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Original Article

Association Between the rs538089 of the LMNA Gene and Dilated Cardiomyopathy in Iranian Patients

Hosein Ali Yousefi[1], MS; Nastaran Asghari Moghaddam[1*], PhD; Mehrdad Jafari Fesharaki[2], MD

ABSTRACT

Background: Dilated cardiomyopathy (DCM) is one of the most common causes of heart failure. More than 40 genes with different strengths are involved in its pathogenesis. The second most important gene in DCM pathogenesis is the *LMNA* gene. *LMNA* has 12 exons and encodes Lamin A and Lamin C. This study aimed to screen any mutation that occurs in exons 4 and 5 of this gene in patients suffering from DCM.

Methods: Thirty patients with DCM were enrolled in this study. A control group was formed from 30 normal participants. After DNA extraction, polymerase chain reaction (PCR) was performed to amplify desired DNA fragments. Then, the amplified fragments were sequenced via the Sanger technique. The obtained sequences were statistically analyzed using the SPSS software, version 24.

Results: In exon 5, in 23.3% (n = 7) of the patients, 1 substitution mutation (c.861 T>C; rs538089) was detected. All the patients were heterozygous for this variant. The frequency for mutated alleles was significantly higher in the patients than in the normal controls ($\chi^2 = 4.821$; $P = 0.028$). No mutation was observed in exon 4 both in the patient and control groups.

Conclusions: Although rs538089 is a synonymous mutation, its predominant existence in the *LMNA* gene of our patients was interesting, as was its association with the female gender. It could be assumed that this variant may play a potential role in DCM. ***(Iranian Heart Journal 2020; 21(4): 103-110)***

KEYWORDS: Dilated cardiomyopathy, LMNA, rs538089

[1] Department of Biology, Islamic Azad University, Central Tehran Branch, Tehran, IR Iran.
[2] Department of Cardiology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, IR Iran.

***Corresponding Author:** Nastaran Asghari Moghaddam, MD; Department of Biology, Islamic Azad University, Central Tehran Branch, Tehran, IR Iran.
Email: nas.asgharimoghaddam@iauctb.ac.ir

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Cardiomyopathies are defined as myocardial disorders frequently causing heart failure and death. [1] Three subtypes have been described according to their phenotypes: dilated cardiomyopathy (DCM), hypertrophic cardiomyopathy, and restricted cardiomyopathy. [2] DCM is the most prevalent kind of cardiomyopathy in that it accounts for between 30% and 40% of all cases of heart failure; further, it is the leading

cause of heart transplantation. [3–5] DCM is characterized by left ventricular enlargement and systolic dysfunction without the existence of coronary disease, valvular disease, or hypertension. [6,7]

DCM can develop in either gender, at any age, and in individuals from any ethnic group. [8] Although in a high percentage of DCM cases, the etiology remains unknown, it is estimated that nearly 30% of idiopathic DCM cases are familial. [9] It is now well-established that genetics is of great importance in the etiology of this disease. [10] Studying the genetics of familial DCM has led scientists to hypothesize that both sporadic and familial cases of DCM have genetic bases. If this assumption is true, examining DCM via the genetic approach could be beneficial for the diagnosis, prevention, and management of DCM. [9]

Genetic heterogeneity is common in DCM more than in the other types of cardiomyopathy. It has been estimated that more than 40 genes contribute to the development of DCM. [10] The most common inherited form of DCM is autosomal dominant, and it usually commences in the second or third decade of life. [11-15] One of the most reviewed genes involved in the progression of DCM is *LMNA*: it accounts for 5% to 8% of familial cases. [16,17] The *LMNA* gene is located on 1q22 (ID: 4000); it consists of 12 exons and encodes nuclear proteins Lamin A and Lamin C. [18] Lamin filaments are responsible for the integrity and stability of nuclear membranes and nuclear pores. [19] In addition, in association with LEM proteins, lamins serve as a scaffold to facilitate proper gene expression and to assist efficient DNA repair. [20] Three polypeptides, namely Lamin A (664 amino acids), Lamin B, and Lamin C (574 amino acids), compose lamina filaments. Lamin B is produced by a gene, *LMNB*. Lamin A and Lamin C are encoded by a single gene, *LMNA*, via alternative splicing. [21] Through the disruption of the integrity of the lamina, the aforementioned processes become dysregulated and may have disastrous outcomes. [20] More than 460 mutations in the *LMNA* gene have been reported; they cause at least 10 different diseases called "laminopathies" in patients carrying them. [22] *LMNA* is considered to be the second most important gene involved in DCM development, with often an autosomal dominant pattern of inheritance. [23] Pathogenic variants of *LMNA* account for 7.5% of familial and up to 11% of sporadic cases. To date, 114 pathogenic variants in *LMNA* have been shown to be associated with DCM. Extensively variable phenotypes can be detected in patients with DCM carrying the pathogenic variants of *LMNA*; nonetheless, the existence of an alteration in the conduction system has been frequently observed. Normally, Lamin A/C DCM has poor prognoses and serious cardiovascular complications and frequently necessitates heart transplantation. [12]

