVDs include conditions such as coronary heart disease (angina and heart attack) and stroke. The common types of CVD are cardiomyopathies.1-4

Cardiomyopathy defines as a myocardial disorder in which the structure and function of heart muscle is abnormal. Although several mechanisms reported to be involved in the development and progression of cardiomyopathies, their molecular pathophysiology is not fully understood.5 Cardiomyopathies are classified into the different groups. Hypertrophic cardiomyopathy (HCM), which is one of the most common inherited cardiac diseases, occurring about 1 in 500 people6,7 and is the main cause of sudden cardiac death in young people.8 It is characterized by thickening of the left ventricle that more common affects the septum.9,10 Common symptoms include angina, dyspnea, palpitation, syncope, and exercise limitation, which is inherited as an autosomal dominant trait.7,8

Dilated cardiomyopathy (DCM) occurs in 5-8 per 100 000 and is defined by the left ventricular dilatation and disturbed systolic function.5,9,11 The most common form of its inheritance is autosomal dominant; however, recessive, X-linked, and mitochondrial inheritances have also been reported.7,12,13 Ischemic cardiomyopathy (ICM) is the most common type of DCM. ICM is the term used to describe patients whose heart can no longer pump enough blood to the rest of their bodies.14

More than 50 genes are identified that are associated with the different types of cardiomyopathies10,15 among them; more than 200 mutations are reported to be in sarcomeric protein genes.8,16 These genes encode cardiac sarcomere proteins, components of thick and thin filaments with contractile, structural or regulatory functions (Table 1).7
It has been suggested that the α-cardiac actin gene (ACTC) deficiency plays a destructive role in cardiac function and identified to cause both HCM and DCM. ACTC1 gene spans about 7.7 K bp and contains 7 exons encoding a protein with 377 amino acids, located on 15q14 chromosome. Several point mutations in this gene seem to be associated with different types of cardiomyopathies (Table 2).

Recent studies have reported that Glu 101 Lys (E101K) mutation of ACTC1 leads to HCM, DCM and left ventricular non-compaction. Furthermore, both of the E101K and Met 123 Val (M123V) mutations cause atrial septal defects (ASD) distinguished by incomplete closure of the ostium secundum that enables blood flow between the left and right atria via the interatrial septum. Here, we investigated the association of M123V and E101K mutations with cardiomyopathy in a sample of Iranian patients and healthy individuals.

Materials and Methods

A total of 130 healthy individuals and 130 patients (30 HCM and 100 dilated cardiomyopathy) were participated in this study. All the patients and healthy controls were diagnosed based on echocardiography examination by a heart specialist. All the patients and controls were selected from Tehran Heart Center outpatient clinic from 2009 to 2011. The Medical Ethics Committee of Tarbiat Modares University, Tehran, Iran, approved the study. Written informed consents were obtained from all subjects in accordance with the declaration of Helsinki and prior to sampling. Table 3 summarizes the demographic characteristics of the patient and control individuals.

Table 1. Some prevalent genes, which their deficiencies are reported to be associated with cardiomyopathies

| Genes                        | Ref |
|------------------------------|-----|
| TNNT2 (troponin T)           | [11]|
| MYL3 (essential myosin light chain) | [15]|
| MYBPC3 (myosin binding protein C) | [11]|
| MYL2 (regulatory myosin light chain) | [15]|
| MYH7 (β-myosin heavy chain)   | [15]|
| TPM1 (α-tropomyosin)         | [12]|
| TNNI3 (troponin I)           | [14]|
| ACTC (α-cardiac actin)       | [11]|

Table 2. Common ACTC1 point mutations, reported to be associated with cardiomyopathy

| Mutations     | Ref |
|---------------|-----|
| Met 305 Leu   | [10]|
| Tyr 166 Cys   | [10]|
| Ala 295 Ser   | [10]|
| Pro 164 Ala   | [16]|
| Ala 331 Pro   | [16]|
| Glu 99 Lys    | [16,10]|
| Met 123 Val   | [12]|
| Glu 101 Lys   | [11,15]|

Genomic DNA was extracted from blood samples using a DNP™ Kit (Cinnagen, Iran). Briefly, lysis solution was used to lyse blood cells and then genomic DNA from white blood cells selectively precipitated by isopropanol. The precipitated DNA was washed and desalted by ethanol and dissolved in TE buffer and stored in −20 °C. The quantity and quality of extracted DNA were examined spectrophotometrically or visually by electrophoresis of samples on 1% agarose gel.

The value of 100 ng of purified genomic DNA used for genotyping. The genotypes for mutations of E101K and M123V were determined by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) and a mismatch PCR-RFLP method, respectively. Specific primer pairs for genotype determination of each mutation was designed by Gene Runner software (version 5, University of Wisconsin, Madison, WI, USA) and confirmed by Amplify software (Table 4). Since there was no restriction site in the M123V site, the forward primer was designed as a mismatch primer to introduce a Bcl I restriction site. E101K and M123V mutations abolish the recognition site for Ava I (Takara, Japan) and Bcl I (Fermentas, Canada) restriction enzymes, respectively. A gradient PCR used to find the best annealing temperature of 58 °C for primers in DNA amplification. The genotyping PCR was performed through following instruction: an initial denaturation at 95 °C for 5 minutes, followed by 40 cycles of denaturation at 95 °C for 30 seconds, and annealing at 58 °C for 45 seconds, extension at 72 °C for 75 seconds and a final extension of 5 minutes at 72 °C.

