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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

Table 1
Primers for Ion PGM™ sequencing.

Primer ID	Start position (Without the common tail)	End position	Amplicon Id	Start position	End position
*LMNA*_PP1_1F	156084537	156084556	*LMNA*_PP1_1	156084557	156084748
*LMNA*_PP1_230R	156084749	156084767			
*LMNA*_PP1_162F	156084698	156084716	*LMNA*_PP1_162	156084717	156084906
*LMNA*_PP1_390R	156084907	156084926			
*LMNA*_PP1_346F	156084882	156084900	*LMNA*_PP1_346	156084901	156085090
*LMNA*_PP1_574R	156085091	156085110			
*LMNA*_PP2_1F	156100366	156100394	*LMNA*_PP2_1	156100395	156100550
*LMNA*_PP2_202R	156100551	156100567			
*LMNA*_PP2_52F	156100417	156100439	*LMNA*_PP2_52	156100440	156100600
*LMNA*_PP2_267R	156100601	156100633			
*LMNA*_PP3_1F	156104135	156104160	*LMNA*_PP3_1	156104161	156104365
*LMNA*_PP3_54F	156104187	156104208	*LMNA*_PP3_54	156104209	156104365
*LMNA*_PP3_250R	156104366	156104385			
*LMNA*_PP4_1F	156104537	156104559	*LMNA*_PP4_1	156104560	156104723
*LMNA*_PP4_210R	156104724	156104751			
*LMNA*_PP4_122F	156104659	156104679	*LMNA*_PP4_122	156104680	156104852
*LMNA*_PP4_334R	156104853	156104872			
*LMNA*_PP5_1F	156104895	156104916	*LMNA*_PP5_1	156104917	156105093
*LMNA*_PP5_220R	156105094	156105115			
*LMNA*_PP5_183F	156105074	156105094	*LMNA*_PP5_183	156105095	156105304
*LMNA*_PP5_230F	156105123	156105146	*LMNA*_PP5_230	156105147	156105304
*LMNA*_PP5_432R	156105305	156105326			
*LMNA*_PP6_1F	156105515	156105540	*LMNA*_PP6_1	156105541	156105739
*LMNA*_PP6_242R	156105740	156105757			
*LMNA*_PP6_204F	156105715	156105738	*LMNA*_PP6_204	156105739	156105958
*LMNA*_PP6_466R	156105959	156105981			
*LMNA*_PP7_1F	156105981	156106000	*LMNA*_PP7_1	156106001	156106170
*LMNA*_PP7_210R	156106171	156106190			
*LMNA*_PP7_81F	156106052	156106081	*LMNA*_PP7_81	156106082	156106251
*LMNA*_PP7_291R	156106252	156106271			
*LMNA*_PP8_1F	156106608	156106626	*LMNA*_PP8_1	156106627	156106797
*LMNA*_PP8_208R	156106798	156106815			
*LMNA*_PP8_82F	156106688	156106709	*LMNA*_PP8_82	156106710	156106871
*LMNA*_PP8_286R	156106872	156106893			
*LMNA*_PP9_1F	156106868	156106887	*LMNA*_PP9_1	156106888	156107040
*LMNA*_PP9_192R	156107041	156107059			
*LMNA*_PP10_1F	156107323	156107342	*LMNA*_PP10_1	156107343	156107531
*LMNA*_PP10_228R	156107532	156107550			
*LMNA*_PP10_147F	156107463	156107483	*LMNA*_PP10_147	156107484	156107652
*LMNA*_PP10_350R	156107653	156107672			
*LMNA*_PP10_230F	156107552	156107570	*LMNA*_PP10_230	156107571	156107756
*LMNA*_PP10_459R	156107757	156107788			
*LMNA*_PP11_1F	156108192	156108209	*LMNA*_PP11_1	156108210	156108396
*LMNA*_PP11_223R	156108397	156108415			
*LMNA*_PP11_176F	156108367	156108384	*LMNA*_PP11_176	156108385	156108547
*LMNA*_PP11_381R	156108548	156108575			
*LMNA*_PP11_241F	156108432	156108451	*LMNA*_PP11_241	156108452	156108637
*LMNA*_PP11_465R	156108638	156108657			
*LMNA*_PP12_1F	156108478	156108495	*LMNA*_PP12_1	156108496	156108677
*LMNA*_PP12_225R	156108678	156108763			
*LMNA*_PP12_161F	156108638	156108657	*LMNA*_PP12_161	156108658	156108828
*LMNA*_PP12_371R	156108829	156108848			
*LMNA*_PP12_301F	156108788	156108801	*LMNA*_PP12_301	156108802	156108977
*LMNA*_PP12_519R	156108978	156108997			
*LMNA*_PP12_469F	156108946	156108964	*LMNA*_PP12_469	156108965	156109136
*LMNA*_PP12_682R	156109137	156109159			

