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Novel linkage of *LMNA* Single Nucleotide Polymorphism with Dilated Cardiomyopathy in an Indian case study

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ABSTRACT

Background: Dilated Cardiomyopathy (DCM) is one of the most commonly encountered heart diseases reported globally. It is characterized by enlarged ventricles with impaired systolic and diastolic functions. Mutations in *LMNA* gene are one of the causative factors to precipitate the disease. However, association of SNPs of *LMNA* with DCM in particular has not been well documented.
Method: Here we present a limited and restricted case study of patients from south eastern part of India afflicted with idiopathic DCM and conduction defects. By using next generation sequencing we have sequenced the exons of *LMNA* gene from genomic DNA isolated from patients.
Result: We have identified the linkage of 8 different *LMNA* SNPs with idiopathic DCM viz. rs121117552, rs538089, rs505058, rs4641, rs646840, rs534807, rs80356803 and rs7339. These SNPs are scattered throughout the gene with prevalence for the region encoding the central rod domain of lamin A/C.
Conclusion: Most of these SNPs in *LMNA* were previously reported to be involved in various disorders other than DCM. We conclude that, variation in *LMNA* is one of the major underlying genetic causes for the pathogenesis of DCM, as observed in few Indian populations.

1. Introduction

Dilated Cardiomyopathy (DCM) is a disease of the heart muscle which is characterized by ventricular dilation and reduced myocardial contractility thereby resulting into impaired systolic and diastolic function [1]. Clinical symptoms include heart failure, thromboembolism and sudden cardiac death. DCM is the most common among the five commonly characterized cardiomyopathies. It has an estimated prevalence of 1/2500 among different populations [2,3]. The pattern of the disease inheritance is mostly autosomal dominant [4]. However, genetic screening shows only 30–35% familial DCM follow the Mendelian mode of disease inheritance [5] while the remaining idiopathic origin DCM follows a complex multivariant origin. Extensive epidemiological studies are still limited to US, European, and Australian populations and also to some Asian countries like Japan, China and Korea where a total of 165 *LMNA* mutations have been reported (http://www.umd.be/LMNA/) [6–13].

More than 40 genes have been reported so far to be associated with the pathogenesis of DCM which is a heterogeneous disease [14]. 6% of all DCM cases are caused by mutations in lamin A/C gene (*LMNA*). Fatkin et al. in 1999 [15] first showed the involvement of *LMNA* mutations with DCM and conduction diseases. All these studies suggest that the *LMNA* related DCM patients portend a high risk of sudden cardiac death.

LMNA consists of 12 exons and encodes two splice variants lamin A and C which maps in the long arm of chromosome 1 (1q21.2–q21.3) [16]. Mutations in *LMNA* are known to cause a wide spectrum of diseases other than DCM, in a tissue specific manner collectively termed as laminopathies namely Lipodystrophy, Limb–girdle muscular dystrophy, Emery–Dreifuss muscular dystrophy and many more [17,18]. Most *LMNA* mutations causing striated muscle disorder are missense mutations distributed throughout all the exons of the gene. Along with mutations in *LMNA*, various Single Nucleotide Polymorphisms (SNPs) in *LMNA* were reported to be associated with different disorders other than laminopathies. A total of 40 *LMNA* SNPs are reported in Leiden Open Variation Database (www.dmd.nl/lmna_seqvar.html). Out of 40, 30 are silent mutations and the rest are missense mutations [19]. A frequently occurring *LMNA* SNP, rs4641 at exon 10 was found to be associated with various disorders such as adipose tissue metabolism and obesity related phenotypes [20–22]. In spite of having such a high prevalence rate, still *LMNA* related DCM patients suffer from poor

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prognosis [11,23], high risk of sudden cardiac death and life threatening arrhythmias. The underlying cause of DCM due to *LMNA* mutations is still largely unknown and it lacks proper genotype–phenotype correlation. Therefore, the severity of *LMNA* mutations or variations in DCM patients calls for the genetic testing of *LMNA* in patients for early prognosis and to clinically manage complications of the disease on a wider population.

Increasing number of patients in West Bengal, India is diagnosed with DCM each year which is a serious health concern. Patients come to the clinic complaining of respiratory distress, cough and chest pain, edema of distal extremities, palpitations and syncopal or presyncopal attack. A combination of investigations according to the recommendations of American Heart Association (AHA) [2] and World Health Organization (WHO) [24] form the major diagnostic approaches for DCM patients. We have specifically focused into such a tertiary care center at Kolkata-N.R.S. Medical College and Hospital which receives a number of patients from Kolkata and its surrounding districts afflicted with DCM. We have confined our studies on IDCM. We report for the very first time the association of *LMNA* SNPs with IDCM patients of eastern zone of India. Through genetic analysis we have revealed the association of 8 different *LMNA* SNPs with IDCM patients. Among these 8 SNPs, SNPs rs538089, rs505058, and rs4641 were previously reported to be associated with DCM in French population [25]. The rests of the SNPs rs121117552, rs646840, rs534807, rs80356803, and rs7339 were hitherto reported for other diseases but not DCM.

2. Materials and methods

2.1. Clinical assessment and screening of subjects for DCM

The clinical investigation and management of DCM started with the acquisition of patient's history on admission. Following the history of the patients the physicians would diagnose for DCM and screen them, following the recommendations of AHA [2] and WHO guidelines [24]. The investigations included Chest X-ray, ECG and echocardiography and coronary angiography (if needed). Echocardiography is still regarded as the gold standard for diagnosis. Written informed consent was obtained in accordance with the study protocol approved by the local ethical committee. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki. A cohort of 10 unrelated patients with diagnosed IDCM and suitable 12 control individuals were selected for our study from N.R.S. Medical College and Hospital, Kolkata, India.