To evaluate the significance of mutations in the *LMNA* gene in patients suffering from DCM in Iran, we performed the present study with the primary aim of determining whether a mutation in exons 4 and 5 of *LMNA* could be causative and whether a novel variant would be responsible for DCM in Iranian patients.

METHODS

Clinical Assessment

In the current case-control study, 30 patients were blindly selected after a diagnosis of DCM by physicians in keeping with the recommendations of the World Health Organization. [24] The investigation included chest X-ray, electrocardiography, and echocardiography (as the gold standard), as well as coronary angiography (for patients aged > 35 y). Patients with a history of hypertension, valvular disease, or ischemic heart disease were excluded. Thirty healthy individuals were chosen as members of the control group after medical examinations in

order to confirm the absence of heart disease. They were selected by specialists from among patients who were admitted to Masih Daneshvari, Shahid Labbafi-Nejad, and Imam Hossein hospitals in Tehran, Iran. The participants completed an informed consent form voluntarily. The sample collection period lasted 6 months (from July to December 2018). The study was designed and conducted in accordance with the Helsinki declaration. This project received the ethical approval number of IR.SBMU.MSP.REC.1396.701.

Genetic Analysis

The sample under investigation comprised 5 mL of the peripheral blood of the participants. The blood samples were transported in ice from the hospital to the laboratory in sterile vials containing EDTA and stored at −20 °C. The isolation of genomic DNA was performed using a KBC kit® as the protocol provided. The process was followed by testing the quality and quantity of the extracted DNA via gel electrophoresis and spectrophotometry using the NanoDrop (Berthold). For the amplification of the exons 4 and 5 of the *LMNA* gene, 2 sets of primers were used. The Gene Runner software, version 3.05, (Hastings Software Inc, USA) was used to design the primers. The NCBI Primer-Blast was employed to check the primers for specificity. The sequences of the designed primers for exon 4 were as follows: 5'-GGGTGATGACAGACTTGGGC-3' (forward) and 5'-AGTTGGGCATCACTGCTAC-3' (reverse) with the product size of 560 bps. The primers for the amplification of exon 5 had the following sequences: 5'-CCCTACCCTTACCCACGCTG-3' (forward) and 5'-TGACTCCACATCCTGCGACC-3' (reverse), which produced a 456-bp fragment. The 25-µL reaction mixture consisted of 1.0 µL of genomic DNA, 0.5 µL of both forward and reverse primers, and 12.5 µL of the Ampliqon *Taq* 2X master mix. Afterward, the amplification of the desired *LMNA* exons was done using the SensoQuest LabCycler (Germany) polymerase chain reaction (PCR) condition, consisting of an initial denaturation at 95 °C for 45 seconds, followed by denaturation at 95 °C for 30 seconds, annealing at 63 °C for 40 seconds, and extension at 72 °C for 30 seconds. The process was repeated for 30 cycles, and the final extension was done at 72 °C for 1 minute. In the next step, the amplified fragments of DNA were sent to Macrogen (South Korea) for sequencing via the Sanger sequencing method.