and rs7339 was found once in patient S7; rs80356803 along with rs4641 were found once in S9 (Table 3). All these SNPs were absent in 12 healthy controls except rs4641 which was identified in one control individual. The loci of the SNPs have been depicted in the schematic diagram of the protein demarcating their respective positions (Fig. 3).

Results obtained from next generation sequencing were further validated by Sanger sequencing. The desired exons of *LMNA* were amplified by PCR as reported in Perrot et al. [9] and were sent for Sanger sequencing. The SNPs residing in the exons of *LMNA* were all confirmed from the Sanger sequencing, 2 representative chromatograms showing the presence of polymorphism at the desired region are shown in Fig. 4.

4. Discussion

Laminopathies are principally caused by sporadic mutations in *LMNA* gene in individuals. Nevertheless, numerous SNPs have been reported from population based studies which are known to cause specific laminopathies like Charcot–Marie–Tooth Disorder, familial partial Lipodystrophy and Emery–Dreifus Muscular dystrophy (EDMD) [17,18]. 165 *LMNA* mutations have been known to be associated with DCM till date based on studies from Europe, USA and some South East Asian countries but, there are no reports till date establishing any association of SNPs in *LMNA* with IDCM barring one [25]. However, in a recent report from a Genome Wide Association Study (GWAS) with DCM patients identified two different loci 1) rs10927875 and

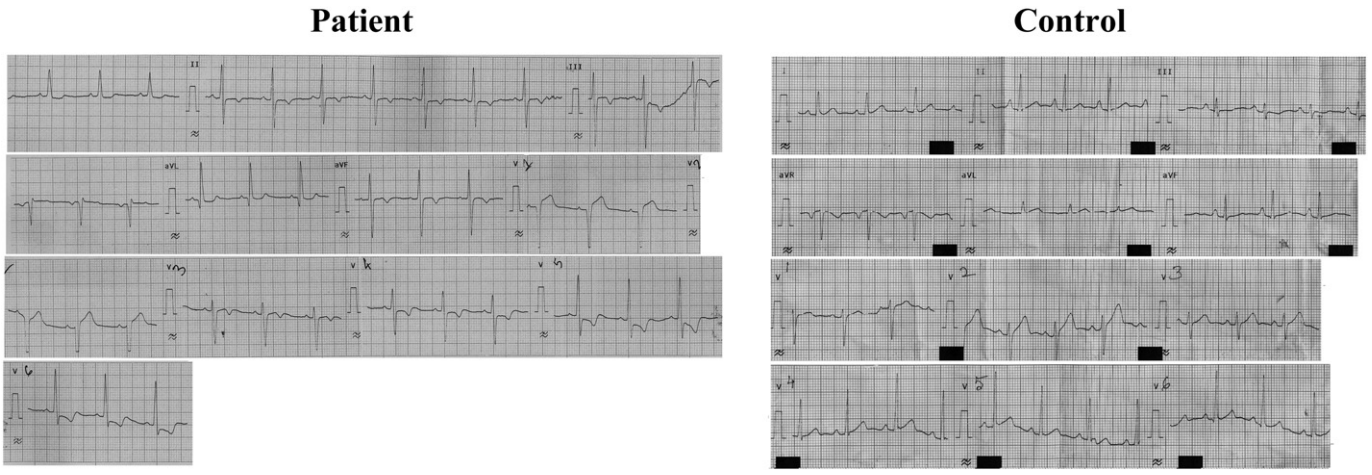

Fig. 1. A representative ECG of a DCM patient and a control subject. ECG of the patient showed features of LVH with strain pattern.