2.2. Isolation of genomic DNA from peripheral blood samples

4–5 ml of blood was drawn from the *antecubital* vein and transported in ice from the hospital to the laboratory in a 6 ml sterile EDTA containing vial. The blood was then transferred into the 15 ml polypropylene conical centrifuge tube and the volume was adjusted to 15 ml by adding RBC lysis buffer (150 mM NH_4Cl, 1 mM $NaHCO_3$) followed by incubation at room temperature for 15 min. The cells were pelleted at 3000 rpm in a clinical centrifuge. This step was repeated 3–4 times until WBC was found. The supernatant was carefully decanted and 3 ml of nucleic acid lysis buffer (10 mM TRIS pH 8, 400 mM NaCl, 2 mM Na_2EDTA, SDS 0.5%) was added followed by the addition of 100 μl proteinase K (10 mg/ml) and vortexed. The sample was then incubated at 56 °C for 1–2 h. Then equal volume of water saturated phenol was added and mixed. The mixture was centrifuged at 12,000 rpm for 12 min and this step was repeated 2–3 times followed by the addition of equal volume of chloroform. It was centrifuged at 12,000 rpm for 12 min and the supernatant was transferred to a fresh tube to which 1/10 volume of 10 M ammonium acetate and 2.5 times ice cold 100% ethanol was added and mixed gently until the precipitate formed. It was then centrifuged at 12,000 rpm for 12 min and the supernatant discarded. The pellet that was formed was washed with 1 ml ice-cold 70% ethanol. After centrifugation the ethanol was carefully aspirated as not to dislodge the pellet which was air dried and subsequently dissolved in 300–500 μl of TE buffer. This constituted the genomic DNA.

2.3. Genetic testing

Genomic DNA isolated from peripheral lymphocytes of subjects was used as a template for genetic testing of the *LMNA* gene. The *LMNA* gene was amplified using 29 sets of primers which cover the entire coding region of the *LMNA* (Table 1); 12 coding exons as well as the immediate intronic regions. Sequencing was performed in Ion Personal Genome Machine® (PGM™) System using Ion PGM™ Sequencing 200 Kit v2 (following the manufacture's protocol). The SNPs obtained from next generation sequencing were further validated by Sanger sequencing. The 12 sets of primers reported in Perrot et al. [9], were used for sequencing the desired exons of *LMNA* by Sanger sequencing.

3. Results

3.1. Clinical status

A cohort of 10 subjects coded by S1 through S10, diagnosed with IDCM, from a tertiary care center at Kolkata-N.R.S. Medical College and Hospital were selected randomly irrespective of age and sex. The age group in the cohort varied from 12 to 80 years. The presence of the disease was classified as sporadic in all the patients. Subjects diagnosed with DCM usually showed symptoms of respiratory distress on exertion and also on rest, cough, fatigability and edema. Irregular pulses, narrow pulse pressure, atrial fibrillation, and elevation of jugular venous pressure were routinely observed. Cardiac examinations of the decompensated DCM patients revealed muffled heart sound with gallop rhythm (LVS3 or RVS3). The apex was down and out and there was the presence of systolic murmur at apex which was indicative of mitral regurgitation. Chest X-ray in patients revealed cardiomegaly with or without pulmonary congestion. ECG showed the following abnormalities – LBBB (left bundle branch block), LVH, ST-T and atrial ectopic, ventricular ectopic and also atrial fibrillation as shown in Fig. 1. Echocardiography which is regarded as the gold standard showed dilatation of cardiac chambers, generalized hypokinesia, low ejection fraction and occasional mitral and tricuspid regurgitation as shown in Fig. 2. Detailed clinical statuses of DCM patients are summarized in Table 2.

3.2. Genetic analysis of LMNA

Genetic analysis of the subjects was performed by screening 12 coding exons as well as the immediate intronic regions of *LMNA* gene, which were amplified by PCR using 29 sets of primers as detailed in Table 1. Using next generation sequencing technique, we identified the association of 8 different *LMNA* SNPs with 5 out of 10 DCM subjects in our study. All the subjects were found to be heterozygous for the identified SNPs. The identified *LMNA* SNPs (as shown in Table 3) were (a) rs121117552 c.612G>A L204L located at exon 3, (b) rs538089 c.816T>C A287A located at exon 5, (c) rs505058 c.1338T>C D446D located at exon 7, (d) rs4641 c.1698 C>T H566H located at exon 10 (e) rs646840 c.937-83G>T located in the intron region, (f) rs534807 c.1157+16G>A also residing in the intron region, (g) rs80356803 c.128T>C located in 5′UTR and (h) rs7339 c.76G>C located in the 3′ UTR (Fig. 3). Out of the 10 patients surveyed, we observed 4 cases viz. S1, S3, S7 and S9 with multiple *LMNA* SNPs. Patient S1 scored maximum for 3 SNPs rs505058, rs646840 and rs534807 and remaining 3 patients with 2 SNPs each. However, SNP rs534807 was also identified in another patient named S3. Patient S3 also harbored rs4641 (Table 3). rs4641 was the most frequently encountered SNP in this cohort, which was identified in three different individuals S3, S4 and S9 (Table 3). SNP rs121117552 was identified in one single patient named S8; rs538089