Statistical Analysis

All the statistical analyses were performed using the SPSS software, version 24, (SPSS Inc, Chicago, IL, USA). The parametrical variables were presented as the mean ± the standard deviation (SD). The normal distribution of the continuous variables was calculated using the Kolmogorov–Smirnov test and the Shapiro–Wilk test, and the post hoc Mann–Whitney test was utilized for the variables with non-normal distributions. The categorical variables were displayed as percentages. The differences between the groups were assessed using the Pearson χ^2 test. For the analysis of the Hardy–Weinberg equilibrium in the distribution of genetic polymorphisms, the Pearson χ^2 test was applied. Odds ratios (ORs) were given with their respective 95% confidence intervals (CIs). For the estimation of the dominant or recessive effects of the found alleles on DCM risk, a multiplicative model was used. A *P*-value of less than 0.05 was considered statistically significant.

RESULTS

Clinical Status

Our study population was comprised of 30 patients with DCM who were randomly selected from 3 hospitals regardless of their age and 30 healthy individuals. The patients were aged between 21 and 75 years. The

average age of the entire study population was 51.63 ± 13.89 years. Gender impact on the study was avoided through the participation of equal numbers of female and male subjects in the research (15 men and 15 women). Fifteen patients declared a familial history of heart conditions, including heart attacks, DCM, atherosclerotic cardiovascular disease, hypertension, and mitral insufficiency. The average age at disease onset was 43.83 ± 16.98 years. The minimum age at disease onset was 6 years. Among the patients participating in the current study, 8 (26%) cases were diabetic (P = 0.009). All the patients received medication. Four (13%) cases had undergone heart transplantation before the commencement of the current study. Three other patients (10%) had implanted pacemakers. The members of the control group were chosen based on the age and gender of the corresponding subjects in attending in the patient group. The demographic data of the study population are presented in Table 1.

Table 1: Characteristics of the cases carrying both TC and TT genotypes

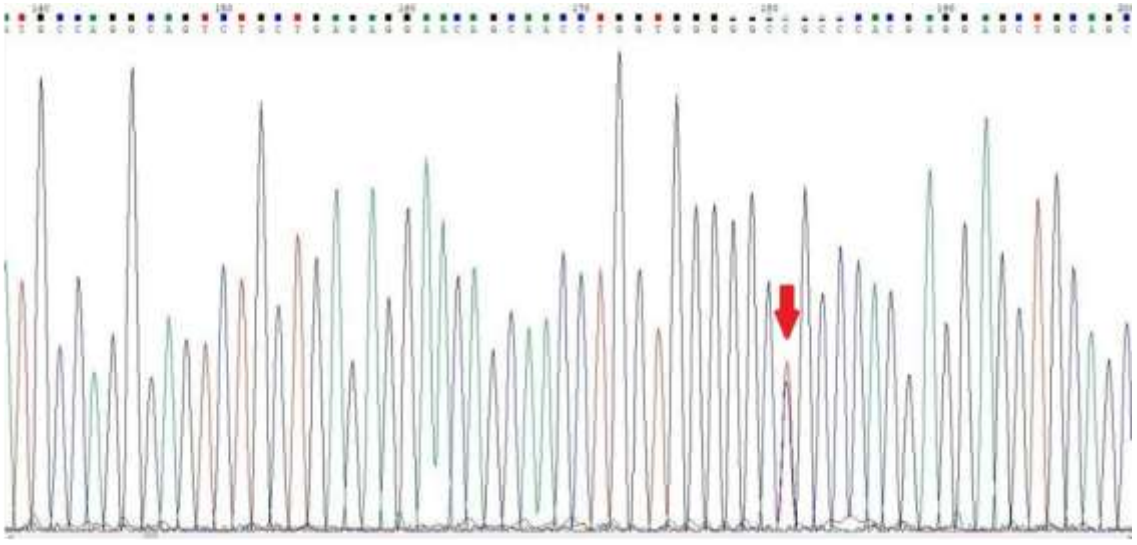
Characteristic	Patients Carrying TC Genotypes	Patients Carrying TT Genotypes
Age at disease onset, y	39	45.3
Male, n (%)	2 (28.6%)	13 (56.5%)
Female, n (%)	5 (71.4%)	10 (43.5%)
Diabetes, n (%)	2 (28.6%)	6 (26.1%)
Implanted pacemaker, n (%)	1 (14.3)	3 (13%)
Heart transplanted, n (%)	2 (28.6%)	4 (17.4%)
Family history of heart disease, n (%)	4 (57.1%)	11 (47.8%)