2) rs2234962. rs10927875 maps to a region on chromosome 1p36.13. This region encompasses several genes among which *HSPB7* was previously suggested to be implicated in DCM whereas rs2234962 was a non-synonymous SNP (c.T757C, p. C151R) which is located within the sequence of *BAG3* on chromosome 10q26 [26]. To date, there are very few reports regarding the genetic background of *LMNA* in Indian population [27,28]. In the present study, we showed for the first time the association of variations in *LMNA* gene with IDCM in Indian population. We identified 8 *LMNA* SNPs in subjects with IDCM in a small cohort of Indian sub-population. All these SNPs were shown to be associated with various other disorders in ethnically distinct population but not with DCM.

We identified 4 different SNPs which reside in the exon region of *LMNA*; the affected exons are 3, 5, 7 and 10 at codon positions 204, 287, 446 and 566 respectively. SNP rs12117552 located in exon 3 of *LMNA* which was identified in one patient of our study was first reported to be associated with Werner's syndrome in an African-American female [29]. rs12117552 in exon 3 which corresponds to silent substitution from G to A at the third base of codon 204 results in same sense variation (rs121117552; L204L). This exon also harbors a mutation hotspot at codon 190, which is critically linked with DCM [9,10]; and thus justifies the development of DCM in patients harboring this variation. Previous reports state the involvement of SNP rs538089 which mapped at exon 5 in *LMNA* with both Werner's syndrome as well as in a subtype

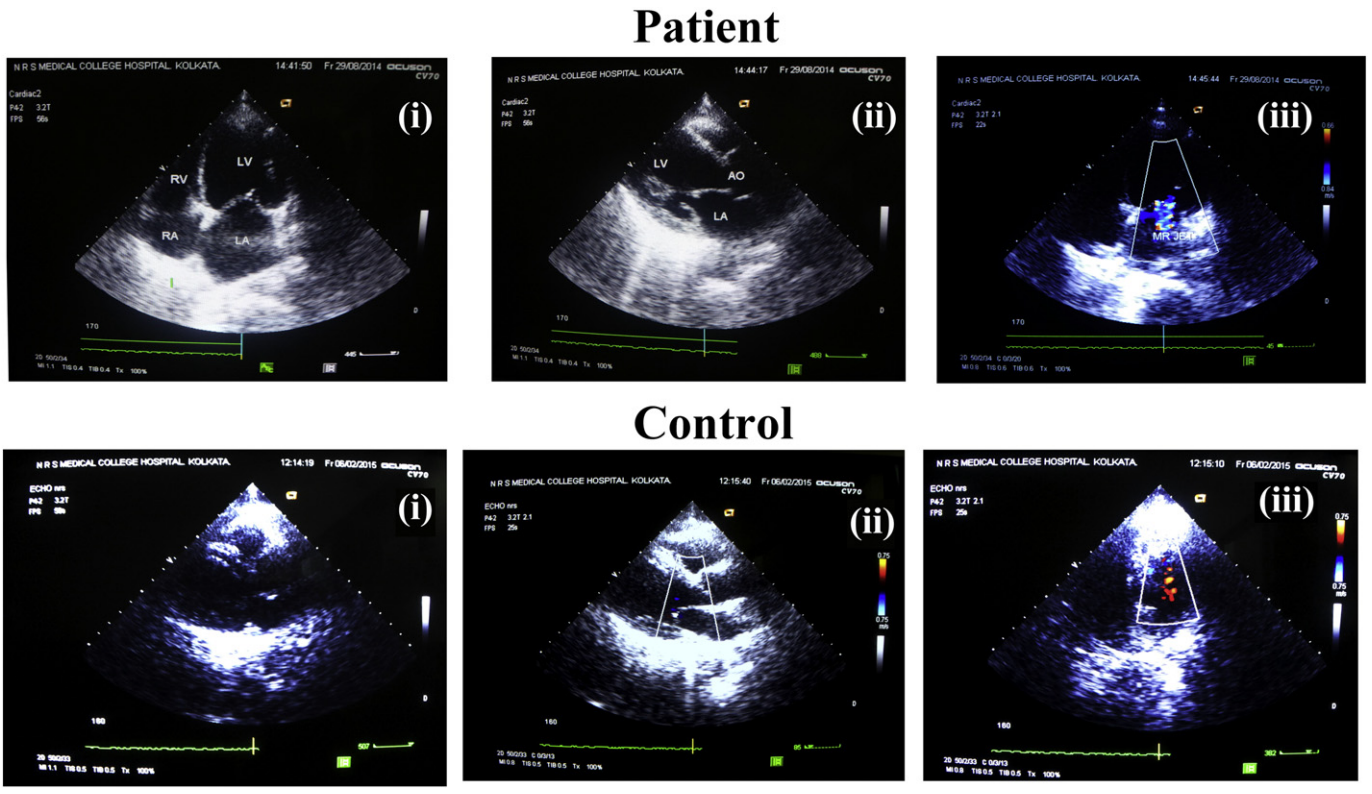

Fig. 2. A representative Echocardiogram of a DCM patient and a control subject. ECHO for the patient sample showed the dilation of LV and presence of mitral regurgitation and global hypokinesia.