Table 3. Demographic and clinical characteristics of patient and control individuals

| Characteristic | Patients | Controls |
|---------------|----------|----------|
| Numbers (HCM/DCM) | 130 (100/30) | 130 |
| Age (mean ± SD) | 54 ± 5.1 | 44 ± 4.6 |
| Sex (M/F) | 98/34 | 104/26 |
| Abnormal ECG (%) | 61 | 0 |
| Family history (%) | 30 | 0 |

HCM: Hypertrophic cardiomyopathy; DCM: Dilated cardiomyopathy; ECG: Electrocardiogram; SD: Standard deviation
Table 4. Designed primers used for genotyping of selected ACTC1 mutations. The genotype for E101K mutation was examined by a PCR-RFLP technique, while the M123V mutation was determined by a mismatch PCR-RLFP. The mismatch base has been shown in bold. The primers were designed using Gene Runner Software

| Mutation | Primer | Sequence | GC% | Mw (g/mol) | The length of amplicon |
|----------|--------|----------|-----|------------|------------------------|
| E101K    | Forward| 5’-AATTATACATCTTTGGGGGAGTGG-3’ | 41.7 | 7462.7     | 708 bp                |
|          | Reverse| 5’-TAATTGTGCTCCGAAACTAACCCTC-3’ | 41.7 | 7271.6     |                        |
| M123V    | Forward| 5’-AGGCCAACCGGGAGAAGATGACCTGATC-3’ | 55.2 | 8960.7     | 290 bp                |
|          | Reverse| 5’-TAATTGTGCTCCGAAACTAACCCTC-3’ | 41.7 | 7271.6     |                        |

PCR products were digested with Ava I or Bcl I restriction enzymes in a total volume of 20 µl at 37°C or 55°C in overnight according to manufacturer’s instructions, respectively. The resulted products of digestion for E101K and M123V were analyzed by electrophoresis on a 1.2% agarose gel or 12.5% polyacrylamide gel respectively, following ethidium bromide staining. The authenticity of used techniques was investigated by sequencing the 30% randomly selected samples of both cases and control samples for each mutation.

The Chi-square test was performed by SPSS software (version 16, SPSS Inc., Chicago, IL, USA) to analysis the Hardy Weinberg equilibrium and the difference of mutation frequency between patients and healthy individuals. A conventional P ≤ 0.05 was considered significant.

Results

**E101K and M123V mutations were not detected in case and control samples**

We included 130 patients and 130 matched healthy individuals as controls in this study. In wild type genotype, Ava I enzyme cuts 708 base pair fragment into two fragments of 331 bp and 373 bp. While in the presence of E101K mutation, a change from G to A, abolishes the recognition site and the enzyme cannot cut the 708 bp fragment. In our study, the 708 bp fragments of all control and patient samples were cut into two fragments, thus, genotypes of all patients were GG (Figure 1). Table 5 shows the genotype of all control and patient samples for E101K mutation. No statistical difference was observed between the genotype and allelic frequency in patient and control groups.

In screening of genomic DNA for detection of M123V, a mismatch primer was used to introduce a Bcl I restriction enzyme including mutation nucleotide. Therefore in the case of wild type genotype, this enzyme can cut the 290 base pair amplicon to two fragments with the length of 261 bp and 25 bp (Figure 2). M123V mutation cause a change of A to G that abolishes the recognition site of enzyme and the amplicon of 290 bp remain intact (Figure 2).

**Figure 1.** The results of digestion of polymerase chain reaction (PCR) product for detection of E101K mutation in ACTC1 gene. A PCR-restriction fragment length polymorphism technique was used for the genotyping. In the case of wild type genotype Ava I enzyme cuts the 708 bp PCR product into two fragments with 331 bp and 373 bp length

**Figure 2.** The results of digestion of polymerase chain reaction (PCR) product for screening of amplicon for M123V mutation. A mismatch PCR-restriction fragment length polymorphism technique was used for genotyping of ACTC1 gene for this mutation. In presence of wild type genotype the restriction enzyme of Bcl I cuts the amplicon with 290 bp into two fragments of 261 bp and 25 bp in the length
Table 5. The genotyping screening results of patients and healthy individuals for E101K (a) and M123V (b) mutations in ACTC1 gene. All samples had wild type homozygote genotypes for both mutations.

| Category | Genotype |
|----------|----------|
| a        |          |
| E101K    | AA       | AG       | GG       |
| Healthy  | 130      | 0        | 0        |
| Patients | 130      | 0        | 0        |
| b        |          |
| M123V    | AA       | AG       | GG       |
| Healthy  | 130      | 0        | 0        |
| Patients | 130      | 0        | 0        |

Figure 3. The sequencing result of the polymerase chain reaction (PCR) products that contain two studied mutations in ACTC1 gene. The results of DNA sequencing were consistent with determined genotypes by PCR-restriction fragment length polymorphism and mismatch PCR-RLFP techniques and no mutations were detected in all of case and control samples. (A) Chromatogram of sequencing of E101K. (B) Chromatogram of sequencing of M123V.