Table 2: Genotype frequencies among the cases and controls

Genotype	Patients (n%)	Controls (n%)	OR (95% CI)	*P*- value
TT	23 (76.7%)	29 (96.7%)	0.224 (0.035 to 1.424)	0.023
TC	7 (23.3%)	1 (3.3%)	1.978 (1.323 to 2.958)	

Table 3: Allele frequencies among the cases and controls

Allele	Patients (n%)	Controls (n%)	OR (95% CI)	*P*- value
T	59 (98.3%)	1 (1.7%)	0.237 (0.038 to 1.497)	0.028
C	53 (88.3)	7 (11.7%)	1.849 (1.334 to 2.564)	



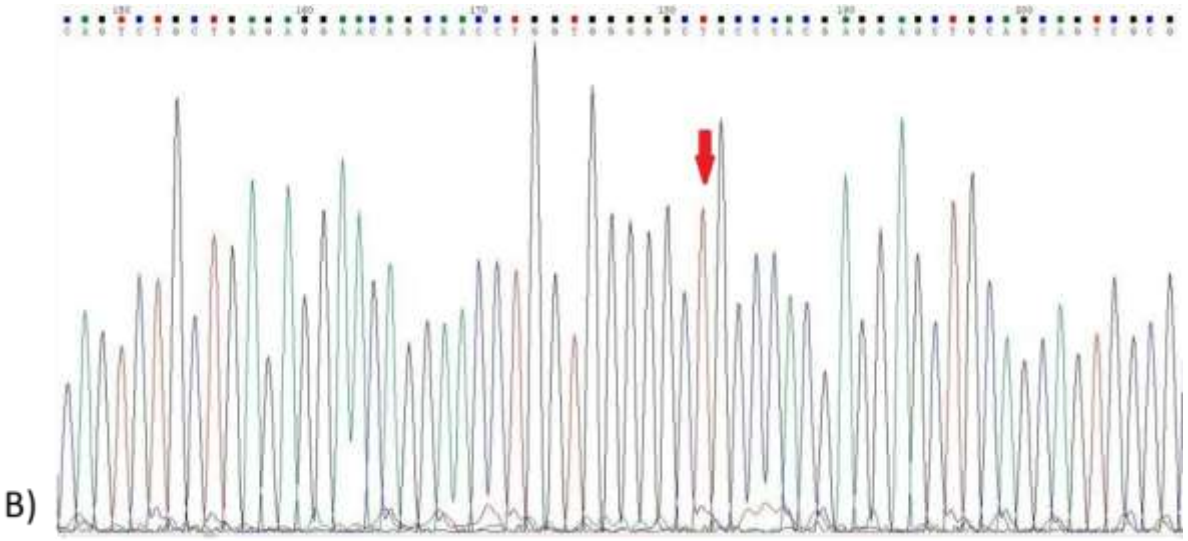


Figure 1: A) Chromatogram of a heterozygous TC patient and B) chromatogram of a homozygous TT patient are illustrated herein.

Genetic Analysis of *LMNA*

The genetic analysis of the participants was conducted by surveying exons 4 and 5 and their immediate intronic regions of *LMNA*. The desired fragments of the given gene were amplified by PCR and sequenced via the Sanger method (Fig. 1). In exon 4 and its surrounding intronic regions, no mutation was found; however, in exon 5, in 7 cases and 1 control, 1 substitution mutation was observed. The mutation was c.816T>C, which is known as "rs538089" and is a synonymous mutation (A287A). No homozygous genotypes were observed for the given mutation in this study. Table 2 summarizes the characteristics of the patients' genotypes and depicts the distribution of the cases and controls by heterozygous genotype.

In our study, genotype-carrying polymorphic alleles were significantly correlated with women suffering from DCM (P = 0.021). No relationship was found between DCM in patients with rs538089 and diabetes (P = 0.7). Concerning the existence of this variant in ethnic groups, it can be concluded that rs538089 could not be assigned to a specific ethnic group (P = 0.894).