Table 2
Clinical status.

Serial no.	Patient ID	Chest X-ray	ECG	Echocardiography			Coronary angiography
				Dilated cardiac chamber	Global hypokinesia	LV ejection fraction	
1	S1	Cardiomegaly	Sinus tachycardia	√	√	35%	Normal
2	S2	Cardiomegaly, pulmonary plethora	Sinus tachycardia, left ventricular hypertrophy	√	√	22%	Normal
3	S3	Cardiomegaly, patchy pneumonia	LBBB	√	√	30%	Normal
4	S4	Cardiomegaly	LBBB	√	√	28%	Normal
5	S5	Cardiomegaly	LBBB	√	√	35%	Normal
6	S6	Cardiomegaly	LBBB, complete heart block	√	√	30%	Normal
7	S7	Cardiomegaly	LBBB	√	√	20%	Normal
8	S8	Cardiomegaly, patchy pneumonia	LBBB	√	√	28%	Normal
9	S9	Cardiomegaly	2:1 AV block	√	√	32%	Normal
10	S10	Cardiomegaly	LBBB	√	√	30%	Normal
11	Control	No cardiomegaly	Normal	Normal cardiac chamber	Good LV systolic function	60%–65%	Normal

of Charcot–Marie–Tooth disease CMT2B1 in North Western African population [29,30]. SNP rs538089 results from a change of nucleotide from T to C at the third base of codon 287; this codon of *LMNA* is also reported to be associated with a frame shift mutation which causes DCM [25]. Thus, a simple variation but not mutation at codon 287 of *LMNA* justifies its association with DCM. Next, we identified SNP rs505058 in our study. In rs505058 an alteration at the third base of codon 446 from T to C results in same sense variation to Aspartic acid at exon 7. In other reports from GWAS this SNP was found to be associated with patients afflicted with Late Onset of Alzheimer's disease (LOAD) in elderly males [31,32]. It was also found to be involved with Werner's syndrome and CMT2B1. The codon 446 was shown to be involved with a mutation D446V in patients suffering from EDMD [33]. EDMD is a condition characterized by weakness of the muscles used for movement (skeletal muscles) and the heart (cardiac) muscle. Consequently, patients suffering with DCM also show weak cardiac muscles, thus a variation at codon position 446 might contribute to the diseased phenotype of DCM.

The most widely studied *LMNA* SNP in literature is rs4641, which is also the most frequently encountered SNP in our study. A substitution of nucleotide C to T at the third base of codon 566 (exon 10) results in the development of SNP rs4641 [21,29,31,32,34] which still codes for Histidine. Previous studies in ethnically distinct population have shown its association with Type 2 Diabetes (T2D), the metabolic abnormalities, and obesity-related traits in some [20,21,34–36]; but not all studies [37,38]. The position associated with rs4641 is very important as it is located adjacent to a splice site at exon 10; alternative splicing at this site leads to the production of either lamin A or C transcripts or protein. A recent report emphasizes the role of T- allele at position 566 in regulating the lamin A to C mRNA ratio in T2D patients [35]. Other reports have shown decreased level of lamin A mRNA in patients suffering from DCM [39]. Thus, it can be hypothesized that rs4641 might contribute to the DCM phenotype by regulating the lamin A to C mRNA ratio although the fact that this is definitely not to be the sole player leading to the pathogenesis of the disease. Interestingly, rs4641 is further associated with Werner's syndrome [29]. This makes rs4641 a candidate gene locus as a biomarker for various kind of disorder along with DCM. The other two SNPs rs534807 and rs7339 were reported to be associated with CMT2B1 a commonly known laminopathy. Most of these SNPs were previously reported to be involved with various kinds of disorders except; rs80356803 and rs646840 which were not reported earlier to be involved with any disorders. We showed for the first time the association of these two SNPs with DCM in Indian population and reside in the intron of *LMNA*. rs538089, rs505058 and rs4641 are the three SNPs identified in Indian population from our study; were also previously known to be involved with DCM in a French population [25]. However we did not observe any other associated symptoms of laminopathies e.g. muscle degeneration, sensory and motor neuron mediated voluntary movement of muscles or unusual accumulation of adipocytes as observed in EDMD, CMT2B1 and FPLD respectively [40–43]. Although all patients were admitted based on symptoms which included cough and respiratory distress, these symptoms are common observations for patients of decompensated Dilated Cardiomyopathy.