Our results showed that all of control and patient samples were cut with Bcl I and had a wild type genotype. Thus, no significant difference was observed in genotype and allelic frequencies between the patients and healthy individuals for M123 V mutation.

Sequencing confirms the molecular analysis results

To validate the obtained results of genotyping by molecular techniques, the DNA sequences of amplicons for 30% of randomly selected of PCR products in the patients and controls groups was determined in two directions (Macrogen, Korea). The result of sequencing was compatible with obtained results from other molecular techniques (Figure 3).

Discussion

In this research, we could not detect any mutations of E101K and M123V in cardiomyopathy patients. Actions are extremely conserved proteins with 90% similarity in their amino acid sequence and constitute 10-20% of cellular proteins. The important roles of actions in cellular processes consist of muscle contraction, gene transcription, chromosome morphology, cell cycle control, modulation of a variety of membrane responses, translation of several mRNA species, and cellular apoptosis. In higher vertebrates, six main isoforms of actin have been identified. The main isoform in adult heart is ACTC1. Furthermore, the genetic damage in ACTC1 that only express in cardiomyocyte induces CVD phenotypes. Several microsatellite markers,
polymorphic repeats, and mutation were reported to affect ACTC1 function.\textsuperscript{18,19} Therefore in the present study, we investigated the association of two ACTC1 mutations (E101K and M123V) with cardiomyopathy.

Some previous studies shown that E101K mutation in ACTC1 gene is associated with apical HCM, left ventricular non-compaction and septal defects. Monserrat et al. didn’t find any mutant carrier without pathological manifestation due to high frequency of this mutation. The E101K mutation is associated with a typical ventricular morphology with some variation in the degree of wall thickening and trabeculation.\textsuperscript{18}

The second functional mutation of Glu101Lys in \(\alpha\)-cardiac actin filament locates adjacent to the myosin head and establishes a weak actomyosin-binding site. The result of this event is slower motility, reduced mediocre force, and a weak interaction with cardiac myosin in the presence of ATP. These defects at the molecular level are sufficient to trigger the disease phenotype.\textsuperscript{15,18} This mutation is usually benign, and sudden death is an exception that occurs in patients with more severe wall thickening and/or systolic dysfunction.\textsuperscript{18}

Previous studies\textsuperscript{17,19} have reported that M123V, a missense mutation in exon 2 of ACTC1 (change from A to G at cDNA position 373) have been observed in secundum ASD patients.\textsuperscript{17,19} It seems M123V mutation also prevents the assembly of actin monomers into a filamentous polymer that can support the movement by myosin. Therefore M123V actin has a reduced affinity for myosin in the presence of ATP. The M123V is located in close proximity to the Glu-101, so it seems the two polymorphisms influence similarly actin interaction with myosin.\textsuperscript{19,22} Despite previous reports for the role of two mutations E101K and M123V in pathogenesis of cardiomyopathies,\textsuperscript{15,16,18,19} we could not detect any of these in our HCM or DCM patients even in heterozygous form.

To our knowledge, this is the first study to investigate the possibility involvement of genetic variations in ACTC1 gene in pathogenesis of cardiomyopathy in Iranian population. However, according to pivotal function of actin in cardiac muscle, it’s possible that other polymorphisms or genetic changes in ACTC1 are associated with cardiomyopathy in the Iranian population.

This study suffers from some limitations such as focusing on only two mutations of E101K and M123V and low number in the case and control groups. So, further studies with higher number of samples should be performed to verify the biological role of ACTC1 deficiency in the pathogenesis of cardiomyopathy in our population.

**Conclusion**

ACTC1 is one of the main components of the sarcomeric thin filaments and is necessary for normal heart morphogenesis and cardiac muscle contraction. Although previous studies documented that genetic mutations of E101K and M123V in ACTC1 are associated with different types of cardiomyopathies, still to but our study no mutations were found in the patients with defined symptoms. Therefore, this result suggests that there is no possible association between the investigated mutations of ACTC1 with cardiomyopathy in the Iranian population. Altogether, despite limitations of this study, it is necessary to investigate for the other mutations and functional polymorphisms of ACTC1 gene in pathogenesis of heart muscle diseases in our population with larger sample size. Also, examination of genetic changes in other sarcomere protein coding genes to find the genetic basis of these genes in pathogenesis of cardiomyopathy in Iranian patients would be interesting.

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**Conflict of Interests**

Authors have no conflict of interests.

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