The frequency of TC heterozygous genotype was 0.13 in the whole cohort and 0.23 in the patient group, which was statistically significant (P = 0.023). The frequency of pathologically suspected alleles was 0.067. The calculated OR using the χ^2 and logistic regression for the TC genotype was 8.826 (95% CI: 1.012 to 76.960), exhibiting the role of the heterozygous genotype in the probability of DCM phenotype appearance. The genotype distributions were consistent with the Hardy–Weinberg equilibrium in the whole population and in the case and control groups, separately. The frequencies of the related allele in the patients and the healthy controls are represented in Table 3. The frequency of the T allele and the C allele in the study population was 93.3% and 6.7%, respectively. The estimated OR was 7.792 (5% CI: 0.928 to 65.433), showing the pathogenesis potency of the C allele in inducing DCM.

DISCUSSION

Lamins are structural proteins that stabilize the inner nucleus membrane and also play a crucial role in the regulation of gene expression. [20] This gene codes 2 related

protein Lamin A and Lamin C by alternative splicing. The 2 proteins share a common structure that consists of 3 domains: head, central rod, and carboxy-terminal tail. [21] Mutation in the *LMNA* gene is involved in at least 10 different allelic disorders termed "laminopathies", including Emery–Dreifuss muscular dystrophy, an inherited disorder causing heart and skeletal muscle abnormalities. [21] It is known that mutations in *LMNA* play a role in DCM pathology (5–8% of familial cases). [25] Moreover, patients with mutations in the *LMNA* gene tend to show progressive heart failure and variable involvements of skeletal muscles. [17] By considering these backgrounds, in the current research, we investigated the existence of mutations in exons 4 and 5 of *LMNA* in patients with DCM to shed a light on the prevalence of *LMNA*-related DCM in patients of Iranian origin.

The only mutation observed in exons 4 and 5 was a T-to-C substitution (rs538089). This polymorphism occurs in codon 287, and it is a substitution at the third base of the codon. Because it does not change the amino acid, the protein is not altered. It is located in the central rod-domain coding sequence. The possible pathogenic variant that has C in this site also has been seen in at least 3 other studies on *LMNA*-related DCM. One of these investigations was conducted on familial DCM in Germany, and the allele was observed in both control and familial DCM cases. [26] Another report on this polymorphism in DCM came in a study done in France. [27] A research similar to ours was the work of Banerjee et al, [28] who reported this polymorphism alongside other polymorphisms in the *LMNA* of 10 patients.

The novelty of our study is that only the heterozygous genotype was observed in the patient group. The negative effect of this polymorphism may be observed if 2 copies of the given allele occur in the loci of *LMNA*. The query that remains is why a synonymous mutation is related to the disease. The polymorphism might not be harmful by itself, but it could be accompanied by another menacing polymorphism as a haplotype. To examine this hypothesis, researchers need to screen the other exons of *LMNA* and the regions in its vicinity, too.

Another fascinating finding in the present study was the association between rs538089 and female patients. Because most of the mutations in *LMNA* affect skeletal muscles, further research is required on the condition of muscles in patients with *LMNA*-related DCM.

The goal of genetic testing is to determine the probability of the occurrence of a given disease at the early stage and to prevent or manage the illness more appropriately. To that end, we performed the present study on mutations in the *LMNA* gene among Iranian patients suffering from DCM. Such genetic tests on the *LMNA* gene could be done in the relatives of patients to confirm hereditary penetrance. Finally, it can be concluded that the rs538089 polymorphism indirectly plays a significant role in the pathogenesis of DCM; still, this conclusion needs confirmation through research on a large cohort of patients with DCM.

There are some limitations to the present study, the most notable of which is its small sample size. Larger case and control groups could confer a better statistical estimation of the role of the found mutation among patients with DCM. Another salient weakness is the lack of information about other exonic mutations in our cases. Our team is currently studying the other exons of the *LMNA* gene, and the results will be published in due course.

CONCLUSIONS

In the current study, the rs538089 polymorphism was significantly associated with DCM in a group of Iranian patients.

Although this variant does not directly cause any alteration in lamina protein, it may be accompanied by another DNA sequence change in the *LMNA* gene, causing DCM. Further research on this polymorphism in Iranian patients will definitely be more informative.

Conflict of Interest

The authors declare no conflicts of interest.

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