Currently, there are no special criteria to distinguish *LMNA* mutation associated Cardiomyopathy and other forms of idiopathic Cardiomyopathy. Genetic heterogeneity is a hallmark for autosomal dominant DCM–Conduction Defects (DCM–CD) where nearly 40 genes have

Table 3
Genetic analysis of *LMNA*.

dbSNP	Amplicon ID	Position of SNP in amplicons	Exon	Type	Zygosity	Genotype	Ref	Variant	Var freq	Ref coverage	Var coverage	Location	Translation impact	Protein variant	Patient ID
rs121117552	*LMNA*_PP3_1	167	3	SNP	Het	G/A	G	A	44.44444	130	104	Exon	L204L	WT	S8
rs538089	*LMNA*_PP5_1	144	5	SNP	Het	T/C	T	C	60.71429	55	85	Exon	A287A	WT	S7
rs505058	*LMNA*_PP7_81	135	7	SNP	Het	T/C	T	C	55.69493	883	1110	Exon	D446D	WT	S1
rs4641	*LMNA*_PP10_147	82	10	SNP	Het	C/T	C	T	51.00671	292	304	Exon	H566H	WT	S3,S4 & S9
rs646840	*LMNA*_PP6_204	221	NA	SNP	Het	G/T	G	T	81.12642	315	1354	Intron			S1
rs534807	*LMNA*_PP6_204	221	NA	SNP	Het	G/A	G	A	6.190476	197	13	Intron			S1 & S3
rs80356803	*LMNA*_PP1_1	56	NA	SNP	Het	T/C	T	C	7.54717	49	4	Utr_5			S9
rs7339	*LMNA*_PP12_301	209	NA	SNP	Het	G/C	G	C	50.65327	982	1008	Utr_3			S7

Het SNPs = number of called heterozygous SNPs in target regions or loci, ref = genotype in the reference gene, variant= genotype obtained in the patient, var freq = frequency of the variant allele, ref coverage or var coverage = the total reads covering the position, location = position in the gene.

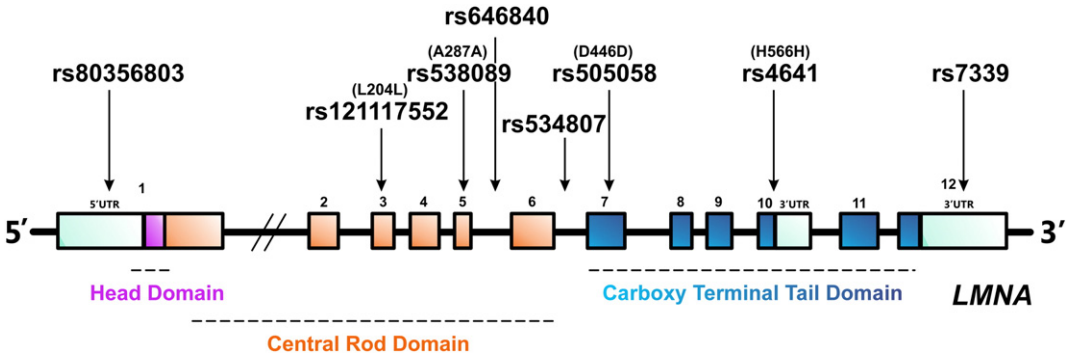

Fig. 3. Schematic representation of 12 exons of *LMNA*. The SNPs identified in our genetic analysis are displayed at their respective position in the gene with arrows.

been uncovered to produce disease phenotype [44–48]. Interestingly majority of these genes encode cytoskeletal and sarcomeric proteins. However, mutations in *LMNA* coding for lamin A/C, DES coding for Desmin and SCN5A encoding cardiac sodium channel protein are still regarded as primary causes for DCM–CD. Therefore, the genes involved in mechanical force transduction and propagation are mutated thereby precipitating the disease. DCM associated with all levels of conduction system defects like sick sinus syndrome, atrioventricular block or bundle branch blocks could be traced back to mutations in *LMNA*. The conduction disease defects call for pacemaker implantation in patients with *LMNA* mutations. In young healthy *LMNA* carriers, arrhythmias including atrial ectopy, atrial fibrillation, non-sustained ventricular tachycardia and ventricular arrhythmias can be the earliest manifestation of the *LMNA* mutation prior to chamber dilatation [10,23]. Once DCM is clinically confirmed the management follows standard care for heart failure including ACE inhibitors, beta blockers, diuretics and aldosterone antagonists as per the recommendation of New York Heart Association functional class. However, it is not clear whether early administration of these therapeutic agents prior to confirmation of the disease can modify the aggressive nature of *LMNA* Cardiomyopathy. As *LMNA* Cardiomyopathy is diagnosed genetically, the sequencing of exons and intron–exon junctions has been reported in various case studies. Due to the advent of deep sequencing technology these sequencing data present a reliable database for cataloging mutations or SNPs in the *LMNA* gene which might lead to the disease.

The ultimate goal of medical research is to uncover novel diagnostic and therapeutic modalities which will be of clinical utility. Therefore, detailed genetic analysis of the *LMNA* gene in the affected patients at the early stage might help in better management and hence therapeutic intervention. Such genetic screening based on *LMNA* gene could be also extended to the nearest kins of the patient to verify any hereditary penetrance which could be then treated as cases of familial DCM. We

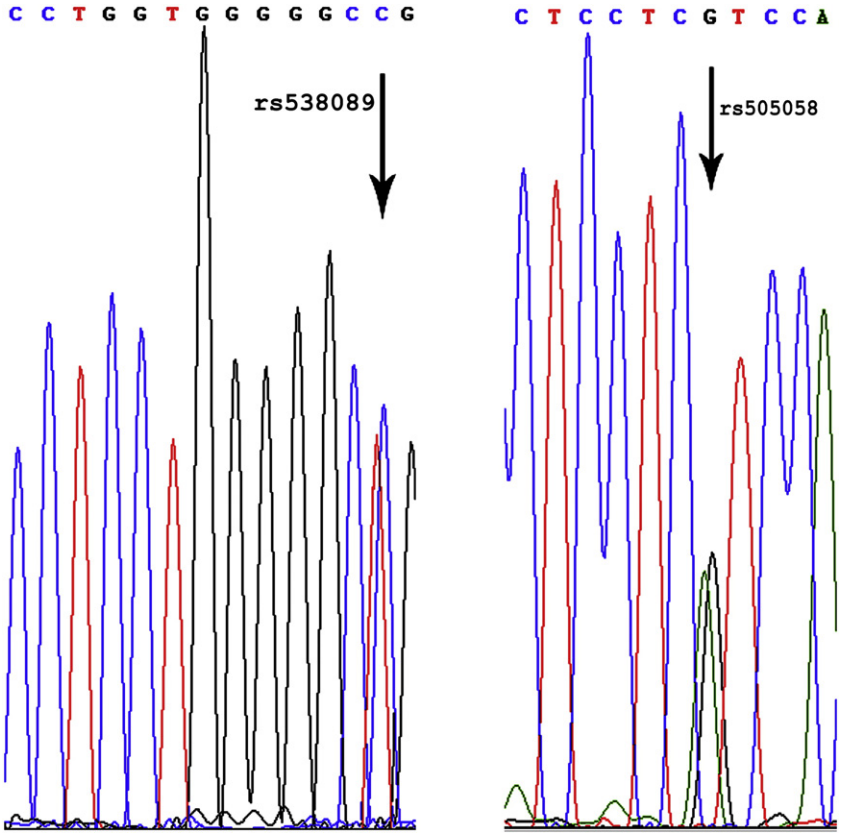

Fig. 4. A representative chromatogram of 2 SNPs identified in DCM patients validated by Sanger sequencing; rs538089 validated using forward primer for exon 5; rs505058 validated using reverse primer for exon 7.

conclude that polymorphism in *LMNA* is one of the major genetic risk factor for the pathogenesis of DCM other than mutation in *LMNA*.

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Conflict of interest

Authors declare no conflict of interest.

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